Roles of Fungal Sensitization in Severe Asthmatic Patients
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RATIONALE: Fungal exposure is an important trigger of asthma exacerbation. However, little is known about the effects of fungal sensitization on the characteristics of severe asthma.

METHODS: We collected data from 146 severe asthmatic patients and analyzed the data including sensitization to fungi (Aspergillus, Alternaria, Cladosporium, Penicillium, and Trichophyton) and non-fungal antigens (house dust mite, dog, cat, cockroach, moth, and chironomidae) as well as Asthma Control Test (ACT) score, pulmonary function, and fractional exhaled nitric oxide (FeNO) value. The diagnosis of sensitization was made by detecting an increase in serum IgE specific to these allergens. We defined atopic asthma patients sensitized by one or more allergens.

RESULTS: 86 patients (59%) were sensitized to one or more allergens. 35 patients (24%) were sensitized to fungal allergen, among which Aspergillus (22 patients, 15%) and Trichophyton (19 patients, 13%) were most common. In the 86 atopic asthmatics, 5 were only sensitized to fungal (Group F), 51 were only sensitized to non-fungal allergen (Group N), and 30 were sensitized to both fungal and non-fungal antigens (Group F+N). ACT score of Group F+N was significantly lower than that of Group F. FeNO level was much higher in Group F+N compared with Group N. About a half of Group F+N were depending upon oral steroid therapy.

CONCLUSIONS: Fungal sensitization, in addition to non-fungal sensitization, is associated with the poorer asthma control in severe asthmatic patients.

Oral Corticosteroid Use and Health Outcomes in Patients with Severe or Difficult-to-Treat Asthma
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RATIONALE: There are limited studies in asthma examining the association between oral corticosteroid (OCS) use and health outcomes, including death, with especially few studies examining dose-response effects.

METHODS: Patients aged ≥6 years with severe or difficult-to-treat asthma from The Epidemiology and Natural History of Asthma: Outcomes and Treatment Regimens (TENOR) 3-year observational study were included (N=4,756). OCS exposure was examined as a dichotomous (yes/no) variable and per 10mg dose increase. Longitudinal health outcomes were assessed semi-annually. Analyses included patients without a given condition at baseline. Multivariable Cox models assessed associations between baseline OCS exposure and risk of new-onset morbidities or death.

RESULTS: After adjusting for age, sex, education, race/ethnicity, and asthma control, baseline OCS exposure (yes/no) was associated with an over five-fold increased risk of death (hazard ratio (HR): 5.26, p<0.001). Each 10mg increment in baseline OCS dosage was associated with a HR: 1.40 (p<0.001) increased risk of death. OCS exposure was associated with an increased risk of osteoporotic fractures (HR: 2.26, p=0.005); an association with dose was not observed. An association between OCS exposure and risk of cataracts was borderline significant (HR: 1.46, p=0.069); no association with OCS dose was observed. There were no associations between OCS exposure and new-onset diabetes or new-onset obesity.

CONCLUSIONS: OCS exposure was associated with an increased risk of death and osteoporotic fractures in this population, with a dose-response effect observed for death. Although we adjusted for potential confounders, the extent to which these associations were due to OCS itself or the requirement for OCS is uncertain.

Increased Serum Levels of Inflammatory Cytokines in Severe Childhood Asthma
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RATIONALE: Care of children with severe asthma remains a clinical challenge, partly due to the heterogeneity of the disease and the lack of definite biomarkers. In this study, we compared levels of Th1 and Th2 related inflammatory cytokines in serum from children with therapy resistant and controlled asthma.

METHODS: Children with therapy resistant asthma (n=34, mean 13.3 years) and controlled persistent asthma (n=39, mean 13.8 years) were included. The protocol included Asthma control test, methacholine challenge, measurement exhaled nitric oxide (FeNO), and blood sampling. Tryptase, Eosinophilic cationic protein (ECP), Eosinophilic protein x (EPX), Eosinophilic peroxidase (EPO), Human neutrophil lipocalin (HNL) and Myeloperoxidase (MPO) were analysed from serum using multiplex technology.

RESULTS: Severe asthmatic children had inferior asthma control (p<0.001) and increased bronchial hyperresponsiveness (p=0.01) in spite of high doses of inhaled steroids (>800ug budesonide), compared to children with controlled asthma. FeNO (15 vs. 17, p=0.93) and IgE (219 vs. 280, p=0.92) were comparable in these two patient groups. Serum levels of ECP (p=0.017), HNL (p=0.026) and MPO (p=0.050) were higher in severe asthmatic children compared to children with controlled asthma. No significant differences were seen when comparing Tryptase (p=0.72), EPO (p=0.22) and EPX (p=0.30).

CONCLUSIONS: Severe asthmatic children have increased serum levels of cytokines related to both Th1 and Th2 inflammation compared to controlled asthmatics. These results indicate a heterogeneous pattern of inflammation. Further studies are required to validate and clarify the mechanisms behind and the clinical utility of these observations.
271 Chronic Eosinophilia Associated with Strongyloides Infection, Severe Asthma, and Central Bronchiectasis

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RATIONALE: Strongyloides stercoralis infections are often missed because they are asymptomatic, or present with non-gastrointestinal manifestations such as skin rash, chronic eosinophilia or pulmonary involvement. We describe a severe asthmatic patient with abnormal lung imaging who was positive for S. stercoralis serum IgG and whose symptoms improved after ivermectin followed by corticosteroid treatment.

METHODS: Case description.

RESULTS: A 65-year-old female with severe asthma, who emigrated to NYC from the Dominican Republic 18 years ago, was referred to us for the evaluation of possible allergic bronchopulmonary aspergillosis. Prior lung imaging had revealed central bronchiectasis and basal infiltrates, and she had a 2-year history of eosinophilia. On our evaluation, the Asthma Control Test (ACT) score was 12. Percutaneous skin testing was positive for tree and ragweed pollens, but negative for A. fumigatus and other mold species. Laboratory tests revealed eosinophilia of 28% (absolute eosinophil count - 2100), total serum IgE of 364.6 IU/mL (nl <140), normal quantitative immunoglobulins, negative ANCA, and negative serum A. fumigatus IgE and IgG. Further evaluation revealed negative stool studies for parasites, but positive S. stercoralis serum IgG. She received ivermectin (200 mcg/kg/day for 2 days). The asthma symptoms persisted so the patient was treated with a tapering course of prednisone for 2 weeks. Two months later the patient's asthma symptoms had improved (ACT score of 20), and chest x-ray demonstrated resolution of the pulmonary infiltrates.

CONCLUSIONS: Given the variable clinical manifestations and possible dangers (e.g. hyperinfection) associated with strongyloidiasis, clinicians must maintain a high index of suspicion in asthmatics being considered for oral corticosteroid therapy.

272 Distinct Phenotypes of Childhood Asthma: Cluster Analysis in a Longitudinal Birth Cohort

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RATIONALE: Distinct phenotypes of adult asthma have been defined, but fewer studies have described childhood asthma phenotypes. We hypothesized that cluster analysis could differentiate phenotypes of childhood asthma in a longitudinal birth cohort.

METHODS: Children enrolled in Cincinnati Childhood Allergy and Air Pollution Study (CCAPS), a birth cohort study, underwent clinical assessments at ages 1, 2, 3, 4, and 7. Asthma was defined at age 7 by parental report of asthma symptoms with confirmed airway reversibility or positive methacholine testing. An unsupervised, hierarchical clustering method was applied to 72 children with asthma to identify subgroups having similar characteristics with respect to sex, race, peak flow, FEV1%, FVC%, FEF25-75%, early aeroallergen and food sensitization, and wheezing at ages 1, 2, 3, 4, and 7.

RESULTS: Four clusters of asthmatics were identified and described by gender, atopy (based on positive aeroallergen skin testing), wheezing history, and lung function. Cluster 1 (n=23) included atopic (70%) males (91%) with late-onset wheezing. Cluster 2 (n=13) contained non-atopic (92%) males (70%) with early-onset wheeze and reduced FEV1 at age 7. Cluster 3 (n=19) was characterized by atopy (74%) and persistent childhood wheezing. Cluster 4 (n=17) included females (82%) with infrequent wheeze and normal FEV1.

CONCLUSIONS: Cluster analysis can be used to define phenotypes of childhood asthma in a birth cohort. Future directions include assessing whether environmental exposures, including traffic exposure, are associated with specific subtypes of asthma. Asthma phenotypes may also be useful to predict asthma severity and guide clinical management of asthmatic children.

273 Relationship Between Asthma Phenotypes and Hypersensitivity Vasculitis

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RATIONALE: Development of vasculitis in asthmatics may be related to the phenotype of asthma in adults. The relationship among asthma phenotypic markers including total IgE level, eosinophil counts, skin prick test positivity was and vasculitis markers including ANCA titers were assessed.

METHODS: Total IgE level, inhalant allergen skin prick tests, and eosinophils counts, and ANCA levels were assessed in 60 patients with asthma and also in 60 systemic vasculitis patients.

RESULTS: IgE-mediated asthma occurred with moderate and severe eosinophilia and positive skin prick test in 83% of pANCA-positive patients with vasculitis, often consistent with Churg-Strauss Syndrome. IgE-independent asthma occurred with mild eosinophilia in 17% of pANCA-positive vasculitis, especially with drug-induced vasculitis. ANCA titers were significantly higher in patients with hypersensitivity vasculitis (Churg-Strauss Syndrome) than in patients with other types of systemic vasculitis for both male and female adults. Skin prick test positivity particularly to pollen allergens was significantly related to total IgE levels in IgE mediated asthma subtypes while very high pANCA titers were related to eosinophil counts only in patients with hypersensitivity vasculitis.

CONCLUSIONS: Vasculitis subtypes seen in select asthma patients were associated with allergic activity including high IgE level and eosinophil count and with autoimmune activity including pANCA titer.
Pediatric Asthma Deaths in North Carolina, 1999-2012
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RATIONALE: Pediatric asthma fatalities are considered largely preventable. However, while not common, they continue to occur. The purpose of our study is to evaluate the prevalence of previously-described demographic risk factors for asthma fatalities and new factors such as obesity, in order to identify common features and areas needed for further research.

METHODS: We performed a retrospective review of asthma fatality cases referred to North Carolina’s Office of the Chief Medical Examiner. We reviewed cases from 1999-2012 with decedents 1-18 years old, and abstracted information on demographics, timing, location, and pathology.

RESULTS: We identified 34 pediatric asthma deaths (range: 15 months-17 years; average: 9) from 1999-2012. Twenty-nine decedents (85%) were black, and two were Hispanic. Fifty-three percent were female. Deaths were nearly equal during colder and warmer months. Twenty-two deaths (65%) occurred during daytime hours (7am to 9pm). BMI for 32 decedents identified four as obese, five overweight, and three underweight using CDC guidelines for pediatric BMI percentiles. Pathology results mentioned eosinophils in 28 of 29 cases (93%), while no reports mentioned neutrophils. Other common features included basement membrane thickening and mucus plugging, with 18(60%) and 19 cases (63%), respectively.

CONCLUSIONS: Eighty-five percent of our cohort was black. Deaths were evenly distributed between colder and warmer months, and neutrophils were not mentioned in pathology. Obesity prevalence among our cohort was lower than national pediatric obesity prevalence. These comparisons are limited by our small sample size. Possible directions for further study include socioeconomic differences, rural versus urban environment, and weather factors.

Impact of Self-Identified Race and Genetic Ancestry on Airway Inflammation in Asthma
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RATIONALE: African-Americans suffer a disproportionate burden of asthma morbidity compared to Caucasians. Airway inflammation, as measured by sputum cytology, is associated with increased asthma exacerbation risk and poor treatment responses to inhaled corticosteroids. Whether African-American race and more specifically African ancestry contributes to airway inflammatory patterns in asthma is not clear.

METHODS: Self-identified African-Americans (n=23) and Caucasians (n=16) with asthma taking inhaled corticosteroids were included in the analysis. All subjects underwent induced sputum and peripheral blood collection. Sputum was processed and cell differentials were calculated as the % of non-squamous epithelial cells in the whole sputum expectorated. DNA was extracted from whole blood or peripheral blood mononuclear cells of African-American subjects. Genetic ancestry assessment was performed from DNA samples using an enriched panel of 100 unlinked Ancestry Informative Markers (AIMs) spanning 22 chromosomes and the program STRUCTURE.

RESULTS: There was no difference found in the percent of eosinophilic asthma (17% sputum eosinophils) among self-identified African-Americans and Caucasians (17% vs. 13%, respectively, p>0.09). The average African ancestry estimate for self-identified African-Americans was 77±0.1%. Using a Spearman’s correlation statistic, no correlation was found between percent African ancestry and percent sputum eosinophils in self-identified African-Americans with asthma (r=0.20, p=0.4).

CONCLUSIONS: Our analysis did not identify a relationship between self-identified race or African ancestry with eosinophilic airway inflammation in subjects with asthma. Our findings suggest differences in airway inflammation are unlikely to contribute to the greater asthma morbidity seen in African-Americans and could be used to inform the design of an adequately powered study to answer the question definitively.
**277** Asthma Severity in Korean Children Assessed By the 12 Pediatric Allergists Working at Different Hospitals

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**RATIONALE:** Information on the exact distribution of severity is critical. However no data is available on the Korean asthmatic children.

**METHODS:** We have requested 12 pediatric allergists to review the medical records on asthmatic children who visited their own clinic during the most recent 3 months. Based on the subjects’ symptoms, signs and their medications to maintain control, their asthma severities were assessed according to both the Global Initiative for Asthma (GINA) criteria and the Japanese Pediatric Guidelines for the Treatment and Management of Bronchial Asthma (JPGL) criteria. Thereafter, the disparity between severities assessed by the two criteria were evaluated.

**RESULTS:** A total of 906 cases (less than 3 years, 22.3%; 3 to 6 years, 21.3%, more than 6 years, 56.4%) were reviewed. When we assess the severities of asthma via GINA criteria, 328/906 cases (36.2%) were mild intermittent, 327 (36.1%) cases were mild persistent and 227 (25.1%) cases were moderate persistent. Whereas only 24 (2.6%) cases were severe persistent. On the other hand, when we classify the severity of asthma by the JPGL criteria, 244/906 cases (26.9%) were intermittent, 300 (33.1%) cases were mild persistent and 342 (37.7%) cases were moderate persistent. Whereas only 20 (2.2%) cases were severe persistent.

**CONCLUSIONS:** In Korea, about 1/3 of asthmatic children had intermittent asthma whereas only less than 3% of subjects had severe persistent asthma. Considering the disparity between both guidelines, specific asthma guideline for Korean children based on our own data should be needed.

**278** Relationship Between Breast-Feeding and Wheeze Risk in Early Childhood in Korean Children: Based on the Fifth Korea National Health and Nutrition Examination Survey 2010–2012

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**RATIONALE:** There are conflicting evidences concerning the relationship between breast-feeding and development of wheezing in early childhood. Epidemiological evidence for a role of breast-feeding in risk of wheezing is inconclusive. The objective of this study was to investigate the associations between breast feeding and risk of current wheezing in early childhood in Korea.

**METHODS:** We combined the fifth Korea National Health and Nutrition Examination Survey data collected from 2010 to 2012 and analyzed 1,011 children from 1 to 3 years old who had been surveyed in regards to breast-feeding. Multivariate regression analysis was used to identify association among the following variables: presence of current wheezing, feeding types and duration of breast-feeding.

**RESULTS:** Prevalence of exclusive breast-feeding and current wheezing decreased both annually from 2010 to 2012. In the univariate analysis, breast-feeding, formula-feeding, duration of breast-feeding were not associated significantly with current wheezing of children younger than 3 years old. No measurable statistically significant relationship was observed among breast-feeding, formula-feeding, duration of breast-feeding and risk of current wheezing in the multivariate analysis.

**CONCLUSIONS:** The present study showed no statistically significant relationship between breast-feeding and the risk of wheeze in early childhood in Korean children. National prospective study is needed to clarify the role of breast-feeding in development of current wheezing.

**279** Association Between Asthma-Related Emergency Department Visits, Meteorological Measurements, and Air Quality Concentrations in the Bronx (2001-2008)

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**RATIONALE:** To evaluate the association between asthma-related emergency department visits (AREDV), meteorological measurements, and air quality concentrations in a high asthma prevalence area, the New York City borough of the Bronx.

**METHODS:** We previously investigated AREDV at two Bronx hospitals (Montefiore-Weiler and Moses) from 1/2001 to 12/2008, and found that a spring increase in AREDV closely correlated with high tree pollen counts. In this study we analyzed the association between AREDV and other indices of air quality including pollutants (nitrogen dioxide-NO2, sulfur dioxide-SO2) and meteorological factors (precipitation, atmospheric pressure, humidity, temperature). Daily AREDV numbers were obtained through the CLG software. Daily counts for pollutants and meteorological variables were obtained through the National Climatic Data Center. Data were statistically analyzed and graphed as daily values.

**RESULTS:** From 2001-2008, there were a total of 42,065 AREDV at the two hospitals. We consistently observed three distinct peaks of AREDV: January (winter), May (spring), and November (fall). The winter peak of AREDV correlated with increased NO2 and increased SO2. High precipitation levels in the fall closely associated with the fall peak of AREDV. We did not observe an association between atmospheric pressure or humidity and AREDV. High temperatures in the summer were associated with decreased AREDV.

**CONCLUSIONS:** There is a consistent association between the winter peak of AREDV and increased winter NO2 and SO2 concentrations. Increased precipitation closely associated with the fall peak of AREDV. These findings may help to predict periods of increased asthma exacerbations, although multivariate and prospective analyses are necessary.
280 Two Pathways Leading to Bronchial Asthma from Cough Variant Asthma Characterized By Different Clinical and Genetic Risk Factors
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RATIONALE: Cough variant asthma (CVA) is an inflammatory disease that is considered a preliminary stage of bronchial asthma (BA). The exact factors of the progression of CVA to BA remain unclear.

METHODS: We analyzed the clinical data and genetic polymorphism from 197 BA and 115 CVA patients. We performed multiple logistic regression analysis to determine the factors that contribute to the progression of CVA to BA. Further analysis was carried out on genes that had a significant confounding effect on the most important factors.

RESULTS: None of the analyzed genetic polymorphisms were significantly correlated with disease progression from CVA to BA. Multiple logistic regression showed that disease progression from CVA to BA was significantly correlated with IgE-RAST score (p = 0.009), FEV1.0%pred (p = 0.0002), and fractional exhaled nitric oxide (FeNO) (p = 0.002). The multivariate regression trees showed that the most effective factor that discriminates CVA and BA was elevated FeNO. Genetic polymorphisms associated with the risk of inflammation in BA were single-nucleotide polymorphisms (SNPs) in LT4H, MMP8, and ADAM33. Together with inflammation, these SNPs promoted the progression from CVA to BA.

CONCLUSIONS: Genetic polymorphism alone could not clarify the progression of CVA to BA. Two pathways were suggested for the exacerbation of airway hyperresponsiveness that eventually causes airflow obstruction. One was that airway inflammation due to atopic predisposition enhances airway hyperresponsiveness; the other was that a factor unassociated with inflammation enhances airway hyperresponsiveness and subsequent airflow obstruction. In both cases, progressive exacerbation leads to the development of BA from CVA.

281 Evidence of Wear-Off Effect from Ig Infusion Therapy in Routine Clinical Practice
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RATIONALE: Immunoglobulin infusion therapy in patients with primary immunodeficiency disease (PID) may provide sub-therapeutic immunoglobulin levels towards the end of the infusion cycle. Current literature is unclear as to the extent of clinical outcomes, such as infections and their economic magnitude, during this wear-off period, prior to next infusion.

METHODS: The MarketScan (US) managed care database (2008–2010) was used to identify a cohort of patients with PID receiving Ig infusions over 7 consecutive months. Infections were identified as health care utilization in ambulatory clinics or other facilities, emergency rooms, or hospitals, with infection coded as reason for visit. Volumes were assessed in 9-day intervals, from day of last Ig infusion and to day of first infection. Excess of infected patients/hospitalizations indicative of wear-off effect were calculated by subtracting mean volumes recorded over first two 9-day periods post-infusion from the number in the period before next infusion.

RESULTS: From a cohort of >1,000 patients with PID, 399 patients with infection were reported with 107, 118, 174 occurring during intervals for Days 1–9, 10–18, and >18, respectively. The excess of infected patients suggestive of wear-off was 61.5 (p<0.01). For infection-related hospitalizations, 72 were recorded with 20, 15, and 37 occasions, respectively, for the above time intervals; the wear-off excess was 19.5 (p<0.02).

CONCLUSIONS: Wear-off explains about 15% of infections with 27% of infection-related hospitalizations occurring in the Ig infusion-treated PID population. These results suggest that wear-off from Ig infusion therapy causes burden to both patients and the health care system.

282 Common Variable Immunodeficiency-Runs in the Family
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RATIONALE: Common Variable Immunodeficiency (CVID) is a disorder that involves low levels of most or all of the immunoglobulin classes, poor specific antibody responses and frequent bacterial infections. We report the case of a family with genetic and phenotypic markers in 7 family members with and without the TACI (transmembrane activator and calcium-modulator and cyclophilin ligand interactor) mutation.

METHODS: A retrospective chart review was conducted on 6 patients 8 to 63 years old amongst the same family with and without the genetic mutation TACI characteristic of CVID.

RESULTS: All 6 patients in the family evaluated had underlying immunodeficiency with varying degrees of hypogammaglobulinemia. Of those 6, there were 5 with a mutation suggestive of CVID. One member, the maternal grandmother, was not evaluated for the mutation but died from Lymphoma a common concern in those with CVID. Amongst this family there were 5 family members (and 2 not evaluated in detail) with report of chronic sinusitis, fatigue, infections including bronchiecstasis, pneumonias, otitis media all requiring frequent antibiotic treatment. Due to uncontrolled infections and persistent symptoms patients were started on either intravenous immunoglobulin (IVIG) or subcutaneous immunoglobulin (SCIG) with notable improvement in symptoms and laboratory markers including one member with almost complete resolution of bronchiecstasis.

CONCLUSIONS: This cohort demonstrates the wide phenotypic variability in patients with the same TACI mutation. One patient without the mutation was also symptomatic. All responded well to IVIG or SCIG treatment.

283 Transitional B Cells, CD21low and Plasmoblasts in Patients with Ataxia-Telangiectasia
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RATIONALE: We aim to evaluate the proportion of transitional immature, CD21low B cells and plasmoblasts in patients with Ataxia-telangiectasia (AT), a complex disease with humoral and cellular immune dysfunction.

METHODS: Blood samples were obtained from 18 AT patients and 15 age-sex-matched controls (C). T, B, and NK cells were enumerated from whole blood samples. Peripheral blood mononuclear cells were cryopreserved, thawed and stained with conjugated monoclonal antibodies. Five-color flow cytometric immunophenotyping was performed to characterize: transitional B cells (CD3−CD19+CD24+CD38−), CD21low B cells (CD3−CD19+CD21+CD38−) and plasmoblasts (CD3−CD19+CD27+IgD+).

RESULTS: From 18 patients, 15 were male and 3 female, aged from 5-25 years old. Ten of them are being treated with immunoglobulin replacement therapy. Lymphocyte numbers were reduced in AT patients (AT = 928–4579 cel/mm3; C = 1646–6601 cel/mm3;p = 0.001). Total CD3+ (AT = 1163.8 cel/mm3; C = 2247.2 cel/mm3;p = 0.001), CD4+ (AT = 531.4 cel/mm3; C = 1153.3 cel/mm3;p < 0.001) and CD8+ (AT = 507.6 cel/mm3; C = 880.3 cel/mm3;p = 0.007) numbers were decreased. B cells counts also showed a reduction (AT = 118.7 cel/mm3; C = 649.3 cel/mm3;p < 0.001). By contrast, natural killer numbers were increased (AT = 583.9 cel/mm3; C = 357.3 cel/mm3;p = 0.04). Transitional B cells proportion was reduced compared with those seen in healthy control subjects (AT = 2.2%; C = 7.3%;p = 0.001). On the other hand, CD21low B Cells showed an increased proportion (AT = 25%; C = 4.9%;p < 0.001). Plasmoblasts did not differ (AT = 2.4%; C = 2.4%;p = 0.93).

CONCLUSIONS: Patients with AT had disturbed B-cell homeostasis as evidenced by low transitional B cells and a large proportion CD21low B cells.
**284 Comparison of Clinical Outcomes and Laboratory Measures in Patients with Common Variable Immunodeficiency on Subcutaneous Immunoglobulin Replacement Versus Intravenous Immunoglobulin Replacement**

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**RATIONALE:** Immunoglobulin replacement can be life-saving for certain individuals with immunodeficiencies. Several options for IgG replacement exist, including preparations of both intravenous (IVIG) and subcutaneous IgG (SCIG) replacement. SCIG administration is an increasingly used method of replacement with potential advantages including fewer systemic side effects, no need for IV access, patient-reported improved quality of life, and decreased cost. In addition, patients with associated co-morbidities, for instance, those that cause protein loss, may demonstrate more stable, consistent IgG levels on subcutaneous replacement when compared to intravenous replacement. However, to our knowledge, these experiences are less well validated, and may be of assistance to providers in discussing treatment options with patients.

**METHODS:** Using retrospective chart review, we examined three cases in which SCIG and IVIG was administered to patients with common variable immunodeficiency (CVID) and protein-losing co-morbid disease.

**RESULTS:** All three patients demonstrated improvement in infection rate, IgG levels, and co-morbid disease when on SCIG as compared to IVIG.

**CONCLUSIONS:** These findings suggest that the pharmacokinetics of subcutaneously administered IgG translate into more consistent IgG levels, contributing to clinical improvement in immunodeficient patients with protein-losing co-morbidities. Limitations to this study are small patient numbers, retrospective design, and potential therapeutic bias. Further characterization of the effects of co-morbid conditions on immunoglobulin replacement is critical to providing improved and informed patient care. The final decision of using subcutaneous versus intravenous immunoglobulin continues to be based on an important discussion between the patient and physician, considering multiple factors including cost, patient preference, compliance, and co-morbid disease.

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**285 Sub-Optimal Response to PCV-13 Vaccinations Among Children with Recurrent Sinusitis and Otitis Media**

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**RATIONALE:** Streptococcus pneumoniae is frequently isolated from the upper respiratory tracts of children with acute sinusitis (AS) and / or acute otitis media (AOM). Beginning in the year 2010, immunization of children between the ages of 6 to 24 mos. with the 13-valent pneumococcal vaccine (PCV13) was recommended. Neither the seroconversion rates nor the duration of effective titer preservation (> 1.3 mcg/ml) among infection-prone children has ever been studied.

**METHODS:** Titers to 23 common pneumococcal serotypes were evaluated for 11 consecutive children (avg. age = 35 mo. / range 16-53 mos.) with recurrent AOM and/or AS. (Quest Diagnostics IgG-pneumococcal Panel identifies 12 of 13 serotypes contained in PCV13 and 11 other serotypes contained in PPSV23).

**RESULTS:** 45% of PCV-13 immunized children had less than 7/12 positive PCV-13 pneumococcal serotype titers. The median number of positive titers to non-PCV13 serotypes was 1 out of 11 (mode =1).

**CONCLUSIONS:** Among PCV13 immunized children, age 2-5 yrs. old with recurrent AS and AOM, anti-pneumococcal IgG protect is poor. This observation could be the result of either an initial failure to seroconvert after the 4-injection PCV13 series, or a rapid loss of “protective” titers. The near absence of immunity to common non-PCV13 pneumococcal serotype could be the result of a paucity of natural exposures at an early age, or a relative state of immunodeficiency; in which natural exposures to pneumococci fail to stimulate protective immunity. Evaluation of anti-pneumococcal immunity should be considered if recurrent AS of AOM continues after PCV13 vaccination.

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**286 Pharmacokinetics of RI-002, an Investigational Igiv Preparation**

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**RATIONALE:** IgIV therapy is indicated for the treatment of primary immunodeficiency diseases (PIDD) associated with defects in antibody production. Respiratory pathogens, especially respiratory syncytial virus (RSV) are particularly troublesome for PIDD and other immune compromised patients with T cell defects. Accordingly, we measured levels of antibody against various pathogens as part of the PK evaluation of RI-002, an investigational novel IgIV preparation.

**METHODS:** RI-002, manufactured from plasma collected from donors prescreened for high-titer antibodies to RSV is being studied for treatment of patients with PIDD. Antibody to H. influenzae type b (Hib), cytomegalovirus (CMV), measles, RSV, and selected serotypes of S. pneumoniae (SP) was assayed.

**RESULTS:** PK studies were performed on subjects infused every 3 (3WkSub) or 4 (4WkSub) weeks. 29 subjects (age=3-74), 10 3WkSub and 19 4WkSub received comparable doses of RI-002, 291 to 654 mg/kg and 299 to 760 mg/kg respectively. Mean IgG plasma concentrations and mean Cmax were comparable. AUC was greater for 4WkSub (8,527 ± 2,335 h×g/L), than for 3WkSub (7,322 ± 1,699 h×g/L), a consequence of the 7-day longer period. Specific antibody to Hib, CMV, RSV, and SP serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18, 19F and 23 were uniformly increased, with no substantive differences between the treatment groups.

**CONCLUSIONS:** The infusion of RI-002 appears to have a PK profile similar to commercially available IGIV preparations. Whether the standardized, high-titer antibody to RSV or other polyclonal antibodies against other respiratory and infectious pathogens will provide added clinical benefit requires further study.
B Cell Lymphopenia As a Complication of Remote Rituximab Use in a Patient with Common Variable Immunodeficiency

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RATIONALE: Common variable immunodeficiency (CVID) is characterized by variable manifestations which can include B and T cell lymphopenia. Rituximab is an anti-CD 20 chimeric antibody that has been implicated in prolonged B cell depletion, although this effect rarely persists beyond 1 year. Herein, we present a case of B cell lymphopenia in a patient with CVID, treated with rituximab 8 years prior to presentation.

METHODS: Retrospective chart review was conducted for patient.

RESULTS: A 57 year old female with recurrent sinusitis had a low immunoglobulin profile (IgG 623 mg/dl, IgM 18mg/dl, IgA 34mg/dl) and absent antibody titer to pneumococcus. Lymphocyte subset analysis showed profound B cell lymphopenia and T cell lymphopenia (CD3 661, CD4 459, CD8 237, CD56 250, and undetectable CD19+ B cells). A diagnosis of Good syndrome was considered given the age of presentation and absence of B cells. No thymoma was present on chest imaging. Eight years prior to development of recurrent sinusitis, she received 2 doses of rituximab for treatment of rheumatoid arthritis. Subsequent treatment with replacement immunoglobulin led to resolution of sinus disease.

CONCLUSIONS: Rituximab induced B cell lymphopenia can occur but B cells usually rebound within 6 to 12 months after discontinuation of treatment. The long term affects and significance of rituximab in the realm of immunodeficiency are still not well characterized. Whether B cell lymphopenia in this patient occurred as part of CVID or was secondary to or exacerbated by rituximab remains unclear. However, rituximab is likely a contributing factor in her sustained B cell lymphopenia.

Mutation of the BTK Gene and Genotype-Phenotype Correlation of Chinese Patients with X-Linked Agammaglobulinemia

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RATIONALE: X-linked agammaglobulinemia (XLA) is a primary humoral immunodeficiency in which affected patients have very low levels of peripheral B cells. XLA is caused by mutations in Brutton’s tyrosine kinase gene (BTK).

METHODS: Genetic background and clinical features of one hundred and twenty-nine Chinese XLA patients from 127 families were investigated. Moreover, we reviewed and analyzed all the paper regarding the Chinese BTK mutation study published on Chinese journal, summarized and provided further insight in the genetic background of XLA in China.

RESULTS: Totally 165 mutations in this study and previous studies were identified, including 44 novel mutations and 121 recurrent ones. In this study the mean age at onset was 26.28 years. Incidence of Pneumonia was (77.1%), Incidence of B cell lymphopenia (46.8%) and sinusitis (41.9%). There were 20 patients with arthritis, 15 of whom had severe genotypes (78.9%). The mutations of the patients with arthritis were prone to be more severe.

CONCLUSIONS: A genotype-phenotype correlation was not observed in our study due to the high heterogeneity of BTK gene in XLA patients. Early diagnosis of congenital agammaglobulinemia was possible by molecular analysis of the BTK gene. A large spectrum of BTK gene mutation in China was reported in our study.
Clinical Manifestations, Gene Mutations and B-Cell Subsets Characterization of Autosomal Dominant-Hyper IgE Syndrome Patients in China

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RATIONALE: Domain-negative mutations in the STAT3 gene result in the classical multisystem form of autosomal dominant hyper IgE syndrome (AD-HIES). This study aims to provide a detailed clinical manifestations, STAT3 gene mutations and the changes of B lymphocyte subsets of AD-HIES.

METHODS: We collected 20 patients with suspected HIES from 20 unrelated families on the basis of National Institutes of Health (NIH) score of >15 points. Clinical manifestations and STAT3 genes mutations were analyzed. Peripheral B lymphocyte subsets were analyzed in 8 patients diagnosed with AD-HIES by 5-color flow cytometry and compared with the B lymphocyte subsets in 10 age-matched healthy controls.

RESULTS: Among the 20 patients, 14 patients were identified with STAT3 gene mutations, including three novel mutations. In the B-cell precursors subsets, HSC, CLP, Pro-B, Pre-B and immature B cells have no statistically significance between AD-HIES patients and healthy controls. In the mature B cell subsets, total memory B cell have markedly reduced in AD-HIES patients (2.84±0.44) compared with the healthy controls (13.19±0.94). The number of B1 cells and memory B cells significantly reduced in the patients with AD-HIES (3.37±0.83 vs 7.60±0.92, 7.73±0.71 vs 20.96±1.60, p<0.01). The numbers of class switch B cells were reduced in the AD-HIES patients (27.50±5.20) compared with healthy controls (43.81±4.03, p<0.05). The IgE+B cells were increased (4.05±1.59 vs 0.425±0.09, p<0.05).

CONCLUSIONS: Heterozygous mutations in STAT3 can lead to B cell developmental disorder, especially in the end-stage cell.

The Utility of Preimmunization and Postimmunization Titers to 23 Serotypes of Streptococcus Pneumoniae in the Diagnosis of Specific Antibody Deficiency in Children

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RATIONALE: Specific antibody deficiency (SAD) is diagnosed by lack of response to the 23-valent Pneumococcal Polysaccharide Vaccine (23-PPV). It is unknown whether each of the 23-PPV serotypes has similar diagnostic utility for SAD. We aimed to identify a subset of 23-PPV serotypes with high diagnostic utilities for the diagnosis of SAD.

METHODS: We retrospectively identified 126 patients aged 2-18 years who had two sets of 23-PPV titers drawn 4-12 weeks apart, and who did not have another identifiable cause of immunodeficiency. Adequate postimmunization 23-PPV response was defined as 4-fold rise for >70% of serotypes (>50% in patients 2-5 years) and/or postimmunization titer of ≥1.3 µg/mL. We calculated sensitivity, specificity and area under the curve (AUC) for each serotype.

RESULTS: Mean age at the time of preimmunization titers was 7.8 ± 4.0 years. 53.9% of patients were male and 94.4% were Caucasian. 101 patients had adequate response following 23-PPV, while 25 had inadequate responses and were diagnosed with SAD. Postimmunization titer to serotype 6B provided the best diagnostic utility, with an AUC of 0.91 (sensitivity 84%, specificity 98%). The following serotypes had AUC ≥0.8 with corresponding sensitivity/specificity, 9N (AUC=0.87, 58%/93%), 9V (AUC=0.87, 48%/91%), 18C (AUC=0.85, 56%/92%), 4 (AUC=0.84, 56%/94%), 7F (AUC=0.81, 54%/85%), 17F (AUC=0.80, 54%/87%), 19F (AUC=0.80, 28%/99%), and 5 (AUC=0.80, 71%/76%).

CONCLUSIONS: We identified a subset of pneumococcal serotypes that provide high diagnostic utility for the diagnosis of SAD. A further prospective study is required to investigate whether the diagnosis of SAD could be established based on a limited panel of pneumococcal serotypes.

Characterization of Common Variable Immunodeficiency (CVID) Subgroups through Modulation of Their Interleukin-21 (aCD40/IL-4/IL-21) Pathway in Vitro

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RATIONALE: We previously described that CVID have impaired in vitro capacity to undergo isotype class-switching in response to interleukin-21 (IL-21). The aim is to present our recent findings and further characterization of 2 CVID subgroups.

METHODS: We recruited a CVID cohort with age/sex-matched healthy controls. Medical charts were reviewed and blood samples were obtained for 7days PBMCs in vitro culture with complete media and aCD40 (1mg/mL) +/- IL-4(200U/mL), IL-21(50ng/mL) or IL-4+IL-21. B-cell subpopulations and IgG production were determined by flow cytometry and ELISA. Analyses were performed through descriptive statistics or Student t test.

RESULTS: Fifty subjects were recruited. Their mean age was 42 years old (range 7-81), 93.2% were Caucasian and 62.8% were female. Twenty-two subjects had further B-cell characterization. All exhibited decreased percentages of CD19+CD27+ memory B-cells (p<0.01) and IgG production (p<0.01) compared to controls following aCD40/IL-21 stimulation. In a subset of CVID (group 2), the addition of IL-4 to culture media (aCD40/IL-4/IL-21) allowed significant increases in the percentage of memory B-cells (1.8±5.9%) and IgG production (0.5±3.0 mg/L). These levels were comparable to controls. In group 1, the percentage of memory B-cells and IgG remained low. The 2 subgroups had different baseline B-cell characteristics and group 1 had 15% more infectious and other (bronchiectasis/cytopenia/splenomegaly) complications.

CONCLUSIONS: CVID subjects have impaired in vitro responses to aCD40/IL-21. In a subset of CVID subjects, the percentage of memory B-cells and IgG production can be increased to control levels through the IL-21 pathway (aCD40/IL-4/IL-21). The 2 subgroups had different baseline B-cell characteristics with group 1 experiencing more clinical complications.
294 Adverse Effects of Different Formulations of Intravenous Immunoglobulin

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RATIONALE: Intravenous immunoglobulin (IVIG) replacement therapy is a necessary treatment for hypogammaglobulinemia in many clinical settings. Our institution serves a large population of immunodeficient patients whom we manage with IVIG. There are multiple formulations of IVIG and side effects, anecdotally, can vary with formulation. Our institution recently changed our formulary IVIG from Gammagard to Gammunex, which afforded us the opportunity to compare the side effect profiles in an unbiased fashion.

METHODS: We compared inpatients who received their first dose of IVIG for any indication during one month in 2013 (when all patients received Gammagard, n = 34) to the same month in 2014 (when all patients received Gammagard, n = 34). A chart review was performed to assess side effects noted within the subsequent five days after IVIG administration.

RESULTS: Many of the side effects were seen in similar frequency between the two formulations (fever/rash, abdominal pain, nausea/vomiting). However, there was a significant increase in rigors, which were only seen with Gammagard (10/34 vs. 3/34, p = 0.048). However, bradycardia (2/34 vs. 0/34) and headache (10/43 vs. 3/34) were more common with Gammunex versus Gammagard with a trend towards significance for each (p = 0.095 and 0.11).

CONCLUSIONS: Most adverse reactions are not significantly different between formulations of IVIG, though rigors are significantly more common with Gammagard. We present these findings as a resource for the community to both assist in provider selection of therapeutics and patient education as we could not find comparisons of side effect profiles for Gammagard and Gammunex in the literature.

295 Diagnosis of X-Linked Agammaglobulinemia (XLA) in an Adult with a Novel Mutation of the BTK Gene and the Cost-Benefit of IVIG in Preventing Deterioration of Pulmonary Function

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RATIONALE: XLA is typically diagnosed in childhood but can be delayed. We present a novel BTK mutation in a patient diagnosed at age 46 and discuss the utility of IVIG treatment.

METHODS: Genetic testing at GeneDx.

RESULTS: A 46 year old male ex-smoker with recurrent sinopulmonary bacterial infections as a teenager was referred for primary immunodeficiency evaluation. Immune evaluation showed IgG 314, IgA <5, and IgM 25 mg/dL. Post-vaccination pneumococcal antibody titers indicated absent response to polysaccharide antigens. There was a small population of CD19 absent, CD20 positive (0.2% of CD45 staining cells) B-cells identified in the peripheral blood. CT imaging revealed mild sinonasal disease and generalized bronchiectasis. Pulmonary Function Testing showed normal spirometry, lung volumes and diffusion capacity. Genetic testing of the BTK gene uncovered a novel missense R562W mutation.

CONCLUSIONS: There is debate regarding the use of IVIG to prevent bronchiectasis and preserve pulmonary function in patients with XLA. IVIG therapy in this context is traditionally life-long and costly. We present a patient with a novel mutation in the BTK gene who did not receive IVIG for the majority of his life and at age 46 had only mild sinopulmonary disease. We estimate the cost of IVIG in our patient to this point in his life could have been upwards of $882,180. We use this case to illustrate the importance of identifying additional factors, which might help predict who will benefit from IVIG to prevent bronchiectasis and preserve lung function.

296 Successful Use of 20% Subcutaneous Immunoglobulin in Pregnant Patients with Primary Immune Deficiency

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RATIONALE: Immunoglobulin replacement therapy is commonly prescribed for patients with Common Variable Immunodeficiency (CVID) characterized by hypogammaglobulinemia and/or the inability to make antibodies to recall antigens. The administration of immunoglobulin in such patients by subcutaneous infusion (SCIg) is common but efficacy and safety in pregnancy are unclear.

METHODS: The records of three pregnant patients who received SCIg at two institutions for the diagnosis of CVID were reviewed. Data regarding safety, efficacy, levels of immunoglobulin G, as well as pregnancy outcomes were recorded. A literature review was conducted.

RESULTS: We identified three young (age range 23-28 years) pregnant female subjects with CVID, receiving 20% SCIg. All three subjects delivered full-term healthy infants. Subjects 1 and 2 required a dose adjustment in the third trimester due to subtherapeutic IgG levels. Subject 3 had normal IgG levels during first trimester but experienced 2 minor urinary tract infections. No unique pregnancy-related adverse effects were noted with infusion. None of the subjects experienced any serious or life-threatening infections, premature labor, eclampsia, intrauterine growth retardation, or febrile complications. None of the infants have experienced complications. Long term-follow up of the patients and their offspring continues.

CONCLUSIONS: Based on this small series, 20% SCIg appears safe and effective for pregnant patients with CVID. However, SCIg carries a category C classification in pregnancy meaning that no randomized studies have been carried out but benefit often outweighs risks in selected clinical circumstances. Review of the literature revealed 2 reports of successful use of SCIg in pregnancy. Prospective studies are required.
297 Successful Utilization of an Immunosuppressive B Cell Depleting Regimen in an Adolescent Male with Recalcitrant Non-Infectious Colitis and X-Linked Hyper IgM Syndrome

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RATIONALE: X-linked Hyper IgM Syndrome (HIGM) is a rare disorder due to a mutation in the gene that encodes for CD40 ligand (CD40L), resulting in defective class-switch recombination. Autoimmune complications such as colitis can be seen in a subset of these patients, which can be challenging to manage. We present an adolescent male with X-linked HIGM who developed severe colitis refractory to standard treatment. Successful treatment with an unconventional regimen consisting of rituximab and 6-mercaptopurine (6-MP) induced remission.

METHODS: This 17-year-old male, also with a history of autoimmune neutropenia, developed an 8-month history of severe secretory diarrhea of 3 liters daily with subsequent profound weight loss. Infectious studies and tissue pathology were unrevealing for common and opportunistic pathogens, coexisting inflammatory bowel disease, or autoimmune enteropathy. Treatment with high dose systemic corticosteroids, a tumor necrosis factor blocker infliximab, and high dose oral immunoglobulin failed to yield any improvement.

RESULTS: Given his immune dysregulation thought still to be autoimmune in nature, a B cell depleting regimen with rituximab at 375 mg/m2 once weekly for 4 doses was initiated in addition to 6-MP at 50 mg daily. His diarrhea completely resolved within 4 weeks of his last treatment with rituximab.

CONCLUSIONS: This is the first reported case of an adolescent with X-linked HIGM complicated by severe refractory colitis managed successfully with a unique regimen of rituximab and 6-mercaptopurine. This treatment protocol may prove to be helpful in other HIGM individuals with a similar and challenging complication.

298 Successful Loading and Maintenance Subcutaneous Immunoglobulin (SCIG) Therapy in a Patient with Myasthenia Gravis (MG)

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RATIONALE: MG is an autoimmune neuromuscular disorder characterized by fatigable voluntary skeletal muscle weakness often caused by antibodies to the nicotinic acetylcholine receptor (AchR). Intravenous immunoglobulin (IVIG) is used as a treatment for MG at doses of 1-2g/kg. We present an anti-AchR antibody positive patient who due to a poor clinical response to azathioprine, prednisolone dependence and the wish to become pregnant, was commenced on IVIG. Despite premedication and infusion rate reduction she suffered recurrent severe aseptic meningitis, and a serum IgG of 21.3-22.6g/l. This facilitated a reduction in immunosuppression and improvement.

METHODS: Once weekly for 4 doses was initiated in addition to 6-MP at 50 mg daily. His diarrhea completely resolved within 4 weeks of his last treatment with rituximab.

CONCLUSIONS: This is the first reported case of an adolescent with X-linked HIGM complicated by severe refractory colitis managed successfully with a unique regimen of rituximab and 6-mercaptopurine. This treatment protocol may prove to be helpful in other HIGM individuals with a similar and challenging complication.

300 Hypogammaglobulinemia in Patients on Natalizumab Therapy

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RATIONALE: The risk for development of progressive multifocal leukoencephalopathy (PML) in multiple sclerosis (MS) patients is increased with duration of natalizumab therapy, the presence of JC virus antibodies and a history of prior immunosuppression. Hypogammaglobulinemia has not been described in such patients.

METHODS: The records of patients on long duration of natalizumab therapy for MS and found to have hypogammaglobulinemia were reviewed. Two were referred because of infections and one because of fluctuating JC virus titers. Hypogammaglobulinemia was noted in all.

RESULTS: Three women ages 62, 61 and 52 years, were on long-term therapy: 59, 60, 60 months of natalizumab, respectively for relapsing-remitting MS. All had recent weakly positive JC virus titers. One had no infections, borderline low IgG, low IgG3 and a small MGUS. The second had significant decreases in IgG, IgM and IgA, recurrent bouts of mucopurulent bronchitis and bronchiectasis. The third had a severe CAP and low IgM. CD19 B-cells were increased in the MGUS patient. Two of the three had suboptimal responses to vaccines; the third had low baseline pneumococcal antibodies. Natalizumab was stopped in one patient.

The literature states that flow cytometry is normal and the response to vaccinations is intact in patients on natalizumab. Data on immunoglobulin levels in such patients was not found.

CONCLUSIONS: Hypogammaglobulinemia may be a risk for the development of PML in such patients. It is not clear if JC virus antibody monitoring is reliable in patients with low immunoglobulins. The prevalence of hypogammaglobulinemia in MS patients on natalizumab needs to be defined.
The Prognostic Value of B-Cell and T-Cell Clonality Testing in Common Variable Immunodeficiency

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RATIONALE: Common Variable Immunodeficiency (CVID) is a heterogeneous primary immune deficiency syndrome characterized by impaired antibody responses, recurrent infections, and inflammatory, autoimmune, and malignancy-related conditions. B cell phenotyping has been well studied, and the number of switched memory B cells showed to have significant prognostic value. However, the implications of clonality testing in these patients are unknown. We hypothesized B and T cell clonality testing may help predict a higher incidence of non-infectious complications in CVID patients.

METHODS: A retrospective analysis was performed of adult patients with CVID seen in our outpatient clinic that have had B-cell and T-cell clonality testing performed as part of routine management. Clonality testing was performed at Virginia Commonwealth University using the BIOMED-2 technique. Patients were labeled positive if they had any history of positive clonality testing. Statistical analysis was performed using Fisher’s exact test with SPSS software package (IBM, Armonk, NY). This study was approved by VCU IRB (HM20000683).

RESULTS: B cell (n=18) and T cell (n=17) clonality results were compared to a variety of outcomes including bronchiectasis or lung disease, autoimmunity, GI complications, cytopenias, granulomatous disease, lymphadenopathy, lymphoma, and other malignancy. Positive B cell clonality correlated significantly with the development of GI complications (p=0.044). Positive T cell clonality correlated significantly with the development of cytopenias (p=0.043) and lymphadenopathy (p=0.044).

CONCLUSIONS: B-cell and T-cell clonality may have prognostic value in patients with CVID, specifically in regards to the development of cytopenias, lymphadenopathy, and GI complications.

Complicated By MRSA Abscess Requiring Surgical Intervention

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RATIONALE: Mild local reaction to subcutaneous gammaglobulin therapy is widely noted in the literature. We present a case of MRSA abscesses after subcutaneous gammaglobulin therapy.

METHODS: A 51 year old female with history of common variable immunodeficiency (CVID) was initially on intravenous immunoglobulin (IVIG) therapy. Due to financial constraints, she wanted to be switched to subcutaneous therapy. She was not the best candidate for this regimen due to her history of pyoderma gangrenosum. She received 2 subcutaneous treatments one week apart at 5 different injection sites. After her second week, she noted erythema and later purulent lesions at 3 out of the 5 injection sites.

RESULTS: The patient was admitted for necrotizing infection of the left abdominal wall. She had multiple wound excisional debridements. Culture of the wounds grew out MRSA. She received intravenous (IV) vancomycin and ceftriaxone initially and later was transferred to a tertiary hospital to get IVIG treatment. Labs showed an elevated white count of 19 and an IgG of 491. Her antibiotics were changed to IV vancomycin and meropenem. She underwent wound exploration with repacking of her wounds. Upon discharge, patient had 3 separate open wounds on the anterior and lateral aspects of the abdominal wall measuring 3x4cm, 8x5cm, and 15x4cm. She was discharged with oral clindamycin and is currently stable.

CONCLUSIONS: Subcutaneous gammaglobulin in patients with a history of serious skin infections should be reconsidered. To our knowledge, this is the first reported case of serious skin infection requiring surgery after subcutaneous gammaglobulin administration.

The Immunoglobulin Diagnosis, Evaluation, and Key Learnings (IDEaL) Patient Registry: Analysis of Serum and Subclass IgG Levels, Pneumococcal Vaccine Response, and Therapy Outcomes

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RATIONALE: The IDEaL Patient Registry collects longitudinal information on patients receiving immunoglobulin (Ig) replacement therapy from Coram CVS/specialty infusion services in an alternate care setting. This poster examines the relationship between serum and subclass levels, vaccine response, and therapy outcomes, including infection rates.

METHODS: Patients of our 140 investigators are eligible for the Registry. With patient consent acquired, patient information from July 2010 onward that had been collected by Coram nursing and pharmacy was entered into the IDEaL database.

RESULTS: In the enrolled population, 71% had IgG levels below reference minimums, with an average of 508 mg/dL. Patients with serum and subclass deficiencies constituted 42% of the population, and a pneumococcal vaccine challenge showed an approximate 30% response rate. We noted a weak correlation between serum levels and pneumococcal response; we also found that in subclass-deficient patients with blunted vaccine response, 67% had low IgG2 levels. Overall, patients averaged about three infections per year.

CONCLUSIONS: Primary immune deficiencies have an extremely variable presentation, and the initial lab findings can have an impact on the patient’s outcomes on Ig therapy. We found a weak correlation between serum IgG levels and pneumococcal vaccine response; we also found that specific subclass deficiency, mainly in IgG2, may have a larger role in the initial presentation of PID.
304 Hypogammaglobulinemia in Preschool Children with Allergic Disease
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RATIONALE: Allergic diseases result in recurrent respiratory symptoms. On the other hand, impaired humoral immune response may be a cause of recurrent respiratory symptoms/infections. Preschool children are prone to recurrent respiratory infections. In this study, we investigated the presence of hypogammaglobulinemia in preschool children with allergic disease and recurrent respiratory infections.

METHODS: 62 children (0-5 years) who admitted to the Pediatric Allergy Immunology Department between 2009 and 2014 with the allergic complaints were included to the study. The presence of atopy were confirmed with skin prick test positivity, positive specific IgE levels and high total IgE levels. Serum IgG, IgA, IgM and total IgE levels were measured and evaluated based on age.

RESULTS: 23 of the patients were girls and 39 of them were boys. Mean age was 34.7±17.6 months. Totally 15 patients had hypogammaglobulinemia. 8 of them (12.9 %) had low IgG levels, four of them (6.5%) had low IgM levels and 10 patients (16.1%) had low IgA levels. Among the patients who have hypogammaglobulinemia (8 children), three children have food allergy, three children have urticaria, one child has got asthma, one child has got allergic rhinitis. Among the patients who have low IgM levels (four children), two of them have urticaria, one child has got food allergy and one child has got asthma. Five children who have low IgA levels have food allergy, three of them have urticaria an done of the has got asthma.

CONCLUSIONS: The allergic preschool children with recurrent respiratory infection should be screened for hypogammaglobulinemia.

305 Myelodysplastic Syndrome (MDS) and Acute Lymphocytic Leukemia in Common Variable Immunodeficiency (CVID)
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RATIONALE: Common variable immunodeficiency (CVID) is characterized by hypogammaglobulinemia, impaired antibody responses and an increased susceptibility to infections. It is also associated with other diseases of immune dysregulation including autoimmune disease and malignancy, particularly non-Hodgkin lymphomas. However, few cases of myelodysplastic syndrome (MDS) and acute leukemias associated with CVID have been described.

METHODS: A retrospective chart review was performed of 3 CVID patients diagnosed with MDS and acute leukemia.

RESULTS: Case 1 is a 60 year-old male who was diagnosed with asymptomatic CVID when he was worked up for low globulin fraction. He was later diagnosed with acute lymphocytic leukemia (ALL) with translocation 4:11 and received a stem cell transplant from his HLA identical brother. He had partial B-cell reconstitution but still required IVIG. Case 2 was a 78 year-old male who was diagnosed with CVID after presenting with E.coli urosepsis followed by C.difficile colitis and found with hypoglobulinemia and autoimmune hemolytic anemia. IVIG was initiated. Twelve years later he was diagnosed with high-risk MDS with complex cytogenetics and a RUNX1 mutation. Treatment with azacitidine was initiated but he succumbed to MRSA sepsis 1 month later. Case 3 is a 74 year-old male with CVID complicated by pulmonary hypertension, neutropenia, thrombocytopenia and autoimmune hemolytic anemia which was previously treated with steroids, IVIG, cyclophosphamide, splenectomy and rituximab. He was recently diagnosed with MDS with ASXL1 mutation. He is pending treatment with azacitidine.

CONCLUSIONS: Non-Hodgkin lymphomas are the most common hematologic malignancies seen in CVID. However, MDS and acute leukemias can also be seen in CVID.

306 Vitamin D and Pneumococcal Antibody Titers: A Potential Role of Vitamin D in the Evaluation and Management of Specific Antibody Deficiency?
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RATIONALE: Vitamin D has been shown to enhance humoral immunity to S. pneumoniae (Spn) and to be associated with a reduction in waning of Spn antibody titers over time, especially in individuals with asthma and other atopic conditions. Specific Antibody Deficiency (SAD) is defined as decreased antibody response to Spn with normal responses to protein antigens and normal immunoglobulin levels. The goal of this study is to determine if vitamin D is associated with an initial or sustained response to a Spn vaccine in patients being evaluated for SAD.

METHODS: A prospective observational study was initiated. Pediatric patients being evaluated for SAD received an unconjugated polysaccharide pneumococcal vaccine (Pneumovax). Spn antibody titers were measured at 4 weeks and 6 months post vaccination. Vitamin D levels (25-hydroxyvitamin D) were obtained at the time of the 4 week titer. Spn titers were compared in patients with normal vitamin D compared to those with low vitamin D (< 30 ng/ml).

RESULTS: At the time of submission, 9 patients have been included in the study. Spn antibody titers were protective at 4 weeks post Spn immunization in patients with decreased vitamin D compared to normal vitamin D, 93% versus 82% of serotypes.

CONCLUSIONS: Vitamin D does not appear to be associated with response to a Spn vaccine 4 weeks post immunization. However, additional data needs to be collected at 6 months post vaccination to further assess both vaccine response as well as the effect of vitamin D on the waning of pneumococcal titers over time.

307 Assessment of Vaccine Competency to Recall and Neo-Antigens in a Cohort of Long-Term Successfully Treated HIV Patients
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RATIONALE: HIV therapeutic vaccination represents a potential alternative to chronic antiretroviral administration. Despite highly effective HIV antiretroviral therapy (HAART), residual immune deficiency may limit therapeutic vaccine effectiveness. We therefore vaccinated HIV patients with Streptococcus Pneumoniae (recall-antigen) and bacteriophage phiX174 (neo-antigen) as a first step in determining vaccine competency.

METHODS: Twenty-three adult males with undetectable viral loads for an average 8.2 +/- 4.3 years were administered Pneumovax 23 (PPV23) once and bacteriophage phiX174 antigen on three occasions. PPV23 antibody titers were measured at baseline and 4 weeks post-vaccination and phiX174 antibody levels at 4, 12, and 26 weeks. Responses to PPV23 were defined using AAAAAI recommendations on diagnostic vaccination in primary immune deficiency. A protective serotype cutoff level was 1.3 ug/ml. Responses were considered normal if 70% of the serotypes were above the protective cutoff and had a 2-fold increase in the titers. Neutralizing antibody levels and rate of bacteriophage phiX174 inactivation (Kv) determined neo-antigen responses. Abnormal responses were defined as Kv values falling 2 standard deviations from the geometric mean of healthy controls.

RESULTS: 16/23 (70%) subjects administered PPV23 had poor post-vaccine responses. In contrast, only 4/23 (17%) patients had inadequate responses to bacteriophage phiX174 vaccination.

CONCLUSIONS: Despite well-controlled HIV disease, responses to PPV23 were poor. Unexpectedly, most patients responded normally to the neo-antigen phiX174. Studies prior to HAART demonstrated very low titers to phiX174. Further characterization of neo-antigen responses in larger HIV treated cohorts is required to better define vaccine competence and establish the effectiveness of HAART in immune functional recovery.
308 Analysis of Specific Antibody Levels to Tetanus, Hib and Pneumococcus in Patients with Antibody Deficiency Receiving Immunoglobulin Replacement

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RATIONALE: The adequacy of immunoglobulin (IgG) replacement therapy for primary antibody deficiency is determined both clinically and using laboratory indices such as IgG trough levels. We assessed whether patients who had satisfactory levels of trough IgG also had protective levels of specific antibodies to tetanus, haemophilus influenza type b (Hib) and pneumococcus.

METHODS: Total IgG trough and specific antibody levels were studied in a single centre in patients with antibody deficiency (N = 115) receiving replacement IgG intravenously or subcutaneously. Twelve pneumococcal serotypes were also measured: less than 9 serotypes above protective levels (0.35 μg/mL or 1.3 μg/mL) were considered insufficient.

RESULTS: The levels of tetanus- and Hib-specific antibodies were protective in 99% and 93% of patients, respectively, while only 75% of patients had protective levels of pneumococcal antibodies. Using a protective cut-off level of 1.3 μg/mL for pneumococcal serotypes, 95% of patients were defined as having deficient antibodies but only 20% of patients were considered having deficient antibodies with the 0.35 μg/mL cut-off.

CONCLUSIONS: The assessment of specific antibodies showed that 25% of patients on IgG replacement therapy had low total pneumococcal antibody levels despite apparently adequate IgG trough levels. The measurement of pneumococcal antibodies may be helpful for patients with a diagnosis defined by vaccination responses (e.g. specific antibody deficiency) and for those with high rates of recurrent infections despite adequate trough IgG levels. Pneumococcal serotype specific antibody levels were more closely correlated with replacement adequacy than 1.3 μg/mL.

309 Long-Term Efficacy and Safety of Recombinant Human Hyaluronidase (rHuPH20)- Facilitated Subcutaneous Infusion of Immunoglobulin G (IgG) (HyQvia; IGHy) in Patients with Primary Immunodeficiencies (PI)

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RATIONALE: IGHy is a novel subcutaneous (SC) IG treatment approach that allows administration of IG every 3-4 weeks, at a treatment interval similar to intravenous (IV) IG, by means of facilitation with rHuPH20. We report efficacy, safety, and tolerability of IGHy in pediatric and adult patients treated for up to 172 weeks.

METHODS: Eighty-three patients (age 4-78 years) received IGIV for 3 months followed by IGHy (75 U rHuPH20/g IG administered SC followed by IgG 10% administered through the same needle) every 3-4 weeks for approximately 18 months; 63 patients continued to receive IGHy for up to an additional 21 months. rHuPH20 was discontinued after 3 years of exposure. Assessments included rates of adverse events (AEs), serious AEs (SAEs), and infections; tolerability; and rHuPH20 antibody levels.

RESULTS: IGHy exposure for all patients was 187.69 patient-years. The annual rates/patient-year of validated acute serious bacterial infections and all infections were 0.03 and 2.99, respectively. The rates of temporally associated local and systemic AEs (excluding infections) per patient-year, regardless of causality, were 2.60 and 2.62, respectively. No IGHy-related SAEs were reported. Fifteen (18.1%) patients had anti-rHuPH20 antibody titers ≥1:160 on ≥2 occasions; there were no associated AEs. No patients developed neutralizing anti-rHuPH20 antibodies.

CONCLUSIONS: IGHy administered at a frequency similar to IGIV over 3 years was effective in maintaining low infection rates in patients with PI, and the AE profile was comparable to that previously reported for IGSC at infusion volumes and rates equivalent to IGIV.

310 Low IgG Trough Levels at the End of 4 Week Treatment Cycle Regardless of the Administration Route

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RATIONALE: Antibody deficiency patients on intravenous IgG (IVIG) once 3-4 weeks often report ‘wear-off’, a feeling of decreased well-being and increased susceptibility to infection, when their IgG levels drop at the end of each cycle. This is not observed with frequent subcutaneous IgG (SCIG). The effects of giving SCIG once every 3-4 weeks have not been well-studied, but recently, 3-4 weekly administration of hyaluronidase facilitated subcutaneous Ig (HyIG) has been reported.

METHODS: IgG trough levels recorded with 3-4 weekly IVIG (Privigen) and weekly SCIG (Hizentra) were used to estimate trough levels of SCIG given once every 4 weeks using pharmacokinetic modeling/simulation. Trough levels with HyIG are from Wassermann et al JACT 2012, 130: 951-7.

RESULTS: Dose equivalent switching from 3-4-weekly IVIG to weekly SCIG increased IgG trough levels by 14.7%, from 678 (±133) to 794 (±140) mg/dl. PK modeling/simulation predicts that with SCIG once every 4 weeks, IgG trough levels would be 12% lower (ratio 0.88, 90% CI 0.86-0.92) than with weekly SCIG. This closely mirrors results of a clinical trial of weekly SCIG vs monthly IVIG (Desai et al JACI 2009, 124:854-6). In trials comparing IVIG with HyIG at the same 3-4 week intervals (HyIG at 108% of the IV dose), trough IgG levels were indistinguishable (Means with HyIg = 95-103.8% of IVIG).

CONCLUSIONS: Dosing IgG once every 3-4 weeks results in low trough levels, and a risk of ‘wear-off’, regardless of the route. More frequent dosing results in more constant IgG levels, with higher troughs. Frequent (daily to biweekly) SCIG helps minimize “wear-off” effects.
311 Selective Exclusion of High Titer Donor Plasma and Immunoadsorption Chromatography As Steps to Reduce Isoagglutinin Titters in IVIG

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RATIONALE: Hemolysis is a rare but potentially serious complication of high-dose IVIG therapy. Isoagglutinins originating in donor plasma are believed to play a major role in these reactions, but host factors are also important. We determined the effects of excluding plasma from donors with high isoagglutinin titters from the pools used to prepare IVIG, and of specific anti-A/B immunoaffinity chromatography (IAC), on the anti-A and anti-B titters of IVIG products prepared using the process for Privigen/Hizentra.

METHODS: A high-throughput assay was used to identify plasma donors with the highest anti-A titters. The effects of addition of IAC with A/B-trisaccharide-coupled resins into the manufacturing process were also studied. Anti-A/B isoagglutinin titters in the resulting IVIG preparations were measured by indirect agglutination and flow cytometry.

RESULTS: Exclusion of the 5% of plasma donors with the highest anti-A titters reduced both anti-A and anti-B isoagglutinins in the final product by approx. 50% (a single 2-fold dilution step, i.e. reduction of the titer from 1:16 to 1:8; and 1:8 to 1:4, respectively, N=30 lots). Specific IAC reduced anti-A and anti-B titters by at least two 2-fold dilution steps in the final product (>80% reduction shown by flow cytometry on standard red cells), while the content of antibodies against common microbial antigens remained unchanged.

CONCLUSIONS: Anti-A/B isoagglutinin reduction in IVIG products is feasible using screening and exclusion of a small percentage of high anti-A titter donors and with specific IAC. These approaches may reduce the risk of hemolysis in IgG therapy.

312 (1) Safety of Intravenous Immunoglobulin Therapy in Patients with Probable Alzheimer’s Disease: A Randomized, Placebo-Controlled Clinical Study

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RATIONALE: Intravenous immunoglobulin (IVIG) contains polyclonal human antibodies that bind to Aβ aggregates, foster the dissolution of Aβ fibrils, and enhance microglia-mediated phagocytosis of amyloid deposits in vitro. We hypothesized that IVIG might reduce cognitive decline and preserve functional abilities in Alzheimer’s Disease (AD) patients.

METHODS: This was a placebo-controlled, double-blind, multicenter study in subjects 50-89 years of age with mild to moderate AD (mini mental state examination score 16-26). Subjects were randomized 1:1:1 to receive biweekly infusions of 400 mg/kg, 200 mg/kg IVIG, or 0.25% humanalbumin over 18 months. Clinical assessments and biomarkers measurements were conducted throughout the study.

RESULTS: Primary and secondary efficacy endpoints were not met. Common non-serious adverse events (AEs) in treated subjects included headache, rash, infusion-site extravasation, and diarrhea. Statistically significant risk ratios for IVIG were chills (3.85) and rash (3.08). Of 11 serious AEs considered related to IVIG, a lower proportion occurred in the high-dose group (16.5% versus 23.7% in low-dose group). The rate of thromboembolic events was lower in treated subjects (1.9% versus 5% in control subjects). The rate of new or worsening renal failure was similar in all subjects and there were no cases of respiratory failure. The rate of infections was lower in treated subjects (34.0% versus 47.9% in control subjects).

CONCLUSIONS: Eighteen months of IVIG treatment was well tolerated in elderly patients, and no new safety signals were identified.

313 Response to Conjugated Pneumococcal Vaccine in Patients with Inadequate Immunogenic Response to Polysaccharide Vaccine

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RATIONALE: Pneumococcal vaccination is important in both evaluating and eliciting humoral immune responses. Thirteen-valent pneumococcal conjugate vaccine (PCV13-Prevnar) is comprised of polysaccharides conjugated to a protein carrier that elicits a T-cell dependent immune response. This study evaluates whether PCV13 is an efficacious alternative for patients who fail to respond to 23-valent pneumococcal polysaccharide vaccine (PPSV23-Pneumovax23).

METHODS: A retrospective chart review of 26 patients (22F; 4M; 23-89 yrs old), who failed to have an adequate response to PPSV23, (> 2 fold increase of titer and an absolute value > 2 mcg/ml to pneumococcal IgG serotypes), and then received PCV 13 was conducted. Antibodies to 12 of 13 vaccine serotypes, if available, were evaluated at least 6 weeks after vaccination.

RESULTS: 19.2% of patients (5/26) had 30% improvement in protective titers post PCV13, 42% (11/26) mounted no immune response to PCV13. Only 25% (6/24) of patients had 70% protective titers after vaccination with both PPSV23 and PCV 13.

CONCLUSIONS: Only a quarter of patients who failed PPSV23 and then received PCV13 achieved 70% protective titers to the studied pneumococcal strains with over one third of patients not mounting any additional response at all. This poor response may be reflective of the already existing or suspected specific humoral deficiency. Further studies are needed to evaluate the efficacy of PCV13 in patients who fail to mount a response to PPSV23.

314 Functional Interaction of Mir-155, a Pro-Inflammatory microRNA, and Quaking in the Innate Immune Response

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RATIONALE: mir-155 is a pro-inflammatory microRNA upregulated in human and mouse macrophages exposed to LPS (lipopolysaccharide) that is required to mount an effective immune response. Quaking (QKI) is a tumor-suppressor gene encoding a conserved RNA-binding protein. In silico analyses suggest that QKI transcripts are top predicted targets of mir-155. We hypothesized that mir-155 might carry out its pro-inflammatory signaling at least in part by targeting QKI.

METHODS: Mouse RAW-264.7 macrophages were stimulated with LPS or mock PBS three times over a period of 6 days. qRT-PCR was used to monitor the expression of Qki, mir-155 and Tnf (tumor necrosis factor alpha). Qki expression was correlated with Western blotting. Data from triplicate experiments were used for statistical analyses.

RESULTS: After 8 hours of LPS challenge there was a 2-fold decrease in QKI, while mir-155 and Tnf both increased approximately 10-fold (p<0.05). Qki returned to its initial levels at 2-days, while mir-155 remained high for 3-days. LPS re-stimulation at 3-days, mimicking chronic inflammation, reduced 2.3-fold Qki expression (p<0.05) over a 48-hours period in parallel with a new up-regulation of mir-155. Western blotting confirmed the above observed changes for Qki.

CONCLUSIONS: In mouse macrophages exposed to LPS, increased mir-155 expression inversely correlates with Qki expression.
All abstracts are strictly embargoed until the date of presentation at the 2015 Annual Meeting.

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**315 Exogenous PGI2 Protection Against Respiratory Syncytial Virus (RSV)-Induced IL-13-Producing Th2 Cells and ILC2**

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**RATIONALE:** RSV is the leading cause of hospitalization in infants. Severe RSV infection is also a risk factor for the subsequent development of asthma. During RSV infection, IL-13 mediates mucus production, which directly contributes to airway obstruction and respiratory failure. Our laboratory previously showed that endogenous prostaglandin (PG) I2 reduced RSV-induced illness in mice. To pave the way for a clinical effectiveness study using PGI2 for the treatment of RSV infection, we performed preclinical studies to determine how exogenous PGI2 impacts RSV-induced illness and determine the mechanisms by which exogenous PGI2 modulates host antiviral immunity.

**METHODS:** 8 week old BALB/c WT mice were infected with 1x106 PFU of RSV clinical isolate strain 00/12-35. Beginning 24 hours after infection, mice were treated with the exogenous PGI2 analog cicaprost (2 μg/50 μl) or PBS (vehicle, 50 μl) every 12 hours. Lungs were harvested 4 and 6 days after infection. IL-13, IL-10, and IFN-γ levels were evaluated by ELISA. IL-13+Th2 cells and group 2 innate lymphoid cells (ILC2) were identified by flow cytometry.

**RESULTS:** Compared to vehicle-treated mice, cicaprost-treated mice had significantly decreased lung IL-13 and IL-13+Th2 cells 4 days after RSV infection. Lung IL-10 and IFN-γ were significantly increased, while ILC2s were significantly decreased 6 days following RSV infection in cicaprost-treated mice compared to vehicle-treated mice.

**CONCLUSIONS:** These data suggest that exogenous PGI2 protects against RSV 00/12-35-induced IL-13-producing Th2 cells and ILC2.

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**316 The Use of Radialabelled 18-F-F2-Deoxy-2-Fluro-Glucose (18-FDG) in Combined Positron Emission Tomography-Computed Tomography (PET-CT) to Evaluate Infection: Lessons Learned from a Case Series of 23 Patients with Chronic Granulomatous Disease (CGD)**

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**RATIONALE:** Use of 18-FDG in PET-CT is gaining acceptance in the musculoskeletal system can be due to recent trauma, so clinical correlation should be ascertained before assuming an infectious process. Fifth, clinical resolution of infection is closely associated with diminished 18-FDG uptake.

**CONCLUSIONS:** 18-FDG PET-CT can define areas of infection, and studies are warranted to determine whether biopsy of areas of highest intensity may result in improved yield. CGD lymphadenopathy or granuloma unrelated to infection is surprisingly lacking in significantly increased 18-FDG uptake. Our observations suggest that it may be possible to use 18-FDG PET-CT to identify the scope and intensity of infection in patients with CGD and, in complex multifocal infection, may better assess overall resolution and response to therapy than CT alone.

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**317 (1) Immunopharmacological Characterization of Potent, Selective and Orally Available RORyt Inhibitors for Treatment of Autoimmune Diseases**


**RATIONALE:** Th17 cells, functionally regulated by the transcription factor RORyt, play key roles in the pathogenesis of autoimmune diseases. Here we report the immunopharmacological characterization of our novel RORyt inhibitors in comparison with a JAK inhibitor.

**METHODS:** The effects on Th17 differentiation and Th1/Th17 cytokine production were evaluated using human peripheral mononuclear blood cells or mouse splenocytes stimulated with anti-CD3/anti-CD28 antibodies. Specificity and safety profiles were assessed by BioMAP T cell autoimmunity panel that models immunosuppression, anti-inflammatory action, tissue remodeling, Th1/Th2/Th17 skewing, and cell cytotoxicity in human primary cells. In vivo pharmacological effect was tested in a mouse experimental autoimmune encephalomyelitis (EAE) model.

**RESULTS:** The lead compound MG2905 showed potent inhibitory effects on IL-17A production in human and mouse systems (IC50 values were < 5 nM and < 30 nM, respectively) without affecting IFN-γ production. The IC50 value in human was about < 200 nM in the presence of 50% human serum. In contrast, a JAK inhibitor Tofacitinib enhanced IL-17A production in a bell-shaped fashion although it inhibited IFN-γ production in a dose-dependent manner. IL-17-specific profile of MG2905 was also supported by human T cell autoimmune panel, where other immunosuppressants and drugs e.g. Tofacitinib broadly inhibited immune responses. In mouse EAE model, oral administration of MG2905 resulted in suppression of clinical score.

**CONCLUSIONS:** We developed potent, selective, and orally-available RORyt inhibitors possessing clean Th17-specific immunopharmacologic profile which should be useful for the treatment of autoimmune diseases.
319 Cord Blood DNA Methylation of Treg Cytokine Genes Differ with Parity
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RATIONALE: Women undergo immunological changes during pregnancy including changes in the balance of Th1/Th2 cytokines. To investigate whether these changes can influence DNA-Methylation (DNA-M) in cord blood of consecutive births, we analyzed cord blood DNA-M of genes in the Th1, Th2, Th17 and Treg pathways of two consecutive deliveries.

METHODS: In the Isle of Wight birth cohort, umbilical cord blood samples were collected from offspring of cohort participants to measure DNA-M using the Illumina Infinium HumanMethylation450 beadchip. Cord blood DNA-M was available for two consecutive pregnancies in seven mothers. Cytosine-phosphate-guanine (CpG) sites in Tnfα (157 CpGs, 19 genes), Tnfβ (68 CpGs, 12 genes), Tgfb1 (110 CpGs, 15 genes), Treg (10 CpGs, 2 genes) and in a random sample (271 CpGs) were considered. To identify CpGs with significant changes (p<0.05), mixed linear models were applied to compare cord blood DNA-M of two consecutive pregnancies.

RESULTS: In the last of two pregnancies, cord blood DNA-M showed significantly more CpGs of the two Treg genes (60%) with significant differences compared to CpGs of a random sample (32.47%) (p=0.02). Among the six differentially methylated CpGs, three each were from FOXP3 and CTLA4, respectively.

CONCLUSIONS: In cord blood, significant methylation differences in Treg genes between consecutive pregnancies suggest an adaptation in the course of two pregnancies. Future studies need to investigate whether these epigenetic changes are responsible for differential immune responses in offspring with different birth order.

320 A Human Microbiome Enhanced Campylobacter Jejuni Induced Autoantibodies and Th 2 Skewing of Adaptive Immunity after Fecal Transplant
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RATIONALE: Guillain-Barré Syndrome (GBS) is a common cause of acute generalized paralysis. Infection with the enteric pathogen C. jejuni often precedes GBS when bacterial lipo-oligosaccharide resembling host nerve gangliosides activates the immune system to produce autoantibodies. We showed that C. jejuni 11168 from an enteritis patient produced T helper-1/17 responses in C57BL/6 IL-10−/− mice, while a C. jejuni GBS patient strain (260.94) blunted Th-1/17, but enhanced Th-2 responses. Only Th-2 antibodies cross-reacted with nerve gangliosides. We hypothesized that human gut microbiota (Humicrobiota) enhances immunity to C. jejuni infection with elevated C. jejuni-specific and autoantibodies.

METHODS: C57BL/6 germ-free mice were given a human fecal transplant, bled after gut microbiota stabilized, and their offspring used in a 30 day infection trial. Congenic 16microbiota and mouse microbiota (Humicrobiota) mice were inoculated with C. jejuni enteritis strain 11168, GBS strain 260.94 or sham inoculated. Plasma was collected from all mice and levels of Th-1 and Th-2 antibody isotypes to nerve gangliosides and to C. jejuni strains were measured. IL-4 and IFN-γ responses were measured in gut lamina propria cells by RT-PCR.

RESULTS: Autoimmune responses were significantly elevated by the presence of 16microbiota. 16microbiota had significantly higher levels of IgG1 antibodies to C. jejuni 11168 and to GM1 and GD1a nerve gangliosides than infected Humicrobiota mice. Infected 16microbiota mice had a 12-fold increase in IL-4 levels and decreased IFN-γ levels.

CONCLUSIONS: 16microbiota enhanced C. jejuni induced autoantibodies to nerve gangliosides and Th-2 skewing of the adaptive immune response.
**Mast Cell-Expressed TG2 Induces the Development of Mptp-Induced Parkinsonism Via Down-Regulating Treg Cells in Mice**

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**RATIONALE:** This study aimed to investigate the role of transglutaminase 2 (TG2) expressed in mast cells in substantia nigra (SN) of Parkinson’s disease (PD).

**METHODS:** C57BL/6 mice received 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) by i.p. to induce PD. Bone marrow-derived mast cells (BMMCs) were adaptively transferred to TG2-/- (KO) mice by i.v. before MPTP injection. TH+ and DAT were determined by immunohistochemistry and Nissl staining, the population of mast cells by May-Grünwald-Giemsa staining, FcεRI receptor and the Treg cell population by FACS analysis, and the co-localization of mast cells and Treg cells by confocal analysis.

**RESULTS:** TG2-/- mice protected against the loss of TH+ DA neuronal cells and DA transporter, and showed reduced infiltration, expression of c-kit, tryptase, FcεRI receptor, migration and adhesion molecules, and CD4 count (>200 cells/mm3), and positive IGRA were associated with elevated nil IFN- values, but viral load and co-morbid inflammatory conditions (i.e. cancer and autoimmune disease). Multivariate linear regression was used to estimate the relationship between nil IFN- values and HIV, CD4 count, CD8 count, viral load and IGRA result.

**CONCLUSIONS:** Even with well-controlled infection, HIV-positive individuals have higher spontaneous secretion of IFN- by blood cells. This may reflect ongoing immune activation, despite low disease burden.

**Biologic Therapies for Psoriasis and Macrophage Leptin Levels: A Link to Obesity and Atherosclerosis**

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**RATIONALE:** Psoriasis is now considered a systemic immune-mediated inflammatory disease. Use of tumor necrosis factor (TNF)-alpha inhibitors such as adalimumab resulted in a breakthrough in psoriasis management and enhanced our understanding of pathophysiology. Ustekinumab a human monoclonal antibody to the shared p40 subunit of IL-12 and IL-23, is another effective treatment. Leptin, an adipostatic circulating hormone produced in white adipose tissue, may also play a role in the development of psoriasis. This study examines the link between biologics used in the treatment of psoriasis and leptin levels in the THP-1 human monocyte/macrophage cell line.

**METHODS:** THP-1 differentiated macrophages were incubated (18 hours) under the following conditions: 1) untreated control; 2) adalimumab 5μg/mL; 3) ustekinumab 1μg/mL; 4) ustekinumab 5μg/mL. Cellular RNA was isolated and subjected to QRT-PCR for measurement of leptin and leptin receptor expression.

**RESULTS:** At 5μg/mL, ustekinumab increased leptin expression by 180% (n=3, p<0.05) Adalimumab did not increase leptin, but upregulated leptin receptor expression in THP-1 macrophages by 214% (n=3, P<0.05). Ustekinumab upregulated leptin receptor expression in a concentration dependent manner, by 129% at 1μg/mL and 191% at 5μg/mL.

**CONCLUSIONS:** Adipokines such as leptin may link adipose tissue to obesity-related complications of psoriasis. Leptin levels are increased in both male and female patients with psoriatic arthritis and may relate to elevated cardiovascular risk. Our finding that biologics may increase leptin and leptin receptor expression suggests possible unintended effects of these drugs that may be atherogenic and require countermeasures.
**324 Therapeutic Effects of CCL22 Sirna in a Mouse Model of Asthma**

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**RATIONALE:** Macrophage-derived chemokine (CCL22) are responsible for the allergic inflammation. In children With Bronchial Asthma (BA), Serum CCL22 level was higher in children with BA. The aim of the present study was to evaluate the effects of CCL22 suppression in mice with asthma. In this study, we hypothesized the immune suppression using bacteria expressing CCL22 miRNA would be induced therapeutic effects on a diseases.

**METHODS:** The recombinant strain of *Salmonella typhimurium* expressing CCL22 miRNA (ST-miRClC22) was prepared for in vivo knockdown of CCL22. The study was conducted in children and mice with BA. Clinical characteristics chemokine (CCL2) and cytokine of the children were measured. We were investigated function of CCL22 in a mouse model of ovalbumin (OVA)-induced allergic asthma. CCL22 siRNA were treated in mice with BA. Immune responses were tested by ELISA, Microarray and Histological analysis.

**RESULTS:** We constructed a recombinant strain of *Salmonella typhimurium* expressing CCL22 miRNA (ST-miRClC22) for the in vivo knockdown of CCL22. The CCL22 gene was downregulated with CCL22 miRNA in activated lymphocytes. In mice test, administration of CCL22 siRNA significantly reduced airway hyperresponsiveness (AHR), airway eosinophilia and Th2 cytokine production, in bronchoalveolar lavage (BAL) fluids and lungs from OVA-sensitized and -challenged mice. Furthermore, Children with BA showed an increased CCL22 concentration in serum compared with non-atopic healthy subjects.

**CONCLUSIONS:** Children with BA showed an increased CCL22 concentration in serum compared with non-atopic healthy subjects. In mice with asthma, CCL22 suppression reduced the major pathophysiological features of allergic asthma. Thus, CCL22 suppression.

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**325 Generation of Recombinant FcRls of Dog, Cat and Horse for Component-Resolved Allergy Diagnosis in Veterinary Patients**

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**RATIONALE:** Type I allergies may affect human and animals with similar symptoms. Allergy diagnostics in dogs, cats and horses is based on intradermal skin tests with allergen extracts, whereas IgE-based diagnosis has a lower clinical impact. This is possible due to a lack of high-quality IgE detection reagents for these species. We aimed here to express the recombinant canine, feline and equine FcRls chains for high-affinity IgE detection to improve the specificity and spectrum of diagnostic IgE tests in dogs, cats and horses.

**METHODS:** Combined with a custom SV40_Neo mammalian expression vector the Flag-tagged FcRls fusion proteins of canine, feline and equine were expressed in CHO-DUKX B11 cells. The resulting 384 clones of each species were evaluated and selected with respect to their productivity and quality by ELISA and immunoblot. Canine, feline and equine alpha chains were purified via anti-FLAG M2 affinity gel and tested for correct folding in CD spectroscopy. The binding to relevant IgE species was tested in immunoassays.

**RESULTS:** The recombinant products were correctly folded as determined by CD-spectroscopy. ImmunobLOTS and ELISA assays verified integrity of the recombinant canine, feline and equine alpha chains. The recombinant proteins detected allergen-specific serum IgE of the relevant species, but also showed a high degree of crossreactivities to other species including humans.

**CONCLUSIONS:** In this study we established the expression of canine, feline and equine alpha chains for specific IgE detection. These tools will allow introducing IgE-diagnosis, specifically component-resolved diagnosis, in these species.

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**326 Preclinical Assessment of the Effectiveness of α-Dectin-1-Pam3 Conjugate in Controlling Th2 Responses**

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**RATIONALE:** Dectin-1 is a pattern recognition receptor, which contributes to both innate and adaptive immunity against certain fungal and bacterial infections. Previously, we have shown that signals via Dectin-1 and TLR2 synergize to activate dendritic cells (DCs), resulting in decreased Th2 responses. In this study, we have made α-hDectin-1-Pam3CSK4 (Pam3) conjugate and tested its effectiveness in the suppression of Th2 responses in human in vitro and non-human primates (NHP) in vivo.

**METHODS:** An agonistic α-Dectin-1 antibody was chemically conjugated with Pam3. In human in vitro experiments, blood myeloid DCs (mDCs) were treated with α-Dectin-1-Pam3 in the presence or absence of TSLP. Phenotypes and functions of mDCs were assessed. In then NHP in vivo experiment, rhesus macaques were sensitized with house dust mite allergens and then treated with α-Dectin-1-Pam3. Serum IgE and skin reactions were assessed.

**RESULTS:** Conjugating Pam3 to α-Dectin-1 antibody creates no alteration in α-hDectin Ab binding capacity to PBMCs. mDCs given α-hDectin-1-Pam3 can significantly decrease OX40L expression, even in the presence of TSLP, and can thus decrease Th2 responses, while slightly increasing IL-17 and IFNγ. Data from the NHP experiment, which is currently ongoing, will be discussed.

**CONCLUSIONS:** Concomitant activation of DCs via Dectin-1 and TLR2 can significantly decrease Th2 responses while slightly enhancing Th1 and Th17 responses. This suggests that α-hDectin-pam3 conjugate could be a novel therapeutic candidate for Th2-driven inflammatory diseases, including allergy and allergic asthma as well as certain types of cancers.
AB102 Abstracts

Influence of Infant Gut Microbiome on Development of Infant Regulatory T Cells

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RATIONALE: The purpose of this study was to determine if the infant gut microbiome impacted regulatory T-cell (Treg) development in a population-based birth cohort.

METHODS: Treg and gut microbiome (stool) profiles (298 infants) from the WHEALS birth cohort, from metropolitan Detroit, were analyzed. Whole blood samples were stained for Treg markers (CD4, CD25, FoxP3). Gut microbiome was measured on stool samples (1 month (n=130), 6 month visits (n=168)) by 16S rRNA sequencing using the Illumina MiSeq platform. Treg association with microbiome composition was evaluated by permutational multivariate analysis of variance. Linear regression was used to test relationships between Tregs and gross community indices (richness, evenness, and diversity); individual bacterial taxa and Treg associations were tested using a zero-inflated negative binomial model, accounting for multiple tests using false discovery rate q-values.

RESULTS: Microbiome composition of stool samples (6 month visit) was associated with Tregs (6 month visit) (p=0.027). Increasing bacterial richness, diversity, and evenness in these samples were positively associated with increasing Tregs (p≤0.025). Breast-feeding modified the association between microbiome and Tregs. Significant composition associations were restricted to breastfed infants (at 1 month) and explained by 91 taxa (significant q-value <0.05), 48% were members of Lachnospiraceae.

CONCLUSIONS: This is the first study in human infants to demonstrate association between gut microbiome composition and peripheral Treg numbers. Breast feeding appears to modify these effects, selectively enriching for taxa positively associated with Treg induction, suggesting a role for maternal factors in shaping the infant immune system via the gut microbiome.

Universal qPCR Duplex Detection of miRNA and mRNA

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RATIONALE: MicroRNAs (miRNA) are a class of 21-23 ribonucleotides single strand RNAs and function as translation repressors by down-regulating target mRNAs in a sequence-specific manner. Deregulation of miRNAs contributes to many diseases and can be used as robust biomarkers for the early diagnosis, staging, prognosis and response to therapy of various human diseases. However, a method for accurate and sensitive detection of all miRNAs is lacking.

METHODS: A universal quantitative polymerase chain reaction (qPCR) duplex detection method was developed to simultaneously detect the expression levels of a specific miRNA and mRNA from two samples or two different RNAs in a PCR well with two distinctive universal fluorescent dual labeled hydrolysis probes.

RESULTS: The expression levels of 96 miRNAs from the same sample quantitated by the fluorescein amidite (FAM) probe and the VIC probe are highly correlated (R2=0.98) indicating that this duplex detection method is highly reproducible and can be used to detect an identical miRNA in two different samples in a PCR well.

CONCLUSIONS: A universal qPCR duplex detection of miRNAs and mRNAs were developed to detect differential expressions of miRNAs or mRNAs with high specificity and sensitivity in paired samples such as normal and diseased tissues. The well to well and plate to plate experimental variations can be greatly reduced, as the expression levels of an identical miRNA in two different samples are detected in the same well at the same time. The differential expression in samples detected by this method is highly reproducible.
**330** Interrogating VCAM-1 Mediated Tumor Immune Evasion in Murine Cervical Cancer

**Jenna R. Bergerson, MD, MPH; Northwestern University, Chicago, IL.**

**RATIONALE:** Vascular Cell Adhesion Molecule-1 (VCAM-1), an adhesion molecule most commonly expressed on the vascular endothelium, mediates extravasations of leukocytes in inflamed tissues through receptor interactions. Metastatic cancer cells over-express VCAM-1 on their surface as a mechanism to provide pro-survival advantages. Previous research found that cervical cancer cell lines with increased expression of VCAM-1 had more metastatic potential than their parental cell lines in both immune competent and immune compromised mice, suggesting that cells other than T cells were responsible for the observed phenotype. Other investigations demonstrated that macrophage interactions with VCAM-1 provide a pro-survival advantage to metastatic breast cancer cells in bone and lungs. We sought to look for the presence of macrophages and associated cytokines in the lung metastases of mice injected with either metastatic cervical cancer or the parental cervical cancer cell line.

**METHODS:** qPCR was used to assess levels of macrophages and associated cytokines in tumor samples from mice injected with metastatic vs. parental cervical cancer cell lines.

**RESULTS:** Arginase-1, a marker of M2a macrophages, and IL-6, a pro-inflammatory cytokine, had higher expression in the lung parenchyma of mice injected with the metastatic model, as compared to mice injected with the parental cell line.

**CONCLUSIONS:** VCAM-1 over-expressing cells acquire a macrophage M2 polarization state in response to micro-environment signals, which results in more immune suppression as compared to the pro-inflammatory tumor micro-environment found in the parental cervical cancer cell line. Future work will characterize VCAM-1 dependent immune responses to establish if VCAM-1 expression on tumor cells affects immune dysfunction.

**331** Development of a Simple, Rapid Microneutralization Test for Respiratory Syncytial Virus Subgroup B

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**RATIONALE:** The standard assay to measure the development of anti-RSV immunity is the plaque reduction neutralization test (PRNT). However, the PRNT assay is time-consuming and labor-intensive, requiring several days of culture and visual inspection of plaques. We have previously developed a microneutralization test (MNT) for RSV subgroup A based on a Renilla luciferase-expressing recombinant RSV (rA2-Rluc). We have now developed a similar assay system for neutralization of RSV subgroup B.

**METHODS:** Recombinant RSV based on the B1 prototype virus (rB1) was generated by synthesis of a full-length RSV B1 antigenomic cDNA, under the control of a T7 promoter and flanked by ribozymes at both the 3' and 5' ends. An additional transcription unit encoding Renilla luciferase was inserted upstream of the NS1 gene. rB1 and rB1-Rluc were recovered by reverse genetics and amplified in Vero and HEp-2 cells. Luciferase activity and PRNT were performed as previously described.

**RESULTS:** rB1-Rluc expressed Renilla luciferase activity in infected cells, showing a broad linear range similar to rA2-Rluc. Anti-RSV F antibodies that cross-react between the A and B subgroups neutralized rB1-Rluc, as determined by both PRNT and MNT. The rB1-Rluc MNT displayed similar sensitivity to neutralization as the PRNT.

**CONCLUSIONS:** We have developed a simple, rapid assay for anti-RSV B subgroup antibodies to complement our existing RSV A MNT. These assays have similar sensitivity to the standard PRNT and can be adapted for high-throughput screening. The combination of these assays will allow for the rapid determination of cross-neutralizing antibody responses in vaccinated individuals.

**332** Adherence to Therapy in Chronic Granulomatous Disease and Disease Outcomes: A Possibility for Development of New Therapeutic Delivery Devices

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**RATIONALE:** Infections in chronic granulomatous disease (CGD) are prevented through the administration of antibiotic and antifungal agents in addition to subcutaneous interferon-γ (IFN-γ) therapy; however, medication adherence remains a barrier.

**METHODS:** Measurements of adherence including frequency of hospitalizations, number of hospital days, number of days of medication adherence by self report, and medication side effects were reviewed for 5 patients with CGD.

**RESULTS:** Median age is 13 years of age (range 5–17 years). Three patients have autosomal recessive CGD; 2 patients have X-linked CGD. Patient A had 8 admissions while non-adherent to IFN-γ (53 hospital days over 40 months) and 1 admission while adherent. Patient B had 7 admissions while non-adherent (47 hospital days over 27 months) and zero admissions while adherent. Patient C had 12 admissions while non-adherent (57 hospital days over 36 months) and 4 admissions while adherent. Patient D had no admissions prior to or during periods of adherence. Patient E had 2 admissions while non-adherent (38 hospital days over 41 months) and no admissions during 28 months of adherence. Patients reported injection site pain and inconvenience as frequent reasons for non-adherence. One patient reported having fevers after every injection and a febrile seizure.

**CONCLUSIONS:** Patients with CGD frequently report non-adherence to IFN-γ for extended periods which result in frequent and prolonged hospitalizations for infections. In the 5 years of this review, patients were more frequently non-adherent than adherent to therapy. Barriers to therapy and possible technological solutions should be pursued for this medication.
In silico Analysis of Vaccination Adverse Events
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RATIONALE: Vaccination associated adverse events may develop by mechanisms of autoimmune or autoreactive diseases (AD). These events are rare, diverse, and challenging to evaluate given high rates of vaccination, background incidence of AD, and low incidence and variable times for AD onset after vaccinations. A bioinformatics, systems biology approach was introduced to assess the biological plausibility of vaccine-related AD’s.

METHODS: The Vaccine Adverse Event Reporting System (VAERS) identified the most common vaccine-associated AD’s. Multiple curated databases and automated text mining of PubMed provided information on innate and adaptive immune mechanisms active in AD’s, infectious diseases targeted by vaccines, and cellular reactions to vaccine antigens, adjuvants, preservatives, and stabilizers. Gene interaction networks for each AD were displayed using CytoScape software.

RESULTS: Rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), Guillain-Barre syndrome (GBS), and idiopathic thrombocytopenic purpura (ITP) were the most frequently reported vaccine-related autoimmune adverse events. RA was associated with 667 genes, SLE with 448, GBS with 73, and ITP with 49. Only 6 genes were shared by the 4 ADs. Cluster analysis for RA identified 12 immune system categories including “Chemokine plus Receptors,” “Th17 T-cell,” and one module with 10 CXC motif chemokines that were neutrophil chemotactic factors. Genes mined from GBS, GBS peptide autoantigens, influenza A infection, and influenza vaccination-induced leukocyte transcriptomes formed a network of 16 genes that inferred MAPK signaling in influenza vaccine–related GBS.

CONCLUSIONS: Bioinformatics data mining and network analysis identified plausible mechanisms to explain vaccination-related AD’s. This data-driven outcomes generate testable hypotheses to model vaccine safety and efficacy.

Production of Secretory Leucocyte Proteinase Inhibitor in Children with ACUTE and Chronic Pyelonephritis
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RATIONALE: he levels of the secretory leucocyte proteinase inhibitor (SLPI) in blood and urine of pediatric patients having acute (A) and chronic (Ch) pyelonephritis (PN) were assessed.

METHODS: 26 patients with ChPN in remission and 12 patients with ChPN in exacerbation, and 19 patients with APN had levels of SLPI in blood and urine assessed by enzyme-linked immunosorbent assay (“Hycult biotechnology” Human SLPI).

RESULTS: Statistically significant differences were noted between the concentration of SLPI in children with APN (3454.7 [2911; 3740.9] in blood and 150.7 [55.7; 316] in urine) and ChPN (3263.9 [2395.9; 3483.3] in blood and 81.8 [40; 194] in urine), and in the acute stage (3235.3 [2314.8; 3554.9] and 178.65 [48.8; 449.7]) and in remission (3397.5 [2677.3; 3421.3] and 81 [36.4; 170.7]) of ChPN. There is a correlation between the concentration of SLPI and the number of lymphocytes in the peripheral blood (r<0.05) in patients with APN (rK=0.605) and with ChPN in exacerbation (rK=0.536). Correlations between the level of SLPI, C-reactive protein and erythrocyte sedimentation rate and the numbers of neutrophils were not seen (p>0.05).

CONCLUSIONS: SLPI participates in the pathogenesis of APN and ChPN during exacerbation.

Sensitization of a Child to Cyanobacteria after Recreational Swimming in a Lake
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RATIONALE: Recently phycocyanin from the cyanobacteria species, Microcystis aeruginosa (Ma), was found to be the peptide responsible for causing sensitization to cyanobacteria in a subset of chronic rhinitis patients. Here, we report a case of an 11 yr-old girl who developed an allergic reaction manifesting as an erythematous rash over here entire body and severe facial swelling with periorbital edema after swimming in Lake Ontario, Canada. Her mother reported what looked like algae around and on the lake which was subsequently confirmed to be freshwater cyanobacteria by environmental health officials.

METHODS: Patient serum was analyzed for specific IgE to cyanobacteria using extracts from eight species (Microcystis aeruginosa, Synechocystis, Synechococcus, Pseudanabaena, Oscillatoria, Sytonema, Lyngbya, Arthrospira) by IgE-specific ELISA and ELISA inhibition. A beta-hexosaminidase release assay was performed with humanized rat basophil leukemia (hRBL) cells using cyanobacteria extracts and the patient’s serum. Multiple sequence alignment was performed using ClustalW.

RESULTS: Specific IgE was increased in response to Microcystis aeruginosa and Synechococcus Species which was dose-dependently inhibited by pre-incubation of the child’s serum with Synechococcus. Both extracts induced mediator release from sensitized hRBL cells. The phycocyanin sequences from Microcystis aeruginosa and Synechococcus Species showed >85% sequence similarity.

CONCLUSIONS: This case emphasizes the importance of recognizing that recreational contact to freshwater cyanobacteria can lead to sensitization and subsequent allergic reactions. Further work is warranted to determine the extent to which the US populace is exposed to cyanobacteria and the prevalence of sensitization.

Impact of Exposure Level and Duration on Reducing TMA Specific IgE Responses in Workers over Time
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RATIONALE: Trimellitic Acid anhydride (TMA) immunosurveillance programs in the workplace are essential for preventing occupational disease. The purpose of this study was to determine the effect of magnitude and duration of TMA exposure on persistence of TMA-specific IgE responses.

METHODS: Serum collected from TMA exposed workers as part of an ongoing immunosurveillance program was analyzed for TMA specific IgE by ImmunoCAP in workers with high, moderate and low TMA exposure at different time periods. Workers with TMA specific-IgE >0.34 IU/mL anytime during the surveillance period were further analyzed using quadratic regression models.

RESULTS: TMA specific IgE levels (median values) were highest (13.2 IU/mL) for workers with high TMA (level 4) exposure compared to other groups of exposed and non-exposed workers. The magnitude and duration of exposure were strong predictors of changes in log-transformed TMA specific IgE levels among removed workers; their TMA-specific IgE levels decreased over time compared to active workers with significantly high TMA-specific IgE levels who continued to work in TMA low exposure areas. This implies that early detection of high serum IgE level (>2.0 IU/mL) and complete removal from the source of TMA exposure is more effective in lowering TMA specific IgE levels and reducing sensitization over time compared to when workers with mild elevation of TMA specific IgE (>0.34IU/mL) continue to have persistent low level TMA exposure.

CONCLUSIONS: Early detection and removal of workers from TMA exposure, rather than reducing exposure to a lower level, is most effective at reducing TMA sensitization over time.

All abstracts are strictly embargoed until the date of presentation at the 2015 Annual Meeting.
Trends in Ragweed Pollen Counts in the Midwest  
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RATIONALE: Ragweed is a flowering plant in the genus Ambrosia in the aster family, Asteraceae. It is most prevalent in North America, particularly in the Midwest. Ragweed pollen season typically runs from mid-August through mid-October. Average ragweed pollen counts over the last 15 years were evaluated.

METHODS: Using a Hirst spore trap, pollen was collected on glass slides coated with silicone grease from the roof of a 5 story building in Kansas City, Missouri. Collection was performed daily from July 1st to December 1st, 1999-2013. Slides were stained with Calberlas stain in glycerin jelly. Pollen grains were enumerated microscopically every 4 hours for 15 successive ragweed seasons. Data was entered and analyzed using Microsoft Excel. Means for total yearly ragweed pollen counts were calculated and the start and end dates of each season were evaluated. Pollen counters were certified by the National Allergy Bureau.

RESULTS: Total means of yearly ragweed were much higher between 2004-2008 compared to 1999-2003 and 2009-2013. The average ragweed pollen season started around mid-August, however as the years progressed the season ran longer to late-October and even early-November.

CONCLUSIONS: Ragweed pollen season is starting around mid-August as expected but appears to be running longer in more recent years. The average total ragweed pollen counts were highest in 2004-2008, and there has been less total ragweed pollen in the last 5 years since the season running longer. Climate change, including variation in temperature/precipitation and extreme events such as floods/droughts, may account for these unexpected results, but further studies are needed.

Tree Canopy Cover Modifies the Association Between Daily Tree Pollen Concentrations and Emergency Department Visits for Asthma in New York City  
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RATIONALE: Short-term exposure to allergenic tree pollen is a risk factor for asthma exacerbation. Neighborhood-level characteristics that modify the effect of pollen exposure on asthma exacerbations have not been well investigated.

METHODS: We used distributed lag Poisson generalized linear models to describe the relationship between daily concentrations of four allergenic tree pollen taxa (Platanus spp., Fraxinus spp., Betula spp., Acer spp.) with daily emergency department (ED) visits for asthma in each NYC zip code, adjusting for temperature, rain, ozone, PM2.5, seasonal trends, day of week, and year. We derived city-wide effect estimates and tested for effect modification by zip code-level characteristics in a second-stage analysis using random-effects meta-analysis.

RESULTS: Daily concentrations of all four pollen taxa were associated with an increased rate of ED visits for asthma, with rate ratios (RRs) for the cumulative 7-day effect of the maximum pollen concentration ranging from 1.57 [95% CI: 1.48, 1.67] for Betula to 2.03 [95% CI: 1.89, 2.18] for Fraxinus. Associations were stronger in zip codes with higher tree canopy cover. Estimates for the change in the RR per an interquartile range increase in tree canopy cover range from a 15.54% [95% CI: 2.99%, 28.09%] increase for Platanus to a 23.19% [95% CI: 3.17%, 43.21%] increase for Acer. Zip code level measures of median household income, percent non-Hispanic Black, and estimated black carbon did not modify the associations.

CONCLUSIONS: Tree pollen is an important trigger of springtime asthma exacerbations in NYC. Tree pollen exposure may be higher in zip codes with higher tree canopy cover.

Allergen Characterization of Aedes Aegypti By a Proteomic Approach  
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RATIONALE: Several studies suggest that A. aegypti induces respiratory allergic diseases. However, allergens related to this response are unknown. Since cross-reactivity among mosquitoes and other arthropods exist, participation of mosquitoes as primary sensitizers is an unresolved topic. We hypothesize that A. aegypti contains specie-specific and cross-reactive allergens that may sensitize people.

METHODS: Whole body A. aegypti was used to prepare an allergenic extract and to purify native tropomyosin. Sera were obtained from allergic individuals from the tropical Caribbean island of Martinique with asthma and/or rhinitis and specific IgE to mosquito. IgE reactivity against the extract, or purified tropomyosin from different species, was studied by ELISA. Allergens were identified by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE), followed by western blot with pooled sera. Cross-reactivity between A. aegypti and other arthropods (mites, cockroach and shrimp); or tropomyosin from A. aegypti and rDer p 10, rBlo t 10 and rLit v 1 was evaluated by ELISA competition.

RESULTS: Twenty nine IgE-reactive proteins were identified in the 2D-PAGE. Two tropomyosin isoallergens (Uniprot code: Q17H75 and Q17H80) of 32 kDa reacted with 33% of the mosquito positive sera. Higher inhibition of IgE reactivity against mosquito extract was achieved by L. vannamei (71,19%), followed by D. pteronyssinus (58,77%), P. americana (51,32%), B. tropicalis (36,97%) and D. farinae (19,55%). rBlo t 10, rDer p 10 and rLit v 1, produced between 61-75% inhibition of IgE reactivity against mosquito tropomyosin.

CONCLUSIONS: A. aegypti contains cross-reactive as well as unique allergens, which are strong inducers of allergic responses.

Allergy to Dermestidae: A New Indoor Allergen?  
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RATIONALE: Black carpet beetle (attagenus spp.) is a coleoptera classified within the Dermestidae family. It is a ubiquitous insect, present in about 70% of Spanish households. Scarce cases of allergy due to dermestidae have been reported. However, its importance as an indoor allergen has not been established yet.

METHODS: Five patients with perennial rhinitis or asthma were studied. Symptoms were only referred at their homes. Insects were spotted in all the studied houses, and black carpet beetle was identified in its vacuumed-dust. Extracts to their house’s vacuumed-dust and dermestidae were produced. Skin prick tests to both extracts and common aeroallergens were implemented. Specific nasal and bronchial challenges to dermestidae were also performed in one patient. SDS-PAGE and immunodetection assays were accomplished in all patients’ sera.

RESULTS: SPT were positive to dermestidae and vacuumed-dust extracts in the five evaluated patients. Three of them also showed pollen sensitization. SPT were negative to mites or animal dander. SNC and SBC to dermestidae were positive on the challenged patient. Immunoblotting to both extracts, dermestidae and vacuumed-dust, showed several IgE-binding bands ranging between 8 kDa and 35 kDa with both extracts, including two common bands of 25 kDa and 28 kDa in all patients.

CONCLUSIONS: We present 5 patients with perennial allergic symptoms sensitized to this household-colonizing insect. Allergy to black carpet beetle and dust from an infested house was demonstrated by means of SPT, SNC, SBC and immunodetection assays. Dermestidae can be a significant indoor allergen in houses where these insects are present.
### 341 Short-Term Effect of Temperature Change on the Number of Hospital Visits Secondary to Acute Asthma Exacerbations

**Jennifer Lan, MD**; Jay A. Lieberman, MD; Zhao Yang; Department of Allergy and Immunology, The University of Tennessee Health Science Center, Memphis, TN.

**Methods:** We performed a retrospective review of pediatric (2-17 years) emergency department visits and hospitalizations secondary to asthma exacerbations for the year 2012 at a single pediatric hospital. A quasi-Poisson regression combined with a distributed lag nonlinear model (DLNM) was applied to evaluate the association between inter-day temperature change and visit frequency. The analyses adjusted for daily pollutant variables, other weather variables, and temporal factors to control seasonal and long-term patterns.

**Results:** 2728 encounters were examined with an average of 7.5 daily visits. There is a general non-significant trend that greater negative inter-day temperature change is associated with a greater number of hospital visits, with its effect persisting for 7 days. A one-day 20 degree drop was associated with a relative risk (RR) of 1.24 (95% CI: 0.92 to 1.66). The 7-day cumulative RR resulting from a -10°F and -20°F change are 1.24 (95% CI: 0.74 to 2.10) and 2.80 (95% CI: 0.54 to 14.50), respectively.

**Conclusions:** Although not statistically significant, there is a strong trend suggesting a drop in temperature leads to increased hospital visits for asthma exacerbations. Further studies with inclusion of pollen data and data from multiple years may help improve the validity of this association.

### 342 Is Switchgrass an Emerging Allergy Risk?

**Landon Bunderson, PhD**; Raymond W. Arritt, PhD; Iowa State University, Ames, IA.

**Rationale:** Pennisetum virgatum (switchgrass) is currently receiving a lot of attention as an emerging biofuel crop because of its propensity for high yields and resistance to pests and diseases. Depending on a variety of factors, switchgrass could potentially cover large acreages of “low quality” cropland. Although switchgrass is not considered highly allergenic, increased production could lead to increased exposure resulting increased sensitivity. Determining pollen levels at various distances from the crop could help prepare allergists to consult patients on potential risks.

**Methods:** Two Burkard volumetric pollen traps were placed near a switchgrass field in Ames, IA from July to September of 2013. Samplers were placed 2 m and 500 m from the edge of field. The switchgrass field was surrounded by corn and soybean crops and warm-season grassy weeds were minimal in the area. Standard methods were used in the preparation and analysis of slides.

**Results:** Grass pollen levels were very different at the two different sampling sites. The 2 m sampler recorded 6 “very high” days and peak daily mean concentration occurred on August 21st with a mean concentration of 713 pollen grains/m³. The 500 m sampler experienced no “high” or “very high” days and only experienced one day where pollen concentration was “moderate”. The 500 m sampler peaked on August 30th with a mean concentration of 23 pollen grains/m³.

**Conclusions:** While pollen levels adjacent to the switchgrass field may pose a threat to sensitive individuals, exposure can be drastically reduced by separating the patient from the field by a relatively short distance.
Mulberry - a Chronic Pollen Offender in Las Vegas
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RATIONALE: In Nevada, mulberry pollen is a significant trigger for seasonal allergic diseases. An epidemiologic study showed that over one-third of Nevada children were sensitized to mulberry. In response to very high airborne mulberry pollen concentrations, the Clark County Air Pollution Control Regulations prohibited further planting of fruitless mulberry trees in 1991. The goals of this study were to investigate the current mulberry pollen concentrations in Las Vegas and evaluate the effectiveness of the regulation.

METHODS: Air samples were collected using a Burkard volumetric recording spore trap at the University of Nevada, Las Vegas from January 21 to August 31, 2014. Burkard slides were analyzed by light microscopy at a magnification of 400X. Airborne mulberry pollen concentrations were calculated and compared with those recorded at a nearby site between 1989 and 2009. A one-sample t-test was used to compare the annual peak concentrations.

RESULTS: Between 1989 and 2009, the mean peak mulberry pollen concentration was 5104±3892 grains/m³ and the highest concentration of 14,425 grains/m³ was observed in 2009. In 2014, mulberry pollen concentrations started to increase from mid-February and remained high (>90 grains/m³) until the end of March. The peak concentration of 17,197 grains/m³ is significantly higher than the previous records (p<0.01).

CONCLUSIONS: Despite the implementation of the Air Pollution Control Regulation, airborne mulberry pollen concentrations remain high in Las Vegas and cause allergic reactions during pollen season. Regular aeroallergen monitoring, accurate pollen forecasts, and public health interventions are essential to inform the public and alleviate the burdens of allergy sufferers in Las Vegas.

Allergen, Endotoxin and Protein Levels in Cultured E. Maynei
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RATIONALE: Euroglyphus maynei occurs in homes worldwide and is the source of many allergens. High sensitivity to E. maynei has been reported among patients sensitive to Dermatophagoides farinae and D. pteronyssinus. This mite is not widely cultured for commercial purposes. Here, we measured the allergen, protein and endotoxin levels in growing cultures. These data may be useful for developing standard protocols for culturing E. maynei and standardization of extracts for laboratory and commercial purposes. We compare the E. maynei data to those for D. farinae and D. pteronyssinus cultured under the similar conditions.

METHODS: Fresh E. maynei cultures were started and Eur m 2 allergen, protein and endotoxin levels in these cultures were determined at 2-week intervals as thriving cultures developed and food became limiting in 8-14 weeks.

RESULTS: Beginning 0-4 weeks post inoculation of new culture media, the mite populations grew exponentially in the cultures at 30° and 23°C. Eur m 2 and protein increased exponentially in parallel with the mite population growth at 30°C. In contrast, cultures at 23°C exhibited a decline in Eur m 2 and soluble protein even though the mite population grew exponentially. Endotoxin levels in the cultures at both temperatures were very low and are probably not significant over the duration of culture growth.

CONCLUSIONS: Growth temperature influenced Eur m 2 and protein concentrations in E. maynei cultures. Endotoxin levels in E. maynei cultures were very low and probably insignificant in contrast to endotoxin concentration in cultured D. farinae and D. pteronyssinus.

Probability Models for Daily Occurrence of Allergic Pollens in Korea Exclusively Based on Meteorological Data
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RATIONALE: The increasing number of allergic pollens by the global warming and air pollution causes increasing number of patients with allergic diseases.

METHODS: We investigated the long-term trend of the pollen concentration and the distribution of pollens by meteorological conditions at six regions in Korea. Daily probability models were developed for the detection of daily pollens based on the characteristics of daily existence of the pollens and its relationship with the meteorological variables. The logistic regression model, which is a special case of the generalized linear model, was utilized. The long-term trend showed increasing number of yearly pollens for 18 years.

RESULTS: The accumulated temperature showed strong influence on the daily pollen probability over the other meteorological variables. The odds ratio of accumulated temperature for Ragweed was 44.942. The selected variables for trees were mainly temperature dependent variables: mean temperature for Alnus; mean temperature, accumulated temperature, and 7-day sunshine hours for Pine; mean air temperature and wind speed for Betula; accumulated temperature and 7-day sunshine hours for Quercus. The majority of the selected variables for weeds were also temperature variables with added complexity: mean temperature, accumulated temperature, wind speed, and 7-day sunshine hours for Ragweed and Mugwort; mean temperature, accumulated temperature, daily temperature range, and wind speed for Japanese hop.

CONCLUSIONS: Mean air temperature, accumulated temperature, and 7-day sunshine hours had major influence in these models. The daily probability of pollens occurrence was closely related to the daily meteorological variables including air temperature so that it will be considerably influenced by the climate change of the future.

Probability Models for Daily Occurrence of Allergic Pollens in Korea Exclusively Based on Meteorological Data
Jae-Won Oh, MD, PhD, FAAAAAI1, Kyung Kim1, Hye-Rim Lee2, Byoung-Chael Choi1, National Institute of Meteorological Research, Jeju, South Korea, 2National Institute of Meteorological Research, South Korea.

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CONCLUSIONS: Mean air temperature, accumulated temperature, and 7-day sunshine hours had major influence in these models. The daily probability of pollens occurrence was closely related to the daily meteorological variables including air temperature so that it will be considerably influenced by the climate change of the future.
CONCLUSIONS: The effect of air pollution on validated asthma symptom scores is unknown. After informed consent and IRB approval, subjects recruited within similar regions, and from year-to-year, pollen counts can vary by type, quantity, and timing. Local pollen counting stations are needed to improve allergy care.

METHODS: Pollen data were analyzed from April, May, and June, in 2013 and 2014, for both cities. Pollen counts were obtained from the NAB webpage and from the Dayton Regional Air Pollution Control Agency, which uses a Burkhard sampler. Local Indianapolis pollen count data was obtained via Rotorod and posted daily on a webpage.

RESULTS: For the time frame reviewed, Dayton’s pollen information was available for 62 days in 2013 and 2014. In Indianapolis, pollen data was available for 86 days in 2013, and 89 days in 2014. Tree pollen species were similar in both areas. In Dayton, tree pollen was detected on all monitored days. However, in Indianapolis, tree pollen was detected only 65% of reported days in 2013 and 80% in 2014. In Dayton, grass pollen was detected on 50% of reported days in 2013, and 65% in 2014. In Indianapolis, grass pollen was detected 66% of reported days in 2013 and 52% in 2014. Weed pollen was detected in both sites, with plantain and nettle found in early May 2014 in Indianapolis.

CONCLUSIONS: Within similar regions, and from year-to-year, pollen counts can vary by type, quantity, and timing. Local pollen counting stations are needed to improve allergy care.
Osteopontin (OPN) Plays a Critical Role in Respiratory Syncytial Virus (RSV) Infection

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RATIONALE: OPN has been implicated in several inflammatory diseases including allergic diseases and influenza infection. However, the role of OPN in RSV infection is unknown. Since, OPN was one of the major genes that changed its expression in both RSV infected young and aged mice, we examined OPN as a potential risk factor during RSV infection.

METHODS: RNAs or proteins isolated from epithelial cells at 24, 48 and 72 hours post infection (hpi); with rA2-L19F (1MOI), were examined for OPN expression by qPCR and Western Blot. Also, Wild-type and OPN-KO mice (C57BL6 genetic background) were compared for RSV infection and cytokine production (IL-17a and IL-13) following intranasal exposure with 10^4 plaque forming units (pfu) per mouse. Infected mice were euthanized on days 5 and 8 post-infection. Lungs and serum were collected for marker analyses.

RESULTS: RSV infected epithelial cells showed increased OPN expression after 48 hpi; levels were maintained at 72 hpi. In vivo experiments showed an increase in OPN expression in lungs sections of WT mice after immunostaining with mouse anti-OPN antibody at day 5 pi. RSV-N expression and plaques titters were reduced in OPN-KO mice compared to WT (A2-L19F infected mice. Furthermore, our data suggest that OPN controls IL-17a production in the context of RSV infection.

CONCLUSIONS: Our results thus far suggest that OPN plays a critical role in productive RSV infection in epithelial cells and RSV infected mice. However, the mechanism underlying OPN-regulation of RSV infection remains to be elucidated.

Vitamin D Supplementation and the Risk of Colds in Patients with Asthma

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RATIONALE: Vitamin D insufficiency and asthma exacerbations are both associated with respiratory tract infections (RTIs); however, vitamin D supplementation has inconsistently reduced RTIs in the general population. We asked whether vitamin D supplementation reduces severity and frequency of RTIs in adults with mild to moderate asthma and vitamin D insufficiency.

METHODS: RTIs were assessed in the AsthmaNet VIDA (Vitamin D Add-on Therapy Enhances Corticosteroid Responsiveness) trial, which randomized 408 adult patients to receive placebo or cholecalciferol (100,000 IU load plus 4,000 IU/day) for 28 weeks as add-on therapy. Cold symptoms were assessed using daily URRSS-21 scores. Sun exposure was estimated using a spectrophotometer.

RESULTS: 203 of the 408 (49.8%) participants experienced at least one cold, which was associated with a decline in Asthma Control Test scores relative to subjects without colds (average change -1.3, 95% CI -1.8 to -0.8 vs 0.2, 95% CI -0.4 to 0.8, p < 0.001). Vitamin D supplementation had no effect on the average peak cold scores (58.7 (95% CI 52.4 to 65.0) and 62.0 (95% CI 55.1 to 68.9) respectively), nor did it affect the rate of colds (rate ratio 1.2, 95% CI 0.9 to 1.5). Season influenced the frequency of colds and the degree of sun exposure. The change in 25-hydroxyvitamin D level had no effect on the severity or frequency of colds, adjusting for season and sun exposure.

CONCLUSIONS: Our study suggests that restoration of vitamin D sufficiency does not impact cold severity or frequency in patients with mild to moderate asthma.
355 IFN-beta and IFN-lambda1 Induce Kinetically Distinct Patterns of Transcription Factor Interferon Stimulated Genes in Respiratory Epithelial Cells
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Rationale: Viruses and other pathogenic stimuli induce expression of both types I and III interferons (IFN). Type I IFN include IFN-beta and 12 subtypes of IFN-alpha and type III IFN include IFN-lambda1-4. Type I IFN receptor expression is ubiquitous, while type III IFN receptor expression is largely restricted to epithelial cells. Cooperative expression of types I and III IFN suggest unique roles for each, but their similar activation of STAT1/STAT2 heterodimers and interferon stimulated genes (ISG) suggest that types I and III IFN are redundant.

Methods: We determined unique or cooperative roles for types I and III IFN in cellular activation by stimulating BEAS-2B human respiratory epithelial cells with IFN-beta or IFN-lambda1 alone or together at multiple concentrations and time points. We measured expression of up to 23 transcription factor ISG (TF-ISG) and canonical ISG by qRT-PCR.

Results: While IFN-beta induces strong and early peak expression of TF-ISG, IFN-lambda1 induces a gradual, long-term induction of ISG expression. For a subset of TF-ISG (TRIM22, IRF7, STAT1) the response to IFN-beta is sustained, while for another ISG subset (BATF2, ETV7, TRIM25) the response wanes. At EC50 doses of IFN-beta and IFN-lambda1 together, peak expression of TF-ISG is additive, but their induction rapid and sustained.

Conclusions: The types I and III IFN, IFN-beta and IFN-lambda1, respectively, each induce expression of TF-ISG in unique kinetic patterns. Together, these two IFN coordinate rapid, high, and sustained expression of ISG, including those that may affect subsequent cellular activity through their function as transcription factors.

356 Respiratory Syncytial Virus and Rhinovirus Contribute to the First Wheeze Episodes in Japanese Infants
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Rationale: To identify respiratory viruses in infants experiencing their first episode of wheeze hospitalized for lower respiratory illness.

Methods: We enrolled 70 infants (7.6 ± 6.8 months) hospitalized between August 2009 and June 2012 for acute respiratory symptoms with their first episode of wheeze. Nasopharyngeal swabs were collected after written informed consent was obtained from the patients’ parents, and collected clinical data. The identification of viral genomes, including respiratory syncytial virus (RSV), human rhinovirus (HRV), human metapneumovirus, human parainfluenzavirus, influenza virus, adenovirus, and human bocavirus in the sputum/nasal discharge was performed using RT-PCR technique. Slides of sputum were prepared with a cytospin and stained with hematoxylin-eosin for differential cell counts. The patients were followed up for at least 2 years from the first wheeze episodes by clinical examination or phone interview to patients’ mother whether they experienced recurrent wheezing episodes.

Results: Eighty six % of infants experiencing their first episode of wheeze were infected with RSV, HRV or their co-infection. Peripherial eosinophil count was 1.8 ± 2.6%, and sputum eosinophil count was 47.6 ± 24.6%. Fifty seven % of infants had experienced recurrent wheezing episodes. Family history of asthma, virus types, and counts of sputum eosinophils were not associated with their recurrent wheezing episodes. Infants with food allergy were significantly associated with the recurrent wheezing episodes (P< 0.05).

Conclusions: RSV and HRV are major triggers of first wheeze in infants. Respiratory viruses induce eosinophilic inflammation in their airways. Food allergy is the risk factor of recurrent wheezing episodes.

357 Rhinovirus A and C Wheezing Illness in Infancy and the Development of Asthma
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Rationale: Rhinovirus (RV) wheezing illness in early life is a risk factor for subsequent childhood asthma. Whether the species of rhinovirus causing the wheezing illness differentially affects asthma risk is unknown. We hypothesized that RV-C wheezing would be the best predictor of asthma development.

Methods: Children participating in a high-risk birth cohort (Childhood Origins of Asthma) were followed prospectively to determine wheezing illnesses with specific RV species in the first year of life. A total of 259 children were followed at year 6, 238 at year 8, and 217 at year 11. Asthma was defined by physician diagnosis, the use of SABA, daily ICS, step-up therapy and/or oral corticosteroids. Nasal samples were collected during wheezing illnesses and analyzed for respiratory viruses using multiplex PCR, partial sequencing for RV typing.

Results: Children who wheezed with RV-C in the first year of life had significantly higher rates of asthma at age 6 [OR 3.5 (1.3, 9.7)], but not at ages 8 [OR 1.8 (0.6, 5.2)] or 11 [OR 2.6 (0.9, 7.3)]. Wheezing with RV-A was not associated with asthma at year 6 [OR 1.3 (0.6, 3.1)], year 8 [OR 2.3 (1.0, 5.4)] or year 11 [OR 1.7 (0.7, 4.1)]. There were no wheezing episodes associated with RV-B infections.

Conclusions: This longitudinal analysis suggests that wheezing with RV-C in the first year of life is an indicator of increased risk for the subsequent development of asthma. Additional studies are needed to confirm this observation.
358 House Dust Nicotine Levels, Smoking History and Asthma Indicators
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RATIONALE: Tobacco smoke is a known asthma trigger. Exposure to tobacco smoke is usually determined by history. To examine the relationship of nicotine in house dust with smoke exposure history and asthma indicators we conducted the following.

METHODS: House dust was collected from smoking and non smoking homes either from the bag of the family vacuum or by vacuuming the child’s bedroom using HUD protocol. Tobacco exposure history and history of asthma indicators as asthma diagnosis, steroid use, wheeze, cough and shortness of breath was collected by questionnaire. Nicotine in house dust was assayed using a modified cotinine assay kit with antibodies cross reactive to nicotine.

RESULTS: House dust was recovered from 36 homes of which 33 reported some relation to smoking with 16 homes reporting to at least 1 person who smoked inside the home. Very low nicotine was detected in homes denying any history of smoking. Nicotine was detected in 27 of the home dust samples. Nicotine was detected in 15 of 27 homes with a history of smoking. It correlated poorly with any smoking history (r = 0.42). Nicotine concentration also correlated moderately with several asthma indicators including diagnosis of asthma (r = 0.28), cough (r = 0.27), wheeze (r = 0.29), shortness of breath (r = 0.30) and steroid use (0.32).

CONCLUSIONS: Nicotine levels in house dust can be used to verify smoking history. In addition, nicotine levels in house dust correlate positively with several asthma indicators.

359 Comparative Pharmacovigilance Study of Smoking Cessation Therapies and Suicidality Risk
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RATIONALE: Nicotine and non-nicotine based products, including bupropion and varenicline, are used as pharmacological interventions to aid smoking cessation. There has been a growing concern regarding the psycho-behavioral safety of these agents. However, there is no comparative safety analysis between these products to characterize suicidality risk.

METHODS: Adverse event reports that were spontaneously submitted to the FDA Adverse Event Reporting System between 1997Q3 and 2013Q2 were retrieved. Suicidality custom term was created by the following MedDRA preferred terms that were recorded in the cases: “completed suicide”, “depression suicidal”, intentional self-injury”, “self-injurious behaviour”, “self-injurious ideation”, suicidal behaviour”, “suicidal ideation”, and “suicidal attempt”. Bupropion, varenicline, and nicotine were identified by generic names. Empirical Bayes Geometric Mean (EBGM) with 95% confidence interval (EBGM-EB95) is used as a measure of disproportional reporting of suicidality for smoking cessation therapies. A threshold of EBGM>2.0 was considered a suicidality safety signal.

RESULTS: 10,558 suicidality events were reported for smoking cessation therapies, corresponding to varenicline (n=6,804), bupropion (n=3,425), and nicotine (n=329). Varenicline (EBGM=5.43, EB05-EB95=5.32-5.54) and bupropion (EBGM=3.15, EB05-EB95=3.07-3.24) were associated with disproportionate reporting of suicidality. No signal was detected for nicotine (EBGM=0.34, EB05-EB95=0.31-0.37). Majority of reports were serious (e.g. resulted in death or hospitalization). 55% of varenicline users were females (median age=45, min=6, max=84), 60% of bupropion users were males (median age=41, min=9, max=88), and 56% of nicotine users were females (median age=43, min=15, max=86).

CONCLUSIONS: Suicidal ideation and behavior might be potential risks of smoking cessation therapy with varenicline and bupropion, but not with nicotine replacement therapy.

360 Effective of Second Hand Smoke Exposure (SHS) on Asthma Morbidity and Healthcare Utilization: Systematic Review and Meta-Analysis
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RATIONALE: Asthma is a leading chronic disease in children. Second hand smoke (SHS) exposure can trigger asthma exacerbations but the risk has not been quantified uniformly across studies. We sought to perform a systematic review to evaluate and quantify asthma severity and healthcare utilization in children related to SHS exposure.

METHODS: A systematic review that followed standard procedures as developed by the Cochrane in order to assess asthma severity and SHS in children was undertaken. Random effect models were used to combine the outcomes of interest (hospitalization, ER or urgent care (UC) visits, severe asthma symptoms, wheeze symptoms, and pulmonary function tests) from the included studies.

RESULTS: A total of 1607 studies were identified and 36 studies met inclusion criteria. We found that children with asthma and SHS exposure had higher rates of hospitalization, ER visits and lower pulmonary function tests. These children were nearly twice as likely to be hospitalized as compared to asthmatic children without SHS exposure (OR=1.99, 95% CI: 1.20, 3.31, p<0.01). SHS was also significantly associated with ER or UC visits (OR=1.66, 95% CI: 1.02, 2.69, p=0.04), severe asthma symptoms (OR=1.25, 95% CI: 1.20, 1.30, p<0.001), wheeze symptoms (OR=1.31, 95% CI: 1.23, 1.40, p<0.001) and reduced FEV1/FVC (-3.34, 95% CI: -5.35, -1.33, p=0.001).

CONCLUSIONS: Asthmatic children with SHS exposure are twice as likely to be hospitalized with asthma exacerbation and are more likely to have lower pulmonary function tests. Physicians should actively assess SHS exposure and engage parents in tobacco control, which has potential to improve asthma outcomes.
361 The Association Between Vitamin D Insufficiency and Children with Asthma

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RATIONALE: Recent epidemiologic studies have shown that the lack of vitamin D levels may be associated with higher asthma prevalence. However, little clinical evaluation was performed in Korea. This study aimed to explore the association of vitamin D levels in serum with asthmatic children in Korea.

METHODS: A total of 64 children (34 asthmatic children and 30 healthy children) aged 6-14 years were recruited. Serum 25-hydroxy vitamin D3 (25-OH vitamin D) levels were measured and compared between the two groups. Pulmonary function tests (PFT) as well as questionnaire survey including time of outdoor activity were performed as well. The relationship between serum 25-OH vitamin D levels and the results of PFT as well as time of outdoor activity were examined in asthmatic patients.

RESULTS: Serum 25-OH vitamin D levels in asthmatic patients (16.6 ± 4.2 ng/ml) were significantly lower than that in healthy controls (24.2 ± 6.8 ng/ml) (P<0.05). Also, about 0.8 times increase of the asthma prevalence was observed as 1 ng/ml of vitamin D level decreased (OR, 0.788; 95% CI, 0.707-0.879; P<0.001). The level of vitamin D for the group with greater than 1 hour of outdoor activity (20.5 ng/ml) was higher than that for group with less outdoor activity (18.0 ng/ml), but no significance was found. There were also no associations with vitamin D level and the results of PFT.

CONCLUSIONS: Our findings suggest that the levels of serum vitamin D may be one of risk factors for development or exacerbation of symptoms in children with asthma in Korea.

362 Non-Asthmatic Children Have Higher IgM Anti-Enterovirus 71 and Lower IgE Levels, but Higher IL-2 and IL-4 Levels Than Asthmatic Children

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RATIONALE: Enterovirus 71 (EV71) infection is a main cause of hand, foot, and mouth disease, and is associated with aseptic meningitis and severe neurological complications in young non-asthmatic and asthmatic children. It is unknown if Immunoglobulin (Ig) M anti-EV71 plays a regulatory role in asthma.

METHODS: We measured specific IgM anti-EV71, total serum IgE, and IL-2 and IL-4 cytokine levels in serum of non-asthmatic and asthmatic children (N=35, ages 1-20, N=42, ages 5-19, respectively) (UniCAP Total IgE Fluoroenzymeimmunoassay (IU/mL), enzyme-linked immunosorbent assay (OD value; pg/mL). Data are expressed as median (interquartile range). An analysis of covariance was conducted on log10-transformed IgM anti-EV71 scores, with predictors asthma status and age (N=49).

RESULTS: Non-asthmatic children had significantly higher IgM anti-EV71 Ab levels than asthmatic children (1.10[0.8], 2.51[1.6], P<0.001). Interestingly, despite the relatively low serum IgE levels of non-asthmatic, compared with asthmatic children (82[111], 34[136], P<0.001), the non-asthmatic children produced significantly more IL-2 and IL-4 (P<0.001). The ages of the non-asthmatic, but not the asthmatic children had a significant effect on the levels of IgM anti-EV71 produced (estimated slope (ES):+0.035 log-units/yr, SE= 0.012, P=0.006; ES: -0.007 log-units/yr, SE= 0.016, P=0.660, respectively).

CONCLUSIONS: The role of IgM anti-EV71, Th2 cytokines, and age in non-asthmatic children should be further studied.

363 Breakthrough Reactions during Oxaliplatin Desensitization: An Analysis of 177 Cases

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RATIONALE: Desensitization is a useful method for safely reintroducing oxaliplatin in patients with previous oxaliplatin-hypersensitivity reactions (HSRs). However, breakthrough reactions (BTRs) may occur during desensitization and can be problematic, limiting the application of further courses. We sought to investigate the characteristics and related factors of BTRs during oxaliplatin desensitization.

METHODS: All cases of oxaliplatin desensitization performed at the Seoul National University Hospital from June 2011 through August 2014 were included in this retrospective study. Hypersensitivity reactions were assessed using Common Toxicity Criteria for Adverse Events (CTCAE) ver 4.0.

RESULTS: We performed 177 courses of desensitization in 55 patients with oxaliplatin hypersensitivity (mean age 57.8 yr, range 31-76). The initial HSRs had developed after 5.2±3.8 cycles (mean±SD) at a grade of 2.93±0.86. Desensitization was successful in 173 of the 177 cases (97.7%); However, BTRs did occur in 44.1% of the cases at a grade of 2.00±0.83. They appeared predominantly (79.5%) in the last 2 steps of the protocol at an infusion rate of 40.1±25.2 mg/hr. The occurrence and grade of BTRs showed gradual decline by succession of each desensitization course. BTRs were more frequent in patients with previous remote oxaliplatin exposure (78.3% vs 43.8%, p=0.01), but were not related to patient demographics, CBC, initial infusion rate, oxaliplatin dose, previous HSR grade, or steroid premedication.

CONCLUSIONS: Patients with previous remote exposure to oxaliplatin have an increased risk of BTRs during oxaliplatin desensitization. Desensitization protocols should be adjusted to manage this specific group of patients.
364 Progesterone Autoimmune Dermatitis: Presentation, Diagnosis, Management and Outcomes in 17 Cases

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RATIONALE: Progesterone autoimmune dermatitis (PAD) is a complex syndrome associated with hypersensitivity reaction to exogenous or endogenous progesterone, often with cyclical symptoms correlating with the menstrual cycle. Symptoms can range from dermatitis to anaphylaxis and an increasing number of cases have been reported after the advent of in vitro fertilization (IVF), during which women are exposed to supra-physiological levels of progesterone. We present here a large case series of PAD with novel approaches to skin testing and progesterone desensitization.

METHODS: Seventeen cases of PAD referred to the BWH were reviewed. Symptom presentation, diagnostic modalities, desensitization protocols, and outcomes were analyzed. Indications and efficacy of oral and intramuscular (IM) desensitization protocols are evaluated.

RESULTS: Symptoms were heterogeneous and included cyclical dermatitis, urticaria, angioedema, and bronchospasm. Eight patients (47%) reacted to endogenous progesterone with menstruation or pregnancy triggering symptoms. In the other nine patients (53%), symptoms were triggered by exogenous progesterone used for contraception or fertility treatment. Of patients skin tested, 9/15 (60%) had positive skin prick or intradermal testing to progesterone. Five patients were desensitized to progesterone: one IM and four oral. One patient failed the first desensitization, but was successfully desensitized with an adapted protocol. Desensitization resulted in symptom relief and viable pregnancy through IVF in one case.

CONCLUSIONS: This is the largest case series of PAD patients in the literature to date with successful outcomes. PAD presents heterogeneously and skin testing can be utilized for diagnosis. Women with cyclical allergic symptoms and infertility should be evaluated for PAD and possible progesterone desensitization.

365 A Survey of Aspirin Desensitization Practices Among Allergists and Fellows in Training in the United States

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RATIONALE: Aspirin desensitization is an effective component in the treatment of AERD. The purpose of the survey was to gather data regarding aspirin desensitization practices in order to assess whether it is being utilized to capacity.

METHODS: A 16 question survey was generated and distributed through AAAAI membership to physician members in the United States.

RESULTS: We received 684 responses from 4727 invitations. 62.5% of respondents perform aspirin desensitization for AERD. Perceived safety risks are the primary deterrent. Over half of the respondents performed less than 5 desensitizations during fellowship training. Increasing the exposure to aspirin desensitization procedures during fellowship training will likely increase its utilization among allergists.

CONCLUSIONS: Although generally considered safe and effective, only 62.5% of respondents perform aspirin desensitization for AERD. Perceived safety risks are the primary deterrent. Over half of the respondents performed less than 5 desensitizations during fellowship training. Increasing the exposure to aspirin desensitization procedures during fellowship training will likely increase its utilization among allergists.

366 Penicillin Allergy Label Persists Despite Negative Testing

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RATIONALE: Studies support a public health imperative to de-label patients of penicillin allergy, however, “de-labeling” is only effective if acted upon. We hypothesized that persistence of a penicillin allergy label despite negative penicillin testing (PT) would be prevalent, impairing the utility and cost-effectiveness of PT.

METHODS: A retrospective EMR chart review of 100 patients who underwent PT from January 2010 (due to Pre-Pen availability) to May 2014 in a tertiary outpatient clinic was performed to assess the primary outcome of penicillin allergy labeling changes following PT. Covariates included antibiotic utilization before and after testing, reaction history, number of additional drug allergy labels and beta-lactam exposure following PT. Patients were contacted to complete a survey regarding their interpretation of PT results.

RESULTS: Following negative PT, 26/69 patients (37.7%) remained labeled as penicillin allergic in the EMR. 26 patients tolerated subsequent exposure to penicillin either through oral challenge (n=7) or treatment course (n=19). 19.2% of patients who tolerated penicillin didn’t have their label removed. Although 100% (n=40) of patients contacted could correctly identify the result of their PT, 39% (9/23) with negative PT have either kept their allergy label or continue to avoid penicillins.

CONCLUSIONS: PT impacted future therapy in only 17.3% of patients with negative PT. The benefits of PT in clinical practice will only be realized if the results of negative testing are acknowledged and acted upon. This suggests approaches to standardize PT procedures and reporting must be addressed to close this efficacy-effectiveness gap.
Skin Testing with Betalactam Antibiotics for Diagnosis of Betalactam Hypersensitivity in Children

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Rationale: Betalactam antibiotics are the most common causes of drug hypersensitivity reported in children. Penicilloyl polylysine (PPL) and minor determinant were used for skin test but PPL is not commonly available. This study was to determine negative predictive value (NPV) of skin testing with betalactam antibiotics for diagnosis of betalactam hypersensitivity.

Methods: Patients age 1-18 years old with history of betalactam hypersensitivity were evaluated by skin test (skin prick test, intradermal test) with culprit drugs (penicillin G, ampicillin, amoxicillin-clavulanic acid, cloxacillin and cephalosporin). Patients who have negative skin test were performed drug provocation test (DPT) in 3 dose graded challenge.

Results: 86 patients were evaluated for drug allergy. Only 18 patients (20%) were confirmed allergic to culprit drugs. 11 (61 %) of them confirmed by skin test. Among 75 patients with negative skin test were performed DPT, 7 patients (9.3%) were reacted providing NPV 90.7%. The most common culprit drug was amoxicillin (66.7%), followed by amoxicillin-clavulanic acid (16.7%). The history of immediate reaction was associated with true drug allergy (p value = 0.01). There were no serious systemic reaction in our study. There was only minor reactions which were response to symptomatic treatments.

Conclusions: Among children with history of betalactam hypersensitivity, skin testing with culprit drugs was safe and providing a good NPV when PPL was unavailable. However, skin test with betalactam antibiotics did not provide high sensitivity, thus DPT is necessary to confirm the diagnosis of drug hypersensitivity.

Viral Reactivation and Subsequent Cytotoxic Lymphocyte Activation Associates with Increased Morbidity in Children with Dress Syndrome

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Rationale: DRESS syndrome is a severe drug reaction with a reported mortality approaching 10%. It is classified as a Th2-mediated, type IV hypersensitivity reaction; however, viral reactivation of herpes viruses has been frequently identified in patients. Therefore, we hypothesized that cytotoxic lymphocyte against viral reactivation may also be implicated in the pathologic response.

Methods: Six pediatric patients who met the criteria for Dress per RegiSCAR scoring system were evaluated during their illness. Reactivation of herpes viruses was evaluated in all patients and two markers of T cell activation were measured: expression of granzyme B in CD8 T cells and plasma soluble IL2 receptor levels (sIL2R).

Results: Positive PCR to Human Herpesvirus 6 (HHV6) was noted in three cases; two of these patients were evaluated for hemophagocytic lymphohistiocytosis (HLH) due to significant multi-organ pathology, including liver, kidney and lung involvement. The third patient had a milder clinical course but developed relapse on steroid taper. Granzyme B in CD8 T cell was assessed in 5/6 patients and was elevated in two patients with positive PCR to HHV6. Patients with negative viral PCR had normal expression of granzyme B, and less severe clinical courses. sIL2R was elevated in all cases but more remarkable (>10,000) in patients with severe presentations.

Conclusions: In a case series of 6 children with DRESS syndrome, those patients presenting with both viral reactivation and significant CTL activation tend to suffer a more severe clinical course. We propose that these patients require early recognition and aggressive immunosuppression.

Genetic Variants in Arachidonic Acid Pathway Genes Associated with Nsaids-Exacerbated Respiratory Disease

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Rationale: Non steroidal anti-inflammatory drugs (NSAIDs) are the most frequent cause of hypersensitivity drug reactions (HDR). The mechanism of NSAIDs-exacerbated respiratory disease (NERD) has been attributed to the inhibition of prostaglandin-endoperoxide synthases (PTGS) by NSAIDs in susceptible individuals. This leads to a reduction in prostaglandin E2 production and shunts arachidonic acid metabolism toward the lipooxygenase pathway, resulting in the release of cytotoxic leukotrienes. Here, we have examined the association between NERD and polymorphisms in genes encoding PTGS, lipoxygenases and their receptors. In addition, we have analyzed copy number variants (CNVs) for genes involved in arachidonic acid metabolism.

Methods: We included a total of 250 NERD patients, 260 NSAID-tolerant asthmatic subjects (NTA) and 315 unrelated healthy subjects. A total of thirty three single nucleotide polymorphisms (SNPs) in PTGSI, PTGSI, ALOX5, ALOX5AP, ALOX12, ALOX15, LTC4S, CYSLTR1, CYSLTR2, PTGER1, PTGER2, PTGER3, PTGER4, PTGDR and PTGFR genes were studied. Moreover, CNVs in PTGSI, PTGSI, LTC4S, ALOX5 and PTGER1-4 genes were analyzed.

Results: Significant associations with NERD were identified for the following genes: ALOX15 (rs3892408 and PTGSI-1 (rs10306135 and rs5789). Furthermore, the ALOX15 rs3892408-rs11568131 [T-G] haplotype was shown to be statistically significant associated with NERD. Significant differences in ALOX5CNVs among NERD, NTA and control subjects (NERD vs NTA; OR: 0.17; 95% CI: 0.0–0.8; P=0.010; NERD vs Controls; OR: 0.03; CI: 0.0–0.5; P=0.0001) were also found.

Conclusions: These findings help contribute to a more precise knowledge of the underlying mechanisms of NERD and will be important for identifying predictive genetic markers for this pathology.
370 The Association of HLA-B*5101 and Phenobarbital-Induced Severe Cutaneous Adverse Drug Reactions in Thai Children

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Rationale: Adverse drug reactions to phenobarbital (PB), the first-line aromatic anticonvulsant drug, are maculopapular rash (MP) and severe cutaneous adverse drug reactions (SCARs) including drug rash with eosinophilia and systemic symptoms (DRESS), acute generalized exanthematous pustulosis (AGEP), Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN). These conditions have high mortality rate and are usually unpredictable. This preliminary study aims to investigate the association between variations of HLA genotypes and phenobarbital-induced SCARs among Thai children.

Methods: Thai children aged between 0-18 years who were diagnosed with phenobarbital hypersensitivity from 2004-2014. Control patients were phenobarbital-tolerant Thai children with corresponding age groups. Their HLA-B locus was genotyped using a PCR technique.

Results: A total of 45 Thai children were enrolled. Thirteen children were diagnosed with phenobarbital hypersensitivity (7 with MP and 6 with SCARs). Thirty-two phenobarbital-tolerant children were recruited as control. The frequency of HLA-B*5101 in phenobarbital-induced SCARs was 50% (3/6) while only 6% (2/32) was found in the drug-tolerant children (OR=15.95; CI (1.75-128); p=0.02). No patient with phenobarbital-induced MP was found to carry HLA-B*5101. The frequency of HLA-B*1502 in phenobarbital-induced MP was 14.3%, phenobarbital-induced SCARs was 16.7% and drug tolerant was 12.5%. We did not find any association between phenobarbital-induced MP and SCARs and HLA-B*1502, a known HLA genotype associated with anticonvulsant hypersensitivity in previous study.

Conclusions: Our preliminary study shows an association between HLA-B*5101 and phenobarbital-induced SCARs among Thai children.

371 Antibiotic Allergies in a Birth Cohort from 2007

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Rationale: The epidemiology of antibiotic allergies in pediatric populations is not well characterized.

Methods: With IRB approval and written informed consent from all patients, a cohort of children born at Mayo Clinic in 2007 and subsequently living in Olmsted County, Minnesota were retrospectively identified by chart review. Odds ratios (OR) and 95% confidence intervals (CI) were calculated for developing an adverse drug reaction (ADR) to penicillins compared to other antibiotics. Antibiotic reactions and exposures were identified by searching Allergy and Medication sections of clinical notes between 01/01/2007 and 12/31/2013.

Results: Eighty patients (60.0% male) with 86 ADRs to antibiotics were identified in 925 children (51.6% male). Six patients had two or more ADRs. Among the 80 patients with ADRs, 72 (90.0%) were to penicillins (PCNs), 5 (6.3%) cephalosporins, 3 (3.8%) macrolides, 3 (3.8%) sulfonamides, and 4 (4.9%) fluoroquinolones. Of the 925 patients, 604 were exposed to PCNs, 229 macrolides, 140 cephalosporins, 77 fluoroquinolones, and 39 sulfonamides. When adjusted for these exposures, 11.9% (72/604) reacted to PCNs, 7.7% (3/39) sulfonamides, 3.6% (5/140) cephalosporins, 1.3% (3/229) macrolides, and 1.3% (1/77) fluoroquinolones. PCNs were more likely to cause ADRs compared to other antibiotics [OR 10.0 (95% CI 3.1-32.2) versus macrolides; OR 3.6 (1.4-9.1) cephalosporins; and OR 10.1 (1.4-73.9) fluoroquinolones]. Rashes 68.8% (55/80) and hives 26.3% (21/80) were most commonly documented. No anaphylactic reactions or angioedema occurred. Penicillin skin testing was negative in 9 of 10 tested.

Conclusions: In this cohort, penicillins were most likely to cause an ADR by age 7. Penicillin skin testing was useful to rule out allergy. Future studies should focus on the identification of risk factors contributing to antibiotic allergies.
Abstracts

373 An Immuno-Proteomic Analysis of Seminal Plasma Allergens
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RATIONALE: Sera from women with seminal plasma hypersensitivity (SPH) typically reveals multiple protein bands using IgE-specific western blotting. Thus far, only one seminal plasma (SP) protein, Prostate Specific Antigen (PSA), has been described as a relevant allergen for SPH.
METHODS: Serum samples from women with systemic SPH were obtained. Specific IgE against their respective male partner’s SP was determined by ELISA and IgE isotype-specific immunoblotting. Further analyses were performed using two-dimensional electrophoresis (2D-PAGE), followed by immunoblotting and Mass Spectrometry to identify IgE-reactive proteins in SP. Serum IgE from SP-allergic women recognized SP proteins by ELISA and immunoblotting. SP proteins then were resolved using 2D electrophoresis, followed by IgE-specific immunoblotting and mass-spectrometry. To assess the effect of commonly used denaturing agents on allergen recognition by patient IgE, the 2D-PAGE was performed with or without denaturing agents (urea and thiourea) followed by IgE-specific immunoblotting and Mass Spectrometry.
RESULTS: In addition to previously reported PSA, Prostate-specific acid phosphatase (PAPS) was identified as a major SP allergen as it was recognized by human specific IgE in the absence of denaturing agents.
CONCLUSIONS: Women with systemic SPH have heterogeneous responses to SP proteins as they may recognize PSA or PAPS both of which are secreted from prostate epithelial cells. The fact PAPS was recognized by the woman’s serum IgE under non-denaturing conditions suggests that the relevant SP epitope is conformation-dependent. ELISA-inhibition and mediator release assays are currently being performed to assess the functional relevance of PAPS in women with SPH.

374 Basophil Histamine-Release Test with a Modification Is Useful for Diagnosis of Allergy to Formaldehyde
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RATIONALE: Formaldehyde has long been recognized as both an allergen to sensitized subjects and an irritant to the general population. The reliability of basophil histamine-release test has not been high for this allergen. Here, we report a 28-year-old female case of urticaria induced by paraformaldehyde used during root canal treatment. Formaldehyde-specific IgE assessed by ImmunoCAP was strongly positive (>100 UA/ml), but her basophils showed no histamine-release reaction in response to dialyzed formalin. We thus sought underlying mechanisms that would explain the discrepancy between the basophil test results and the presence of serum formaldehyde–specific IgE.
METHODS: Following days to weeks of storage of a mixture of formaldehyde plus albumin, the solutions were serially diluted and tested for basophil stimulation, without dialysis step.
RESULTS: We found that mixtures stored for 3 days to 2 weeks were potent inducers of histamine release from sensitive basophils, indicating that binding of formaldehyde to albumin is a slow process. Protein-unbound formaldehyde showed no effect on basophil activation, since formaldehyde solution added at 300 or 1000 ng/ml before stimulation with formaldehyde-albumin conjugates failed to modify histamine release from basophils. Formaldehyde-globulin conjugates could not induce basophil histamine release.
CONCLUSIONS: Formaldehyde induced basophil histamine release only when it had been pretreated with albumin for days to weeks, suggesting that this molecule’s slow interaction with proteins may affect the usefulness of this in vitro test.

375 Elevated Serum Tryptase Levels during Rituximab Hypersensitivity Reaction
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RATIONALE: Rituximab is a chimeric anti-CD20 monoclonal antibody that is frequently implicated in hypersensitivity reactions. The mechanism of hypersensitivity is not well defined. Reports have suggested a cytokine-dependent reaction, a Type I IgE-mediated reaction, or a type III immune-complex reaction. Markers to understand the mechanisms of hypersensitivity are lacking. We present two patients with a mast-cell dependent hypersensitivity reaction to rituximab.
METHODS: We retrospectively identified two patients with elevated serum tryptase 30-60 minutes after a grade III hypersensitivity reaction to rituximab.
RESULTS: Each patient had a positive skin test to rituximab at the intradermal level. Patient one had a baseline tryptase of 6.8. He tolerated two infusions well. On his third lifetime infusion, he developed hypotension and hypoxia within 30 minutes of starting the infusion. Tryptase was elevated to 50.7. Patient two had a baseline serum tryptase of 2.7. She developed hypotension and hypoxia on her second lifetime infusion. One hour after the reaction, her tryptase was 26.0. Both patients were administered intramuscular epinephrine with rapid reversal of symptoms. Desensitization was successfully completed in both patients.
CONCLUSIONS: The above cases demonstrate two patients who initially tolerated rituximab infusions but subsequently developed hypersensitivity reactions. This suggests a sensitization phenomenon. In addition, both patients had elevated serum tryptase levels shortly after the reaction, implicating a mast cell dependent mechanism. This provides compelling evidence that in some patients, rituximab hypersensitivity occurs as a Type I IgE and mast cell mediated process. Furthermore, it suggests that these patients are excellent candidates for desensitization.
Basophil Activation Test As a Biomarker in Allergic Patients to Platin Undergoing Rapid Desensitization

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RATIONALE: Desensitization (DST) has become a cornerstone of the management of immediate hypersensitivity reactions (HSRs) to chemo-therapeutic agents. Nevertheless, there are still no good biomarkers to monitor DST safety and effectiveness. The main goal of our study was to assess basophil activation test as a test to monitor allergic patients to platins undergoing rapid DST.

METHODS: We studied 14 oncologic patients who presented platin allergy and 6 healthy volunteers. We performed the BAT immediately before DST to platins, assessing CD203c and CD63 expression on basophils. Several patients were evaluated in at least 2 different DSTs.

RESULTS: BAT was positive in 9 patients (64.3%), with increased expression of CD203c and CD63 in 9 (64.3%) and 5 (35.7%) patients, respectively. The BAT positivity was 54.5% for carboplatin and 100% for oxalaplatin. Subsequent BAT analysis in different DST procedures showed that the test remained positive with an even greater expression of CD203c and CD63 on basophils after platins exposure. Some patients with positive BAT reacted during DST, in spite of being premedicated, showing the correlation between BAT results and clinical outcomes. There was an association between CD63 expression and the severity of the reactions. All controls had negative tests. Further investigation is necessary to determine the predictive values of BAT to platins.

CONCLUSIONS: We standardized a BAT to platins that presented good sensitivity and can predict severe reactions. Short-term DST to platinum drugs does not induce persistent hyporesponsiveness on basophils, highlighting the need to maintain DST in allergic patients to platins.

Desensitization to Monoclonal Antibodies

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RATIONALE: Monoclonal antibodies (mAbs) represent a therapeutic option in patients with chronic inflammatory diseases and oncologic disorders. Desensitization is useful in cases of hypersensitivity reactions (HSRs) without a proper alternative.

The aim was to evaluate the effectiveness and security of desensitization protocols in patients with HSR to mAbs.

METHODS: A retrospective-descriptive study was led at our Allergy Department. Medical records from January 2012-July 2014 were searched for HSRs to mAbs that underwent desensitization.

RESULTS: Sixty-two desensitizations to mAbs were performed in 6 patients. Mean age 42.24 ± 15.5, 50% women, 50% atopic. HSR was immediate in all cases (4 moderate, 2 severe). Two patients showed positive immediate intradermal-test (inflliximab and rituximab). The prescribed mAbs were: cetuximab (28), infliximab (21), rituximab (10) and trastuzumab (3). Initially 4 patients had a 16-step and 2 a 12-step protocol. Four received pretreatment with AAS and/or montelukast based on the severity of the reaction. Sixty-one (98.4%) were successfully completed. Fourteen reactions were observed during 11 desensitizations in 4 patients: fever (6), pruritus/wheals (4), musculoskeletal pain (3), nausea/vomits (3), and hypotension (1), 71.6% were immediate, 78.6% mild. Only 2 required adrenaline. The initial protocol was modified in 4 patients to avoid new reactions. In all four, an extra and/or prolonged steps, had to be added. Three also received treatment during the following infusion, and in one, pretreatment was added (montelukast). There was no need to withdraw the biologic medication in none of the patients.

CONCLUSIONS: Desensitization to mAbs is solidly effective and secure, although most protocols need to be personalized.

Evaluating Clinical Outcomes of Penicillin Skin Testing in Affecting Inpatient Antibiotic Stewardship

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RATIONALE: To review the role of penicillin skin testing (PST) in affecting the outcomes of inpatient antibiotic stewardship.

METHODS: This was a retrospective study of 100 patients with history of penicillin allergy admitted to our two medical centers, St. Mary’s and Methodist hospital, between 1/1/2008 to 1/1/2009 and evaluated by an Allergist. The PST was performed using standard methods utilizing benzylpenicilloyl-polysine, penicillin G, benzilpenicilloate, negative control, and histamine controls. Charts were reviewed for basic demographic data, PST results, and antibiotics used after the allergy evaluation. The IRB approved the study and all subjects signed a written informed consent.

RESULTS: Of the 100 patients studied, 58% were female and all were 18 years of age or older. Most patients had a previous reaction to penicillin (71%), semisynthetic penicillins (14%), and cephalosporins (9%). The most common reactions were urticaria, unspecified rash, shortness of breath, and local angioedema. Results of the PST were positive in 1% of patients, negative in 79%, and indeterminate in 20%. Prior to PST, the most commonly used antibiotics were vancomycin and fluoroquinolones. After PST, vancomycin and fluoroquinolone use decreased from 34% to 11% and 20% to 10%, respectively. PST led to the narrowing of antibiotics with an associated increase in the use of beta-lactam antibiotics from 16% prior to PST to 59% post-PST. The beta-lactams most commonly used post-PST were semisynthetic penicillins that included nafcillin, amoxicillin, ampicillin, and piperacillin-tazobactam.

CONCLUSIONS: PST is an effective tool that can assist physicians in improving inpatient antibiotic stewardship.

Determinants of Placebo Reaction at Oral Provocation Test in Adults

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RATIONALE: Placebo-controlled oral provocation is an essential step in the management of drug allergy. To investigate reactions to placebo and features of placebo reaction was to aim of the study.

METHODS: Data was collected from files of patients undergoing oral drug provocation (OPT) at tertiary level of outpatient adult allergy clinic. Placebo was used in the first day of OPT. On active drug day, either alternative drugs and/or suspected culprit drugs were used. All OPT was performed under strict control of both trained allergy nurse and allergy specialist. Demographic and clinical features of placebo reactors and non- reactors were compared.

RESULTS: A total of 106 patients with 42.6±1.28 y mean age (F/M:68/ 38) were recruited consecutively. Among them, 23 (21.7%) reacted to placebo. Only 3 cases had visible urticarial plaques. Migratory itching with tingling sensation, tickling at throat, rhinorrhea, headache and dizziness was the other symptoms in the remaining placebo reactors. Higher education level (OR:3.15, 95%CI:1.19-8.27, p=0.03) and having comorbidities other than allergic disorders (OR:4.08, 95%CI: 1.46-11.42, p=0.009) was the only significantly related factors with placebo reaction. Gender, having allergic diseases, class of culprit drug, duration of drug allergy, period after last reaction, elapsing time, severity and outcome of index reaction was not identified as related factor with placebo reactions.

CONCLUSIONS: Placebo reactions could have special clinical characteristics related with population and are not uncommon.
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380 A Case of Anaphylaxis to Ranitidine, Confirmed By Challenge
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RATIONALE: Ranitidine is a competitive, reversible inhibitor of histamine at the H2 receptor and is used to treat a variety of gastrointestinal conditions, as well as urticaria. Anaphylaxis to ranitidine has been reported but is rare. We present a case of anaphylaxis and oral challenge to ranitidine.

METHODS: Our patient was referred for evaluation of a possible ranitidine allergy.

RESULTS: A 31 year old woman with a history of urticaria with proton pump inhibitors was placed on ranitidine for gastritis. Within a few hours of taking 150 mg ranitidine, she developed urticaria and angioedema of the lip and throat. She went to a local emergency room and was treated with diphenhydramine and oral steroid course with resolution of symptoms. Although skin and IgE-RAST testing to ranitidine have been reported, there is no known standardized skin testing or commercially available IgE- assay for ranitidine. Therefore, at our office, she was challenged with a 75 mg oral ranitidine tablet, monitored for 30 minutes, and then, the dose was repeated for a total of 150 mg, with an additional 30 minute wait. She was prescribed an epinephrine autoinjector. Two hours after the challenge ended, she developed urticaria on back, as well as eyelid and lip angioedema. She self administered an epinephrine autoinjector with resolution of symptoms and was monitored at an emergency room. In consultation with gastroenterology, an alternative therapy was considered.

CONCLUSIONS: This case demonstrates ranitidine anaphylaxis, which was confirmed by a supervised challenge in the controlled medical setting. If alternative therapies are unavailable, desensitization may be considered.

381 Stevens Johnson Syndrome (SJS)/Dress and Rechallenge to Possible Culprit Drug in Severe Extrapulmonary MAC Infection
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RATIONALE: SJS and DRESS represent a diagnostic and therapeutic dilemma when alternative drugs have inferior efficacy.

METHODS: Incremental drug challenge.

RESULTS: A 43 year old female with a history of SLE developed severe septic arthritis with MAC. Clarithromycin, ethambutol and rifampin were started. Two weeks later rifampin was switched to rifabutin due to susceptibility results. Eleven days later she developed SJS. She had fever, eosinophilia, liver and kidney function abnormalities consistent with DRESS. Antibiotics were stopped and she recovered. Patch testing was positive to rifabutin and ethambutol with an irritant reaction to rifampin. ID consultants felt it was imperative to restart one of the bacterial antibiotics to treat her extrapulmonary MAC infection as the alternatives were bacteriostatic. With her clinical history and patch testing, we decided to conduct an oral challenge with rifampin starting at 1/4 of the dose and doubling the dose every week until the therapeutic dose is reached. CBC/ eosinophils, CMP and acute phase proteins were monitored every 3 days to identify early signs of a reaction. Three days following introduction of rifampin, she developed transaminitis and elevation of CRP that resolved after discontinuation. Eosinophilia and skin manifestations were absent.

CONCLUSIONS: Suspected causative agents should be avoided in SJS/ DRESS whenever possible. However, severe disease and lack of alternative medications may warrant rechallenge when multiple agents are in question. Our case study suggests that a carefully designed incremental drug challenge with frequent monitoring of clinical and laboratory parameters could minimize the risk of overt clinical reaction and help with the therapeutic decision.

382 Drug Desensitization in Children
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RATIONALE: Hypersensitive reactions to necessaries drugs are an exceptional issue in childhood. We reported our experience with drug desensitisation in children.

METHODS: Patient 1: A 3 years old patient with ornithine transcarbamylase deficiency and liver transplantation was diagnosed with acute colanghites, Pseudomonas aeroginosaswas isolated in her blood. In the seventh day of the treatment, coinciding with an infusion of amikacin, she started an episode of angioedema in her hands and lips. Patient 2: A 5 years old boy with enterocolitis and eosinophilia was diagnosed of schistosomiasis. He completed a treatment with praziquantel. As the eosinophilia persisted, he started his second cycle, developing immediately abdominal pain, fever and urticaria. Patient 3: A 17 years old girl with liver transplantation because of cystic fibrosis, received filgastrim treatment as she had an episode of fever and neutropenia. During the filgastrim infusion, the patient started with headache, palpitations, and pruritic erythema in face. With those reactions, drugs were stopped in all patients, and symptoms were controlled with antihistamines and corticoestroids. Skin prick tests with amikacin, praziquantel and filgastrim were performed and three schemes of desensitization according to 12-step 6-hour Castells protocol were carried out. Premedication was used with methylprednisolone and dexclorpheniramine in all patients.

RESULTS: Prick tests with the different solutions of amikacin, praziquantel and filgastrim resulted negative. There was any reaction during the desensitization protocols and the treatment could be successfully completed in each patient.

CONCLUSIONS: Rapid desensitization could be a safe method to deliver the necessary drugs after hypersensitivity reactions in children.

383 An Assessment of Current Practice and Knowledge of Penicillin Allergy at Hospital-Based Pediatric Centers
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RATIONALE: Inappropriate withholding penicillin therapy in a large number of patients mistakenly labeled as penicillin allergic is associated with increased morbidity, mortality, and healthcare costs. This survey was performed to determine current knowledge and practice patterns of pediatricians for patients labeled as penicillin allergic at hospital-based centers.

METHODS: Pediatric providers at hospital-based centers were recruited by email for an online survey to assess current knowledge and management of penicillin allergy. Participants identified their level of training and clinical practice setting.

RESULTS: Most participants were inpatient providers. Only 29% of subjects correctly identified the rate of penicillin allergy to be 10%; 61% of respondents knew that penicillin allergy was not permanent. Only 21% thought skin testing was a reliable tool for assessing for penicillin sensitization. Allergy referral rates were 11% for a history of a rash to penicillin and 36% for a history of anaphylaxis. Most providers (57%) would prescribe a 3rd or 4th generation cephalosporin to a patient with a history of a rash to penicillin, but only 21% would for a history of anaphylaxis.

CONCLUSIONS: Participated pediatric providers demonstrated inadequate knowledge of the natural course of penicillin sensitization, routinely used broad spectrum antibiotics, and tended not to refer to Allergy. Education should be focused on these areas to reduce associated morbidity, mortality, and costs.
**Desensitization to Deferasirox in a Patient with Iron Overload**

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**RATIONALE:** Iron overload is a common complication of frequent blood transfusions often requiring years of treatment with iron chelators. Deferasirox is an oral iron chelator taken daily. It is often preferred to the alternative, deferoxamine, which requires daily subcutaneous infusions over 8-12 hours. Our patient is a 17yo male with relapsed ALL and iron overload who experienced a pruritic maculopapular rash associated with 102°F fever 8-12 hours after taking deferasirox. He required inpatient admission and was treated with systemic steroids, H1 and H2-blockers with resolution of rash over 2-3 weeks. We present our experience of desensitization to deferasirox in this patient.

**METHODS:** We used a step-wise desensitization protocol starting at 1/100,000 of the goal dose (2000mg).

**RESULTS:** A T-cell mediated, non-IgE mechanism was suspected for this reaction given the delayed onset of rash and fever. Initially, we attempted desensitization starting at 1/1000 of the goal dose, however, the patient developed pruritic flushing 3 hours after the second dose. We then decreased the starting dose to 1/100,000 of the goal dose and doubled the dose every 3 days. This was successful, until the 500mg dose when the patient was unable to tolerate a full doubling of the dose due to itching. He instead increased to 750mg, 1000mg, 1500mg and finally 2000mg. We lengthened the interval between dose increases to 7 days. It took 3 months to achieve goal dose. His ferritin level dropped >1000ng/ml during the desensitization period.

**CONCLUSIONS:** Successful desensitization to deferasirox is possible in non-IgE mediated reactions using a slow conservative protocol.

**385 Oral Challenge Tests with Nsais: Evaluation of Patients Attending a Specialty Clinic in Ribeirao Preto, Brazil**

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**RATIONALE:** Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) are among the most common causes of drug-induced hypersensitivity reactions. Oral Challenge Tests (OCT) are useful to determine alternative drugs for patients with hypersensitivity to NSAIDs.

**METHODS:** Prospective study enrolling 116 patients with positive history of hypersensitivity to NSAIDs, between October 2010 and July 2014. Patients completed the European Network for Drug Allergy questionnaire, and underwent single-blind, placebo controlled OCT, in hospital environment. Patients were given 10%, 20%, 30% and 40% of the therapeutic NSAID dosage, selecting an NSAID different from the one(s) implicated in prior allergic reaction(s). Fifteen minutes after each dose, patients were evaluated with Peak Flow and blood pressure measurements, heart and respiratory rates and general examination.

**RESULTS:** Eighty-five of 116 patients (73%) were female. NSAID reactions were: angioedema (58%), urticaria (42%), anaphylaxis (24%), and respiratory symptoms including nasal pruritus, sneezing, rhinorrhea, dyspnea, and cough (22%). The NSAIDs most implicated by history were: Dipirone (70%), Diclofenac (52%), Acetaminophen (29%), Ibuprofen (21%), Cetoprofen (20%) and Nimesulide (19%). Reactions to two or more drugs were reported by 86 patients (74%). 153 OCT were performed, aimed at identified alternative drugs, with 28(18%) positive results. Angioedema, urticaria and/or respiratory symptoms were triggered by Celecoxib (7 tests), Benzydamine (5), Nimesulide (3), Ibuprofen (3), ASA (2), Acetaminophen (2), Etoricoxib (2), Dipirone (1), Diclofenac (1), Meloxicam (1), and Viminol (1). One patient presented anaphylaxis during OCT using Celecoxib.

**CONCLUSIONS:** OCT was an effective method to identify alternative choice(s) of medication to patients with NSAIDs hypersensitivity. Caution is needed when using COX-2 inhibitors in these patients, for the potential of causing reactions including anaphylaxis in a minority of patients.

**386 An Analysis of Intravenous Versus Oral Penicillin Desensitization Data to Determine Which Administration is Safer**

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**RATIONALE:** It is believed that fewer reactions occur with oral desensitization as compared to parenteral desensitization. We analyzed data from published reports of oral and intravenous penicillin desensitization in order to determine which route of administration is safer.

**METHODS:** A retrospective study of published articles on drug desensitization was performed. We restricted our analysis to subjects with a history of penicillin allergy and documentation of positive penicillin skin tests. We compared the frequency and types of reactions during oral and parenteral desensitization to penicillin. We graded the severity of reactions during desensitization utilizing the World Allergy Organization Severity Classification System.

**RESULTS:** Fifteen articles were reviewed with a total of 104 intravenous and 86 oral desensitization attempts. There were 17 penicillin skin test positive patients who were desensitized parenterally and 74 who were desensitized orally. Seventeen out of 17 intravenous desensitization attempts were successful versus 73 out of 74 using the oral method. The frequency of success in the intravenous studies versus the oral studies via a t test showed no statistical difference (p = 0.1820). The intravenous group had seven Grade 1, and one Grade 2 reactions while the oral group had seven Grade 1, one Grade 3, and one Grade 4 reactions. The frequency of reactions in the intravenous versus the oral studies via a t test showed no statistical difference (p = 0.4177).

**CONCLUSIONS:** We showed that there was no statistical difference in terms of reactions and success between oral and intravenous desensitization after analyzing the literature; however, larger studies are required.
Utility and Safety of Skin and Drug Provocation Tests in Children with a History of Penicillin-Induced Rash

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RATIONALE: Penicillin allergy is the most commonly reported drug allergy. A history of skin rash is unreliable to determine a drug allergy. A skin test is a more specific test while the drug provocation test is a standard tool for the diagnosis of drug allergy. We aimed to report the utility and safety of skin and drug provocation tests to confirm the diagnosis of penicillin allergy among Thai children with a history of rash after taking this antibiotic.

METHODS: A prospective study was carried out in children with a history of skin rash during penicillin therapy from the database of Songkhlanaagarind Hospital from 2009 to 2013. Allergologic testing included skin prick test (SPT), intradermal test (IDT) and drug provocation test (DPT) according to the international standard guidelines. The reagents for SPT were benzylpenicilloyl polylysine, minor determinant mixture, penicilloyl-polylysine, penicillin G, ampicillin, Augmentin and any other suspected penicillin. IDT was performed in subjects who had a negative SPT. In subjects who were negative for both SPT and IDT, a DPT with the suspected penicillin was performed.

RESULTS: Sixty-three patients participated in this study. The mean age was 8 years. Amoxicillin was the most common culprit drug (87.3%). A penicillin allergy was confirmed in 5 (7.9%) of these 63 patients. One patient was identified by positive SPT and 4 by DPTs. No severe adverse reaction was found.

CONCLUSIONS: Allergologic tests are important for the definite diagnosis of drug allergy. Our protocol is safe and efficient for the evaluation of children with a history of penicillin-induced rash.

Cefazolin Is a Common Cause of Perioperative Hypersensitivity Reactions

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RATIONALE: Hypersensitivity reactions (HSR) during the perioperative period are unpredictable and can be life threatening. Evidence based guidelines for evaluation of perioperative HSR are lacking and data on causative agents varies among different studies. We propose a standardized protocol to evaluate and manage all patients presenting with perioperative HSR.

METHODS: A prospective study was carried out in children with a history of skin rash during penicillin therapy from the database of Songkhlanaagarind Hospital from 2009 to 2013. Allergologic testing included skin prick test (SPT), intradermal test (IDT) and drug provocation test (DPT) according to the international standard guidelines. The reagents for SPT were benzylpenicilloyl polylysine, minor determinant mixture, penicilloyl-polylysine, penicillin G, ampicillin, Augmentin and any other suspected penicillin. IDT was performed in subjects who had a negative SPT. In subjects who were negative for both SPT and IDT, a DPT with the suspected penicillin was performed.

RESULTS: Sixty-three patients participated in this study. The mean age was 8 years. Amoxicillin was the most common culprit drug (87.3%). A penicillin allergy was confirmed in 5 (7.9%) of these 63 patients. One patient was identified by positive SPT and 4 by DPTs. No severe adverse reaction was found.

CONCLUSIONS: Allergologic tests are important for the definite diagnosis of drug allergy. Our protocol is safe and efficient for the evaluation of children with a history of penicillin-induced rash.

Steroid/ Local Anesthetic Injection Reactions- Which One Is Frequently the Allergic Component?

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RATIONALE: Some patients report adverse reactions to steroid/local anesthetic injections used frequently for pain control. We describe skin testing results of patients presenting with local or systemic reactions to a combined steroid/local anesthetic injection.

METHODS: Retrospective analysis of 105 patients who had local anesthetic and/or steroid skin testing at Mayo Clinic from 01/2004 to 07/2012.

RESULTS: We identified 23 patients who reported either a systemic or local reaction to a steroid/local anesthetic injection. Of these, 5 patients were tested only to local anesthetics and all 5 tests were negative. 18 patients were tested to both steroids and local anesthetics and overall 44% (8/18) of these skin tests were positive. 62.5% (5/8) of positive tests were positive reactions to a steroid, 12.5% (1/8) was positive to a local anesthetic- lidocaine, and 25% (2/5) were positive to both a steroid and a local anesthetic. In only 2 cases (with triamcinolone and lidocaine), the drug causing the historical reaction correlated with the drug resulting in a positive skin test. In 5 cases the medication causing the historical reaction was not the same medication resulting in a positive skin test and in the remaining 1 case the historical steroid and local anesthetic were unknown.

CONCLUSIONS: In cases of adverse reactions to combined steroid/local anesthetic injections the steroid component resulted in a positive skin test significantly more often than the local anesthetic. However in only 25% of patients was there a confirmatory positive skin test to one of the agents causing the historical reaction.
390 Serious Infections of Hospitalized Patients Are Associated with a Higher Prevalence of Reported Beta Lactam Antibiotic Allergy

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RATIONALE: To determine if having a vancomycin resistant enterococcus (VRE), methicillin resistant Staphylococcus aureus (MRSA) and Clostridium difficile (C-diff) infections are associated with a higher prevalence of reported beta lactam antibiotic allergy in hospitalized patients.

METHODS: A retrospective study of the charts of patients with documented VRE, MRSA and C-diff hospitalized in 2013 at Hackensack University Medical Center were evaluated for a reported beta lactam antibiotic allergy. The number of patients hospitalized during this time period and the total number of patients allergic to a beta lactam antibiotic were also determined. Infected patients were evaluated for prevalence of beta lactam antibiotic allergy.

RESULTS: There were 44,733 admissions for 2013 with 5,299 reporting a beta lactam allergy making a prevalence of 11.8%. A total of 798 subjects matched our criteria having these serious infections (prevalence 1.8%), of which 53.9% were male. The study included 41 patients infected with VRE, 390 with MRSA and 367 with C-diff. Of the infected patients, 164 (20.5%) reported beta lactam allergy. This is 1.75 times the prevalence of beta lactam allergy for this hospitalized group. The mean age of patients with a beta lactam allergy was 69.7 years and without was 60.6 years. There were 138 in-hospital deaths with this group of which 21 (12.8%) had a history of beta lactam allergy.

CONCLUSIONS: Serious infections caused by multi-drug resistant organisms in these hospitalized patients during 2013 reflected higher prevalence of patients with a reported beta lactam allergy. Results were not age or sex dependent and all diagnosis’ were included.

391 Safe Administration of Aspirin to High Risk Aspirin-Sensitive Patients

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RATIONALE: Aspirin (ASA) therapy may be indicated for antiplatelet or antimediated effects in unstable patients expected to be at high risk from potential respiratory or cardiovascular adverse effects of ASA desensitization, or those who are difficult to desensitize with established methods. We hypothesized that repeated administration of subthreshold doses of ASA could rapidly produce desired cumulative pharmacologic effects without significant risk, and might facilitate subsequent desensitization to larger single doses of ASA.

METHODS: A hemodynamically unstable patient with acute coronary syndrome, asthma, nasal polyposis and ASA sensitivity initially received ASA 10mg every 3 hours; desensitization subsequently was completed when her condition had stabilized after coronary angioplasty and stenting had been performed. A patient with mast cell activation syndrome with overproduction of prostaglandin D2 who had repeatedly reacted to 25mg doses of ASA initially received 20mg ASA every 6 hours, followed by gradual escalation of ASA dosage over several days to 325mg 3 times daily.

RESULTS: Both patients tolerated repeated subthreshold doses of ASA totaling 80 mg within the initial 24 hours; subsequently both were able to tolerate single doses of 325mg after desensitization.

CONCLUSIONS: Repeated non-escalating administration of subthreshold doses of aspirin is a safe initial approach to achieve desired pharmacologic effects in critically ill ASA- sensitive patients and in those difficult to desensitize. It may facilitate subsequent desensitization, to be carried out after clinical stability has been achieved. Further investigation of this approach in high-risk aspirin-sensitive patients is warranted.

392 Skin Testing, Graded Challenge and Desensitization to Von Willebrand Factor (vWF) Products in Type III Von Willebrand Disease (VWD)

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RATIONALE: Hypersensitivity reactions to vWF replacement products limit their use for prophylaxis and treatment of life-threatening bleeding. Skin testing, graded challenge and desensitization for vWF-containing products have not previously been reported.

METHODS: Two consanguineous siblings, ages 2 and 8, with Type III VWD, required regular vWF infusions. Unfortunately, each had infusion reactions with several brands of vWF products. The younger sibling’s symptoms included urticaria, angioedema, and respiratory distress. The older sibling primarily noted shortness of breath. Efficacy of replacement therapy was also limited by inhibitors detected by mixing studies. Both patients were evaluated by skin testing to two vWF products (Wilate and Humate P), multiple preparations of Factor VIII, and polysorbate 80, based on the ingredients of the products.

RESULTS: Despite reactions consistent with IgE-mediated hypersensitivity, each patient had negative skin testing to vWF products, Factor VIII, and polysorbate 80 at the highest non-irritating concentration based on control subject skin testing. Despite negative skin testing, as the younger patient had severe infusion reactions, he underwent desensitization to Wilate, which was successful. The older patient underwent a successful graded challenge to Wilate, though he had back pain during subsequent infusions, which resolved once infusions were transitioned from QOD to daily. Subsequently, both patients have started regimens for induction of tolerance for their inhibitors.

CONCLUSIONS: This is the first report of skin testing, graded challenge and desensitization for vWF replacement products in patients with VWD. Patients with this condition who have experienced reactions to vWF products may benefit from these protocols.
**393 Drug Hypersensitivity Reactions in Patients with Clonal Mast Cells Disorders**

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**RATIONALE:** The occurrence and characteristics of drug-induced anaphylaxis in patients with clonal mast cell disorders (CMD) is largely unknown. In this single-center study we assessed the prevalence of drug hypersensitivity in a population of mastocytosis patients.

**METHODS:** All patients with ascertained CMD, followed in our clinic were asked to fill a questionnaire about previous adverse drug reactions. Patients with drug hypersensitivity underwent oral challenge tests (OCT) with alternatives drug.

**RESULTS:** 158 patients were enrolled; 89 (56.3%) male, mean age 52±14 years. In 58.9% of patients the diagnosis of CMD was done after hymenoptera sting anaphylaxis, and in 7% after drug anaphylaxis. Fifty-one (32.3%) patients reported a total number of 78 drug hypersensitivity reactions, 16 (31.4%) had 2 or more episodes and 7 (13.7%) with different drug classes. The most frequently involved drugs were NSAIDs (54.9% of patients, 43 episodes), and antibiotics (41.2% of patients, 25 episodes). Anaphylaxis occurred in 11/28 (39%) patients with NSAIDs reactions and in 10/21 (47.6%) patients with antibiotics, mostly amoxicillin (66.7%). Thirty-eight OCT with alternative NSAIDs (13 nimesulide, 14 etoricoxib, 6 meloxicam, 3 paracetamol and 2 aspirin) were performed and only 1 patient had rhinitis during aspirin challenge. Thirty-seven OCTs with antibiotics (15 clarithromycin, 13 ciprofloxacin, 2 clindamycin, 2 doxycyclin, 2 amoxicillin and 3 cefuroxime) were performed without adverse reactions.

**CONCLUSIONS:** In our population the prevalence of self reported drug hypersensitivity was 32.3%, that is higher than in general population. The most involved drugs are antibiotics and NSAIDs. OCT with alternative drugs are safe and useful tools for a diagnosis.

**394 A Case of Aspirin Exacerbated Respiratory Disease (AERD) with Aspirin-Induced Hypersensitivity Vasculitis**

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**RATIONALE:** AERD is a syndrome of asthma, chronic rhinosinusitis with nasal polyps and, acute upper and lower respiratory tract reactions to COX-1 inhibiting NSAIDs. Management involves aspirin desensitization and daily maintenance. Drug-induced hypersensitivity vasculitis, specifically due to aspirin or NSAIDs, has been well documented. To our knowledge, we report the first case of a patient with both AERD and aspirin-induced hypersensitivity vasculitis.

**METHODS:** Aspirin desensitization procedure adapted from The Scripps Clinic protocol, San Diego, CA.

**RESULTS:** A 38-year-old Caucasian female with AERD and history of prior nasal polypectomy and aspirin desensitization without complication, underwent a second polypectomy and aspirin desensitization for recurrent nasal polyposis. After the second aspirin desensitization, she developed an urticarial rash, which persisted despite maximal antihistamine therapy. Additionally, while on daily aspirin, the patient continued to have uncontrolled asthma despite therapy on inhaled corticosteroids, long-acting beta-agonists, inhaled tiotropium, and oral montelukast. Thus, she underwent bronchotherapyplasty, during which she discontinued aspirin and had resolution of her urticaria. However, the urticaria returned with increasing severity after her third aspirin desensitization procedure. Skin biopsy showed perivascular neutrophilic infiltrate with a suggestion of fibrinoid necrosis, suspicious for urticarial vasculitis and urticarial hypersensitivity reaction. Aspirin was discontinued with complete resolution of skin lesions.

**CONCLUSIONS:** To our knowledge, this is the first reported case of aspirin-induced hypersensitivity vasculitis in a patient with AERD. We are currently discussing further treatment options for AERD in this patient who cannot tolerate conventional therapy with aspirin.

**395 Single NSAID – Induced Serum Sickness-like Reaction to Naproxen in a Patient Able to Tolerate Both Aspirin and Ibuprofen**

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**RATIONALE:** Little is known about serum sickness-like reactions after NSAIDs use. Most type III hypersensitivity reactions to cephalosporins are drug-specific rather than class-specific, but no single NSAID-induced serum sickness-like reactions have been reported.

**METHODS:** Case report.

**RESULTS:** A 64-year-old woman was diagnosed with serum sickness-like reaction to either naproxen or cyclobenzaprine three years before presentation in our clinic. Ten days after she received both medications for cervical radiculopathy, she developed severe polyarthritis, fever, and myalgias, and had elevated levels of CRP (79.8 mg/dl, normal <0.9 mg/ dl). At our clinic, patient wanted to know if she could tolerate other NSAIDs. She underwent successful aspirin and ibuprofen challenges in our office in separate visits, and since then she continued aspirin daily and ibuprofen as needed. Complement levels, CRP, chemistry and CBC were normal at weekly follow up appointments. Naproxen challenge was initiated and she tolerated 100mg daily for seven days. Her dose was increased to 250 mg at subsequent visit, and after three days she developed stiff neck, headache, myalgias, arthralgias, and low-grade fever. Laboratory results showed elevated CRP (1.6 mg/dl, baseline 0.1 mg/dl), lymphocytopenia, thrombocytopenia, and mild transaminitis. Findings resolved after naproxen discontinuation and short steroid course. Patient was advised to avoid naproxen in the future.

**CONCLUSIONS:** To our knowledge, this is the first reported case of a single NSAID-induced serum sickness-like reaction. The different chemical structures of naproxen (two benzene rings), ibuprofen and aspirin (each has one benzene ring) probably influence the specific immune response, which may explain the different reaction to these NSAIDs.
396 Pretreatment with IVIG and Corticosteroids for Contrast Media Induced Severe Adverse Drug Reaction
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RATIONALE: Contrast media (CM) is known to cause severe adverse drug reactions (ADR) including delayed reactions such as SJS/TEN and leukocytoclastic vasculitis (LCV). Pretreatment for subsequent administration is not standardized. Here we present a patient with history of severe delayed ADR to CM in whom pretreatment with IVIG and corticosteroids successfully reduced her reactions to repeated exposure.
METHODS: Our patient is a 67 yo female with reported history of SJS to CM who presented with acute coronary syndrome requiring cardiac intervention. For two days prior to catheterization, she was treated with prednisone with one week taper. No further ADRs were observed. RESULTS: Hours after CM exposure, the patient developed a non-blanching purpuric rash on bilateral feet clinically consistent with mild LCV. Protocol was adjusted, changing steroids to methylprednisolone 40mg q6hrs with continuation of IVIG. Due to the necessity of repeat coronary angiography, IVIG and IV steroids were continued until 2 days post repeat catheterization. Thereafter she was transitioned to oral prednisone with one week taper. No further ADRs were observed. CONCLUSIONS: While history suggested SJS, our patient’s subsequent reaction was more consistent with LCV. The improvement of the LCV and lack of recurrence after repeat CM challenge suggests administering IVIG and steroids may be an effective option for preventing severe delayed ADR to CM in patients with a history of severe LCV.

397 Skin Testing, Graded Challenge and Desensitization to the Tetracycline Class of Antimicrobials in Patients with Hypersensitivity Reactions
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RATIONALE: Tetracyclines are becoming more widely utilized. Hypersensitivity reactions to this class of antimicrobials, mostly to doxycycline and minocycline, may limit therapeutic options particularly for tick-borne, respiratory or drug-resistant infections. Skin testing with establishment of highest non-irritating concentrations to these agents has not been previously reported, and graded challenge and oral desensitization protocols are rare.
METHODS: Four patients, aged 12 to 67, developed likely IgE-mediated pruritic rash/urticaria during treatment with one of the tetracycline antimicrobials. Three patients subsequently required further therapy with this group of antimicrobials for respiratory infections or Lyme disease. All patients were evaluated with skin testing to either doxycycline and/or minocycline. Non-allergic control subjects were also tested to determine the highest non-irritating concentration for each drug.
RESULTS: Two patients tested negative for minocycline, and subsequently completed successful graded challenges to the medication. The third patient’s skin testing to doxycycline was negative, however she had a previous reaction to minocycline, thus a graded oral challenge was performed. The patient underwent the challenge without symptoms and subsequently completed an uneventful full treatment course of doxycycline for a respiratory infection. The fourth patient had positive skin testing to doxycycline, and required an oral desensitization procedure which was successfully completed.
CONCLUSIONS: This is the first report of protocols for doxycycline and minocycline skin testing, including utilization of non-allergic control subjects to establish the highest non-irritating concentrations for each drug.

398 Non-Invasive Management of Myocarditis Despite a Negative Gadolinium-Enhanced Cardiac MRI in a 15-Year-Old Boy with Minocycline Triggered Dress Syndrome
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RATIONALE: DRESS Syndrome (DS) is a life-threatening multisystem drug reaction that may cause rash, eosinophilia, hepatitis, interstitial nephritis and myocarditis. We report a 15-year-old boy with minocycline triggered DS and myocarditis managed despite a normal gadolinium-enhanced cardiac MRI (GE-MRI).
METHODS: Case-report.
RESULTS: The diagnosis of myocarditis rests on endomyocardial biopsy (EMCB). However, EMCB may not show patchy inflammation or alter management, and may cause harm. CBC, acute phase reactants, B-type natriuretic peptide (BNP), cardiac enzymes, ECG, echocardiographic fractional shortening (E-FS), and GE-MRI may yield a presumptive diagnosis. We report a 15-yo boy treated for acne with minocycline for 6-weeks, who then developed rash, fever, UGI symptoms, lymphadenopathy, facial swelling, eosinophilia (AEC 4,600), hepatitis (ALT 591, AST 447), and interstitial nephritis (creatinine 1.68). High cardiac output shock without myocarditis (E-FS 44.2 and BNP <10) and rhabdomyolysis (CK 675) ensued, requiring intensive support. High dose corticosteroids (HDCS) yielded improvement. A slow corticosteroid taper followed. Seven weeks after discontinuing minocycline, still on corticosteroids, the patient developed myocarditis and clinical heart failure (E-FS 21%, BNP 1196, troponin-I 7.202) with a normal GE-MRI. HDCS yielded improvement (E-FS 39.8%, BNP 166, troponin-I 4.014). These values rebounded with 2 more corticosteroid taper attempts. IVIG and then cyclosporine were added to the HDCS treatment, with incremental improvement. CONCLUSIONS: DS is a life-threatening multisystem drug reaction, which may cause myocarditis. EMCB is diagnostic, but seldom done because of limited utility and possible harm. E-FS, BNP and troponin-I were useful for diagnosis and therapeutic monitoring in our pediatric patient with minocycline triggered DS myocarditis.
A Fatal Case of Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS)-Stevens Johnson (SJS)/Toxic Epidermal Necrolysis (TEN) in the Setting of Strongyloides Infection: Treatment Considerations

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RATIONALE: In patients with suspected Strongyloides, ivermectin treatment should be considered because corticosteroid therapy poses a serious risk of hyperinfection syndrome.

METHODS: None.

RESULTS: A 29 yo Indian woman developed unremitting fever, rash and diarrhea after eating raw oysters. Despite therapy with prednisone, doxycycline and ceftriaxone, she worsened. By Day 14 of illness, she exhibited a desquamating rash, lymphadenopathy, and eosinophilia (AEC 10000), therefore ciprofloxacin and clindamycin were added for presumed Vibrio infection. Eosinophilia increased (AEC 2800) and IgE spiked (17,561). Skin biopsy confirmed a drug reaction, therefore IVIG (1g/kg) was administered and antibiotics discontinued. Work up for malignancy and infection continued in the setting of persistent liver dysfunction. Serology was strongly positive for Strongyloides both pre and post IVIG, though stool studies and biopsies were negative for parasitic infection. Ivermectin and methylprednisolone were administered on Day 25 after which eosinophilia resolved and she improved dramatically. Within one week she developed a coalescing maculopapular rash with numerous dusky areas consistent with TEN. Additional IVIG and solumedrol were ineffective. She was transferred to a burn unit and succumbed to pseudomonas septicemia.

CONCLUSIONS: We speculate that reactivation of a dormant Strongyloides infection fostered DRESS. Given that ivermectin is not associated with hypersensitivity reactions, its empiric use may be valuable in at risk individuals with DRESS before instituting corticosteroids. Perhaps corticosteroids and ivermectin modified the cytokine milieu from the eotaxin and ILS environment associated with DRESS, such that re-exposure caused SJS-TEN, with characteristic keratinocyte cell death through Fas ligand. Clinical research is ongoing.

Novel Protocol for Successful Intravenous Insulin Desensitization in a Patient with Insulin Dependent Diabetes Mellitus

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RATIONALE: Immune mediated drug allergy to synthesized insulin is rare. Occasionally individuals present with immediate hypersensitivity reactions (HSRs) to multiple forms of insulin and require desensitization. Current protocols rely on subcutaneous administration of insulin. We describe an intravenous desensitization protocol in a 50-year-old woman with adult onset, insulin-dependent diabetes mellitus and diabetic ketoacidosis. She had a history of epinephrine-requiring, anaphylactic reactions to multiple medications including subcutaneous administration of regular, intermediate and long-acting insulin.

METHODS: In-vitro IgE to human insulin was negative and her baseline trypsinase level was normal. Skin prick testing was negative to regular insulin at 1U/mL. Intradermal testing is equivalent to a drug challenge for insulin, but she declined because of her recurrent anaphylaxis and overall medical fragility. Our patient was pre-medicated with montelukast, cetirizine, famotidine and diphenhydramine prior to desensitization. Serial solutions of regular insulin were prepared at 1:10,000, 1:1,000, 1:100, 1:10 and 1:1 of the standard 1U/mL concentration. We began at a dose equivalent to 0.00005 U/hr and doubled doses every 30 minutes until achieving a goal dose of 0.5 U/hr.

RESULTS: The patient completed this protocol without manifestation of HSRs and the transition from intravenous to subcutaneous administration of regular insulin was uneventful.

CONCLUSIONS: Patients with insulin-dependent diabetes mellitus and immediate HSRs to multiple forms of insulin require desensitization. Existing protocols use subcutaneous administration of insulin, but not all patients can tolerate this method. We have demonstrated that this intravenous desensitization protocol can be used to successfully achieve immunological tolerance of subcutaneous insulin.

Specificity and Sensitivity of Benzyl-Penicillin Skin Testing in Patients with Suspected Hypersensitivity to Penicillin

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RATIONALE: Skin testing with benzyl-penicillin (BP) is sometimes performed alone, when penicilloyl-polysylne (PPL) is not available, or together with other penicillin determinants. As the sensitivity and specificity of BP skin testing have not been clearly established, the aim of our study was to determine these parameters in patients with a history of hypersensitivity to penicillin.

METHODS: Patients presenting a history of hypersensitivity to penicillin were evaluated by prick and intradermal skin tests with PPL (5x10^-5), BP (10 000 U/mL), minor determinant mixture (MDM, 2x10^-5), amoxicillin and clavulanic acid. All cases with negative PPL and MDM, independently of the BP skin test result, underwent a graded drug provocation test (DPT) with BP (600 000 U) followed, if negative, by a two-day home DPT with penicillin V.

RESULTS: We evaluated 76 patients with a mean age of 45.4 years (15.8 – 84.1) and a suggestive history of mostly immediate (67.1%) hypersensitivity reaction to penicillin. The penicillin workup confirmed hypersensitivity for 7 patients to BP, 5 patients to amoxicillin and 4 patients to clavulanic acid. Skin testing with BP was positive for two patients and they both tolerated the DPT. The overall specificity and sensitivity of BP skin testing were calculated to 97.4% and 0% respectively. Among the 16 patients with a final diagnosis of allergy this specificity decreased to 93.75%.

CONCLUSIONS: In our population the addition of BP skin testing do not increase specificity of the overall workup. Moreover, possible false positive results have to be taken into account in the decision of performing this test.
402 Corticosteroid-Related Adverse Events in Chronic Idiopathic Urticaria
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RATIONALE: Clinicians commonly use oral corticosteroids (OCS) to treat the symptoms and signs of chronic idiopathic/spontaneous urticaria (CIU/CSU) – hives of uncertain etiology. Quantitative risks of OCS-related adverse events (AE) in CIU/CSU are poorly characterized.

METHODS: This retrospective cohort study analyzed a commercial claims database from 1/1/2008 to 12/31/2012. We used a validated method to identify adult CIU/CSU patients who had either two outpatient urticaria diagnoses 6 weeks apart in the calendar year or one outpatient diagnosis of urticaria plus one diagnosis of angioedema 6 weeks afterwards. AEs of interest included diabetes mellitus, hypertension, lipid disorders, cataracts, depression and mania, skeletal conditions (osteoporosis and fractures), and pneumonia and opportunistic infections. We used time-dependent Cox regression to separately model cumulative oral prednisone-equivalent exposure in milligrams and the risk of developing AEs; we adjusted for age, sex, Charlson Comorbidity Index, and immunomodulator use.

RESULTS: We identified 12,647 CIU/CSU patients. During the first 12 months observed, 55.4% used OCS (mean treatment duration: 16.2 days; mean per-patient prednisone-equivalent dose exposure: 367.5 milligrams). After the initial 12 months, 27.3 new AEs occurred per 100 patient-years. The adjusted risk for developing any AE was 7% higher (hazard ratio 1.07, 95% CI: 1.05-1.08) per additional 1 gram of prednisone-equivalent exposure. As cumulative prednisone-equivalent exposure increased, so did the risks for each studied AE, except cataracts. The highest risks were associated with developing skeletal conditions and infections.

CONCLUSIONS: Increase in cumulative OCS exposure was associated with significantly increased risks of OCS-related adverse events in CIU/CSU patients.

403 The Role of Neutrophils in Difficult-to-Treat Chronic Urticaria
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RATIONALE: Neutrophil predominance may be seen in the skin biopsies of difficult-to-treat chronic urticaria (dCU) patients. Data regarding the clinical course of CU patients based on the tissue infiltrate obtained from skin biopsy is limited. Our study compared serological and clinical parameters between neutrophilic-predominant urticaria (NU) and lymphocytic-predominant urticaria (LU) patients.

METHODS: Fifty-one biopsies from dCU patients from 1999-2011 were reviewed. dCU was defined as treatment failure with at least 2 concurrent anti-histamines, use of oral steroids and/or immunomodulators. All biopsies were reviewed independently by 2 pathologists for cell count. Autoimmune markers (anti-nuclear antibody, anti-thyroid antibodies, rheumatoid factor, CU index), medications given (oral steroids, immuno-modulators) and response to treatment were analyzed.

RESULTS: Seventy-five percent (38/51) of patients were neutrophil predominant on biopsy. Forty-nine percent (25/51) had negative autoimmune markers. Sixty-seven percent (34/51) were on oral steroids and 18% (9/51) were on immunomodulators (dapsone, omalizumab, hydroxychloroquine, sulfasalazine). Twenty-seven percent (14/51) had complete resolution of symptoms, while 73% (37/51) had incomplete resolution. There was no statistically significant difference in the presence of autoimmune markers, resolution of symptoms, steroid and immunomodulator use between NU and LU patients.

CONCLUSIONS: In our study population, neutrophils were the predominant cell type on biopsy among dCU patients. However, this was not significantly associated with the presence of autoimmune markers or the use of steroids or immunomodulators. Further studies are needed to determine the influence of neutrophils and lymphocytes in dCU and its responsiveness to medical treatment.

404 Release of Transglutaminase 2 from Mast Cells May Be Involved in the Pathogenesis of Chronic Urticaria
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RATIONALE: Mast cells and its mediators play an important role in the pathogenesis of chronic urticaria (CU). Transglutaminase 2 (TG2) has been reported to be expressed in mast cells and to contribute to allergic asthma. The aim of this study is to investigate the role of TG2 in CU and the source of TG2 in urticarial skin tissues.

METHODS: Seventy two CU patients and control subjects (52 normal controls and 11 bronchial asthma) were included. Skin biopsies were obtained from 5 CU and 2 normal controls. TG2 activity and inflammatory cytokines such as TNF-a, TGF-β, IL-4, IL-5, IL-6, and IL-13 were measured in serum by ELISA. Co-localization of mast cells and TG2 were determined by immunohistochemistry.

RESULTS: TG2 activity was significantly higher in sera of CU patients than normal controls (P < 0.05), while TG2 activity in asthmatics was significantly higher than CU patients (P < 0.05). Co-localization of mast cell surface marker c-kit and TG2 were significantly increased in wheals of CU patients comparing with normal controls. The levels of TNF-a, TGF-β, IL-4, IL-5, IL-6, and IL-13 were significantly higher in CU patients than normal controls (P < 0.001, respectively), however, the levels of cytokines were significantly higher in asthmatics than in CU (P < 0.05, respectively).

CONCLUSIONS: TG2 released from mast cells play a role in the pathogenesis of CU. We also demonstrated that other inflammatory cells are activated in CU showing increased levels of various cytokines. However, the inflammation in CU dose not seem stronger than in bronchial asthma.
405 Evaluation of Indoleamine 2,3- Dioxgenase Gene Expression and Activation in Chronic Spontaneous Urticaria
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RATIONALE: Lesions of chronic idiopathic urticaria (CIU) demonstrate mast cell (MC) activation and recruitment of lymphocytes, eosinophils, and basophils. CRTH2/DP2 are receptors for Prostaglandin D2 (PGD2) expressed on infiltrating cells and induce chemotaxis and activation. Since activated MCs release Prostaglandin D2 (PGD2), a possible recruitment pathway for these leukocytes is CRTh2, a receptor for PGD2. We compared CRTh2 expression on leukocytes from the blood of CIU and healthy subjects.

METHODS: We recruited adult CIU subjects (n=20) and nonatopic subjects (n=8) and examined basal expression of CRTH2 via flow cytometry. Basophils and eosinophils were gated using scatter and specific markers. Values are reported as Median Net MFI (± SEM). Data were analyzed using Mann-Whitney Test.

RESULTS: CRTh2 expression was significantly decreased on basophils from CIU subjects as compared with healthy controls (30.71 ± 21.11 vs 398.4 ± 29.41, p=0.0395). CRTh2 expression on eosinophils from CIU subjects also trended lower as compared to controls (61.18 ± 4.197 vs 70.88 ± 6.77, p=0.0524). CRTH2 expression levels were more variable on basophils and eosinophils in CIU subjects as compared to controls. There was no difference between the percentage of basophils or eosinophils expressing CRTh2 in CIU compared with controls.

CONCLUSIONS: CRTh2 expression on leukocytes from CIU subjects is more variable than healthy controls. Levels on blood basophils are significantly reduced, compared to nonatopic controls. Eosinophil CRTH2 levels were overall lower than basophils and trended lower in CIU subjects. These findings suggest that the CRTH2 pathway may be engaged in the recruitment of eosinophils and basophils to CIU lesions.

406 The Expression of CRTh2 on Blood Basophils and Eosinophils in Chronic Idiopathic Urticaria
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RATIONALE: Evidence of mast cell (MC) degranulation and infiltration by leukocytes such as basophils, eosinophils, and T lymphocytes is observed in lesions in chronic idiopathic urticaria (CIU). Since activated MCs release Prostaglandin D2 (PGD2), a possible recruitment pathway for these leukocytes is CRTh2, a receptor for PGD2. We compared CRTh2 expression on leukocytes in the blood of CIU and healthy subjects.

METHODS: We recruited adult CIU subjects (n=20) and nonatopic subjects (n=8) and examined basal expression of CRTH2 via flow cytometry. Basophils and eosinophils were gated using scatter and specific markers. Values are reported as Median Net MFI (± SEM). Data were analyzed using Mann-Whitney Test.

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CONCLUSIONS: CRTh2 expression on leukocytes from CIU subjects is more variable than healthy controls. Levels on blood basophils are significantly reduced, compared to nonatopic controls. Eosinophil CRTH2 levels were overall lower than basophils and trended lower in CIU subjects. These findings suggest that the CRTH2 pathway may be engaged in the recruitment of eosinophils and basophils to CIU lesions.

406 Functional Expression of CRTH2 on Blood Eosinophils from Chronic Idiopathic Urticaria Subjects
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RATIONALE: Lesions of chronic idiopathic urticaria (CIU) demonstrate mast cell (MC) activation and recruitment of lymphocytes, eosinophils, and basophils. CRTH2/DP2 are receptors for Prostaglandin D2 (PGD2) expressed on infiltrating cells and induce chemotaxis and activation. Since activated MC release PGD2, we explored the effects of in vitro PGD2 exposure alone or with a CRTH2 receptor antagonist (AZD1981) on the shape change of eosinophils from active CIU patients.

METHODS: Blood was obtained from CIU patients (n=15) using a JH IRB approved protocol. Whole blood samples were incubated with buffer or AZD1981 (0.1 or 1 uM), and then stimulated with PGD2 (10-8 to 10-5 M). Eosinophil shape change was examined using flow cytometry scatter movement.

RESULTS: A net scatter movement at each PGD2 dose was plotted on a concentration curve, and analyzed using an area under the curve (AUC) method. Cells incubated in buffer alone had an AUC value of 119.9. Cells exposed to 1 uM AZD1981 showed a concentration curve of 52.9 AUC (p<0.001 from buffer alone). The cell curve at 0.1 uM AZD1981 was 76.5 AUC (p<0.001). The 50% of maximal response concentration of PGD2 required for eosinophils was 10-7 M (buffer), 10-6 M (1 uM AZD1981), and 10-6.5 M (0.1 uM AZD1981).

CONCLUSIONS: In CIU patients, PGD2 induces eosinophil shape change at concentrations similar to healthy subjects. Preincubation with AZD1981 markedly reduced the PGD2 mediated shape change response indicating functional expression of CRTH2 on circulating CIU eosinophils and suggesting CRTH2 as a therapeutic target in CIU.
Response Patterns in Chronic Idiopathic/Spontaneous Urticaria (CIU/CSU) Patients Treated with Omalizumab for 24 Weeks in Two Randomized, Double-Blind, Placebo-Controlled Clinical Trials (ASTERIA I and GLACIAL)

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RATIONALE: Response patterns in CIU/CSU patients treated with omalizumab are poorly understood.

METHODS: Subjects were randomized and received placebo (PLB) vs omalizumab (OMA) 75mg, 150mg, 300mg (ASTERIA I, n = 318) or PLB vs OMA300 (GLACIAL, n = 335) every 4 weeks for 24 weeks. Response comprised: Well-Controlled (weekly Urticaria Activity Score [UAS7, itch severity plus number of hives] <6) or Complete Response (UAS7=0).

RESULTS: OMA300 demonstrated the highest percentage of responders at week 12 (W12): UAS7<5 25.0%, 29.9%, 36.3%, 61.7% (PLB, OMA75, OMA150, and OMA300, respectively; ASTERIA I) and 16.9%, 55.6% (PLB, OMA300, respectively; GLACIAL); UAS7=0: 8.8%, 11.7%, 15.0%, 35.8% (PLB, OMA75, OMA150, and OMA300, respectively; ASTERIA I) and 4.8%, 33.7% (PLB, OMA300, respectively; GLACIAL). OMA300 demonstrated the highest percentage of responders at W24: UAS7<5: 25.0%, 29.9%, 36.3%, 61.7% (PLB, OMA75, OMA150, and OMA300, respectively; ASTERIA I) and 4.8%, 33.7% (PLB, OMA300, respectively; GLACIAL). UAS7=0: 12.5%, 23.4%, 20.0%, 48.1% (PLB, OMA75, OMA150, and OMA300, respectively; ASTERIA I) and 3.6%, 42.5% (PLB, OMA300, respectively; GLACIAL). Median time to response by W24: UAS7<6: 11 and 6 weeks (OMA150, OMA300, respectively; ASTERIA I) and 6 weeks (OMA300; GLACIAL); UAS7=0: 12 and 13 weeks (OMA300; ASTERIA I and GLACIAL, respectively). Median time to response was not reached by W24 in other treatment arms.

CONCLUSIONS: Response patterns were dose-dependent: OMA300 demonstrated the largest percentage of patients who achieved Complete or Well-Controlled Response. Omalizumab treatment benefits CIU/CSU patients up to W24. These data highlight the likelihood of response to omalizumab in CIU/CSU patients at different time points.

Omalizumab Improves Quality of Life (QoL) in Patients with Refractory Chronic Spontaneous/Idiopathic Urticaria (CSU/CIU) As Assessed By the Dermatology Life Quality Index (DLQI): A Post-Hoc Analysis of Percent Change from Baseline to Week 12

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RATIONALE: Omalizumab, an anti-IgE monoclonal antibody, was evaluated in three Phase III trials (ASTERIA-I/II and GLACIAL); we report DLQI data from all three studies.

METHODS: Patients in ASTERIA-I/II were symptomatic despite approved doses of H1-antihistamines and received omalizumab 75/150/300mg, or placebo. Patients in GLACIAL received omalizumab 300mg or placebo and were symptomatic despite H1-antihistamines (≥4X approved dose) plus H2-antihistamines and/or leukotriene-receptor antagonists. DLQI domains were assessed at baseline and Week 12. Omalizumab 75mg data are not presented.

RESULTS: DLQI domain scores (%absolute) were improved for omalizumab 300mg vs placebo at Week 12:

- Overall: 74[-10.3] vs 47[-6.1]; p<0.0001[ASTERIA-I]; 78[-10.2] vs 44[-6.1]; p<0.0004[ASTERIA-II]; 73[-9.7] vs 22[-5.1]; p<0.0001 [GLACIAL]
- Symptoms and feelings: 78[2.9] vs 37[-1.5]; p<0.0001[ASTERIA-I]; 69[-2.9] vs 39[-1.6]; p<0.0001[ASTERIA-II]; 68[2.8] vs 18[-1.3]; p<0.0001[GLACIAL]
- Daily activities: 79[-2.4] vs 43[-1.3]; p<0.0001[ASTERIA-I]; 76[-2.3] vs 44[-1.3]; p<0.0007[ASTERIA-II]; 76[-2.2] vs 39[-1.1]; p<0.0001 [GLACIAL]
- Leisure: 84[-1.9] vs 51[-1.4]; p=0.0062[ASTERIA-I]; 81[-1.9] vs 51[-1.1]; p=0.0071[ASTERIA-II]; 76[-1.8] vs 37[-1.0]; p=0.0063 [GLACIAL]
- Work and school: 82[-1.2] vs 60[-0.8]; p=0.0546[ASTERIA-I]; 86[-1.1] vs 52[-0.7]; p=0.0349[ASTERIA-II]; 78[-1.0] vs 63[-0.7]; p=0.0118 [GLACIAL]
- Personal relationships: 84[-1.3] vs 55[-1.0]; p=0.0386[ASTERIA-I]; 84[-1.3] vs 46[-1.0]; p=0.1043[ASTERIA-II]; 78[-1.2] vs 47[-0.7]; p=0.00085 [GLACIAL]
- Treatment: 85[-0.7] vs 48[-0.2]; p=0.0013[ASTERIA-I]; 85[-0.6] vs 68[-0.4]; p=0.0149[ASTERIA-II]; 81[-0.7] vs 48[-0.4]; p=0.0030 [GLACIAL].

DLQI domain scores were also improved for omalizumab 150mg:

- Overall: 51[-8.0] vs 47[-6.1]; p=0.2286[ASTERIA-I]; 66[-8.3] vs 44[-6.1]; p=0.0215[ASTERIA-II]
- Symptoms and feelings: 47[-2.0] vs 37[-1.5]; p=0.0741[ASTERIA-I]; 60[-2.4] vs 39[-1.6]; p=0.0057[ASTERIA-II]
- Daily activities: 63[-1.7] vs 43[-1.3]; p=0.1557[ASTERIA-I]; 69[-1.7] vs 44[-1.3]; p=0.0479[ASTERIA-II]
- Leisure: 69[-1.8] vs 51[-1.4]; p=0.1394[ASTERIA-I]; 62[-1.5] vs 51[-1.1]; p=0.6329[ASTERIA-II]
- Work and school: 62[-1.0] vs 60[-0.8]; p=0.5178[ASTERIA-I]; 79[-1.1] vs 52[-0.7]; p=0.2004[ASTERIA-II]
- Personal relationships: 69[-1.1] vs 55[-1.0]; p=0.2341[ASTERIA-I]; 65[-1.1] vs 46[-1.0]; p=0.4986 [ASTERIA-II]
- Treatment: 62[-0.5] vs 48[-0.2]; p=0.2029[ASTERIA-I]; 69[-0.5] vs 68[-0.4]; p=0.4572[ASTERIA-II].

CONCLUSIONS: Omalizumab significantly improved QoL, as measured by DLQI, in patients with CSU/CIU refractory to standard of care.
Successful Omalizumab Treatment of a 6-Year Old Child with Severe Solar Urticaria
Yuval Tal, MD, PhD1, Zvi Dranitzki, MD1, Meir Shalit, MD, FAAAAI1, David Claes Enk2, Assi Levi3; 1Allergy and Clinical Immunology Unit, Department of Medicine, Hadassah Hebrew University Medical Center, Jerusalem, Israel, 2Department of Dermatology, Hadassah Hebrew University Medical Center, Jerusalem, Israel, 3Photodermatosis Clinic, Laser unit, Department of Dermatology, Rabin Medical Center, Petah-Tikva, Israel.

RATIONALE: Solar urticaria is a rare form of physical urticaria usually occurring minutes after sun exposure. Angioedema might accompany the urticaria in severe cases. Treatment is difficult and often unsatisfactory. Here, we present a 6-year old child with severe solar urticaria and angioedema following short exposures to light despite continued combination therapy with high dose anti-histamines and leukotriene-receptor antagonists. As the child faced living in darkness unless an effective therapeutic remedy was found, we initiated omalizumab treatment with cautious dose elevation.

METHODS: Action spectrum and minimal urticaria dose (MUD) were established by UVB (290-320 nm), UVA1 (340-400 nm) and visible light (400-760 nm) photoprovocation tests. Omalizumab treatment was initiated, with dose elevation every two weeks up to a complete remission dose of 300 mg, repeated every four weeks as maintenance.

RESULTS: The action spectrum was established within the visible light spectrum with a MUD of 30 J/cm2 (unchanged, preceding and during non-biological therapy). Omalizumab treatment was added, beginning with a dose of 75 mg. Partial response was noted at a dose of 150 mg, with some recurrence prior to the next dosing. At a maintenance dose of 300 mg every four weeks, the child remains in remission one year after initiation of treatment, with no urticaria episodes even following prolonged exposure to mid-day summer sun. Repeated photoprovocation with visible light was negative, validating the clinical remission.

CONCLUSIONS: Solar urticaria and angioedema are both agonizing and life-threatening conditions. Treatment with omalizumab should be considered when high dose antihistamines and leukotriene-receptor antagonists fail.

Long-Term Efficacy of Omalizumab in Patients with Treatment-Resistant Chronic Spontaneous Urticaria
Mona Sulaiman Al-Ahmad, MD; Department of Microbiology, Faculty of Medicine, Kuwait University, Kuwait.

RATIONALE: Monoclonal anti-IgE antibody omalizumab is a promising therapeutic option in patients with chronic urticaria resistant to non-sedating H1-antihistamines. However, data about its long-term efficacy and safety are still scant, especially from the Middle East. We describe the long-term clinical course of patients with severe recalcitrant chronic urticaria that was treated with omalizumab in our allergy center in Kuwait, for periods up to 4 years.

METHODS: Thirty patients (15 F/15 M) with mean age of 38.6 years were treated for more than 38 months have been evaluated. All patients suffered from persistent symptoms despite receiving high doses of non-sedating antihistamine [4×/day], leukotriene antagonists and courses of prednisolone, and some failed courses of intravenous immunoglobulin. Autologous skin test, total IgE level and autoimmune work up was done in all patients. Response to treatment was assessed using urticaria activity score (UAS) and a combined symptom/medication score.

RESULTS: Half of the patients (15) completed 4 years of therapy. The mean duration of chronic urticarial is 9.5 years. There was a complete remission of disease in nine patients after the second dose of omalizumab. There was a significant improvement in UAS between pre-treatment and first dose, with mean of 3.9, (95% CI 3.45-4.3) (p < 0.0). The improvement was maintained through out 4th year of therapy with statistical significance. The treatment was well tolerated.

CONCLUSIONS: Omalizumab is a safe and effective corticosteroid alternative for refractory urticaria patients. It is equally effective and safe for long-term use up to 4 years.
Clinical Characteristics of Adolescent Patients with Refractory Chronic Idiopathic/Spontaneous Urticaria (CIU/CSU) in Three Phase III Studies with Omalizumab

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RATIONALE: Information on the clinical profile of CIU/CSU in adolescents is limited. Baseline characteristics and demographics of an adolescent subgroup of CIU/CSU patients enrolled in randomized, placebo-controlled omalizumab trials are evaluated.

METHODS: This is a post-hoc descriptive analysis of pooled baseline data from three omalizumab trials in CIU/CSU patients who remained symptomatic despite H1-antihistamine treatment [and H2-antihistamines and/or leukotriene receptor antagonists (LTRA) in one study]. Demographics and disease characteristics are summarized for overall and for adolescent patients (≥12 to <18 years).

RESULTS: Of 975 patients [mean (standard deviation; SD) age, 42.3 (14.1) years], 39 were adolescents [15.1 (1.5) years]. Most patients were female (overall, 73.4%; adolescents, 69.2%) and white (85.4%; 84.6%), with a mean BMI (SD) of 29.6 (7.3) and 24.4 (4.7) kg/m², respectively. A female (overall, 73.4%; adolescents, 69.2%) and white (85.4%; 84.6%) population was enrolled. The mean disease duration (SD) was 17.9 (4.0) years and adolescents had a mean disease duration of 15.3 (4.0) years. Dermatology Life Quality Index over time was 13.2 (6.5) and 12.0 (5.2), respectively. Overall, patients experienced CIU/CSU for mean duration (SD) of 6.9 (9.1) years and adolescents for 3.2 (3.7) years, and received 5.0 (2.8) and 4.2 (2.3) previous CIU/CSU medications, respectively. Except for one adult, all patients received antihistamines. Concomitant medications included: H2-receptor antagonists (overall, 48.4%; adolescents, 48.7%); LTRA (34.5%; 35.9%) and steroids (45.7%; 33.3%).

CONCLUSIONS: Understanding the clinical profile of CIU/CSU in adolescents will guide clinical practice.

Use of Omalizumab for Treatment of Anti-histamine and Steroid Resistant Chronic Idiopathic Urticaria during Pregnancy

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RATIONALE: The treatment of anti-histamine and steroid resistant Chronic Idiopathic Urticaria (CIU) during pregnancy poses a challenge due to teratogenicity of immunosuppressants. Omalizumab is a recently FDA approved therapy for CIU with pregnancy category B and presents an alternative treatment option.

METHODS: We present an initial series of 4 subjects treated for anti-histamine and steroid resistant urticaria with Omalizumab who became pregnant during therapy from April 2011 to February 2012 at a tertiary care center.

RESULTS: Four women 25-28 years of age initiated on Omalizumab for CIU (300 mg subcutaneously every 28 days) became pregnant and continued therapy throughout their pregnancy. Three patients had a history of asthma demonstrated by pulmonary function tests; two had diagnosis of allergic rhinitis with positive skin testing. All patients were uncontrolled on >3 antihistamines and leukotriene antagonists. 3/4 required immunosuppressive therapy with hydroxychloroquine, dapsone and cyclosporine prior to Omalizumab initiation. All subjects received prednisone and 2/4 required chronic steroid therapy at >20mg daily without symptomatic improvement. Within the first month of Omalizumab therapy, they reported significant improvement of their symptoms demonstrated by increased hive-free intervals, decreased medical utilization and weaning of steroids. Subjects had normal prenatal follow ups, full term deliveries and no pregnancy or fetal complications.

CONCLUSIONS: There is a significant benefit with treatment of CIU during pregnancy with Omalizumab. With recent FDA approval for this condition, pregnancy category B rating and favorable safety data from the Xolair pregnancy registry (EXPECT), these findings support future strategies for use of Omalizumab for CIU during pregnancy.

Use of Tacrolimus in the Management of Refractory Chronic Urticaria

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RATIONALE: Chronic urticaria (CU) causes significant morbidity. Many patients do not achieve adequate control with conventional antihistamine therapy; others require long term corticosteroids, with significant adverse effects. Numerous alternatives have been used including calcineurin inhibitors, primarily cyclosporine. We sought to determine the safety and effectiveness of the calcineurin inhibitor tacrolimus.

METHODS: A retrospective chart review was conducted of adult CU patients who were treated with tacrolimus in our allergy clinic. Laboratory values and clinical evidence for toxicity were abstracted as well as physician reports of efficacy.

RESULTS: Thirty four adults with CU treated with tacrolimus were identified. Ninety four percent of patients were refractory to both 1st and 2nd generation antihistamines, and 50% were on daily prednisone (mean dose of >20mg/day). Seventy three percent had failed at least 1 alternative medication, including montelukast, sulfasalazine, dapsone, hydroxychloroquine, mycophenolate, colchicine, omalizumab and thymoxine. Mean duration of symptoms prior to starting tacrolimus was 5.7 years. Twenty eight patients (82%) improved with tacrolimus. Sixty one percent were able to discontinue daily prednisone, and 33% were able to decrease prednisone dose by >50%. Twenty of thirty four reported adverse effects, most commonly gastrointestinal symptoms, headache or dysesthesias. Three discontinued tacrolimus because of adverse effects. At least three patients required a second course of tacrolimus due to recurrent CU, and at least two achieved remission with this second course.

CONCLUSIONS: Tacrolimus appears to be an effective alternative agent for management of refractory chronic urticaria patients. While adverse effects were common, most were benign, dose-related and infrequently required discontinuation of the drug.
Alternative Agents in Chronic Urticaria and Angioedema
Sharon Deol, David A. Khan, MD, FAAAAI; University Texas SW Medical Center, Dallas, TX.

RATIONAL: Patients who have failed traditional treatment of chronic urticaria with angioedema require trials of alternative medications. Safety profiles, continuous laboratory monitoring, and physician comfort are often barriers to treatment.

METHODS: Retrospective chart review of electronic medical records from a single center allergy and immunology clinic in a major academic hospital was conducted. Records were searched for the following prescriptions: dapsone, hydroxychloroquine, sulfasalazine, cyclosporine, mycophenolate,omalizumab. Medical records were reviewed for any self-reported adverse effects including abnormal laboratories.

RESULTS: Two hundred and sixteen patients treated with alternative agents were identified. One hundred and thirty three adult patients had a diagnosis of chronic urticaria. Adverse effects were experienced by 25/74 patients treated with dapsone, 12/39 patients with sulfasalazine, 17/39 patients with tacrolimus, 5/42 patients with hydroxychloroquine, 2/24 patients with mycophenolate, 3/8 patients with cyclosporine, and 1/23 patient with omalizumab. Most of these side effects were mild, did not require discontinuation of the medication, and resolved after stopping the medication or decreasing the dose. The most common adverse effects were asymptomatic anemia with dapsone and gastrointestinal symptoms with various medications.

CONCLUSIONS: The use of alternative agents for the treatment of refractory chronic urticaria and angioedema is generally safe as long as proper laboratory and clinical monitoring is observed.

Treatment of Autoimmune Urticaria with Mycophenolate Mofetil
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RATIONAL: Chronic urticaria is defined as hives which occur on a regular basis for more than six weeks. Patients with autoimmune urticaria generally have more severe and difficult-to-control symptoms. There is little well controlled data guiding the treatment of chronic urticaria after a patient fails maximum therapy with antihistamines.

METHODS: A 35-year-old woman presented with chronic urticarial rash and angioedema for the six years including daily wheals, which were pruritic and changed their location during 24 hours. She had frequent episodes of lip and facial swelling not related to any specific inducing factor. Other family members including her father and brother had experienced similar symptoms and chronic urticaria was diagnosed. The autologous serum skin test was performed several times and was positive. Treatment with antihistaminic drugs in four-fold increased dosages, with leukotriene antagonists and oral corticosteroids were not effective. New approaches were sought based on immune modulation.

RESULTS: The first immune modulating procedure was plasmapheresis done 3 years ago which induced a remission that lasted for 2 years. Last year urticaria and angioedema relapsed. Next, the patient was treated with the immunosuppressive drug mycophenolate mofetil for one month using 2 grams per day. After one week clinical improvement was observed with wheals disappearing and the score of UAS7 decreasing from 5 to 0. There were no adverse effects after the first month of treatment which has subsequently been continued.

CONCLUSIONS: Mycophenolate mofetil is a useful and potentially safe second line immune modulating therapy for treating autoimmune urticaria in whom antihistamines, leukotriene antagonists, oral corticosteroids, and even plasmapheresis have failed.
420 Chronic Urticaria and Parasitic Infections
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RATIONALE: Chronic urticaria (CU) is defined as hives lasting greater than six weeks. The etiology of chronic hives could be idiopathic or associated with conditions such as hormonal disturbances, autoimmune diseases, physical triggers, and rarely, infections.

METHODS: We report a 39 yo female patient with CU secondary to underlying Giardia lamblia infection as well as a 36 yo female with CU secondary to infection with Trichomonas vaginalis.

RESULTS: The first case is a 39 yo female patient who developed CU and angioedema after visiting family in Massachusetts. History revealed frequent diarrhea with abdominal pain which she thought was due to seafood sensitivity. Food allergy skin test (RAST), H. pylori profile, ANA, and thyroid function panel were negative but stool studies revealed Giardia lamblia cysts. The patient was treated with metronidazole and CU resolved. The second case is a 36 yo female who had a six month history of hives with painful swollen feet, aggravated by sexual activities with her male partner. History revealed chronic bacterial vaginosis. CBC, liver/thyroid function tests, serum tryptase, C3, C4, and IgE were normal. H. pylori profile, ANA, latex-IgE, anti-thyroglobulin, anti-thyroid peroxidase antibodies, and stool ova and parasites were negative. Incidentally, CS deficieny was identified. Three months later, Trichomonas vaginalis was identified. Metronidazole treatment led to resolution of CU.

CONCLUSIONS: In both patients with CU, underlying parasitic infections were found. Treatment with appropriate antibiotics led to complete resolution of CU. Existing literature has proposed that parasites induce IgE-mediated mast cell degranulation leading to urticaria.

421 The Prevalence of Physical Urticaria in Patients with Chronic Urticaria: A Systematic Review and Meta-Analysis
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RATIONALE: Physical urticaria (PU) is a subset of chronic urticaria (CU) induced by physical stimuli. To date, there is no consensus in the literature on the prevalence of PU among patients with CU. Our objective was to determine the pooled prevalence of PU in CU patients.

METHODS: We performed a systematic review and meta-analysis to determine the pooled estimates of the prevalence of PU among patients with CU in the literature up to September 2014. We searched 4 databases (OVID, PubMed, Medline, Web of Science) of published work for which full text was available in English or French. Studies were eligible if they measured the prevalence of PU in adults or children with CU worldwide and ineligible if acute urticaria cases were not differentiated from total urticaria cases. Meta-analysis was conducted using Stata, version 12.0 (StataCorp, College Station, TX).

RESULTS: Ten studies were included in our review. Sample sizes ranged from 202–4157 patients. Totals for cholinergic urticaria are included in the prevalence totals for PU. The pooled estimate of the prevalence of PU was 13.0%; 95% CI (12.4, 13.6) using the fixed effects model. The I² (the variation in effect size that is attributable to heterogeneity) was 98.7%.

CONCLUSIONS: Our analysis identified that among patients with CU, PU is not infrequent. Our results must be viewed with circumspection because of the small number of eligible articles and heterogeneity among studies. Even so, the results suggest that PU is an important subset of CU that requires further research.

422 My Hives Diary: An iOS App to Track Urticaria Symptoms
Evgeniya Antonova, MS, PhD, Karina Raimundo, BPharm, MS, James Zazzalli, PhD; Genentech, Inc., South San Francisco, CA.

RATIONALE: Chronic idiopathic/spontaneous urticaria (C1U/CSU) is characterized by itchy hives of uncertain etiology; symptoms may change daily. No electronic tool currently exists to help CIU/CSU patients systematically track their symptoms and the impact on daily activities and their sleep.

METHODS: The contents of this app are based on the Urticaria Patient Diary UPDD used to assess CIU/CSU symptoms and their impact in three randomized double-blind placebo controlled trials of omalizumab in CIU/CSU. The original UPDD content was developed based on interviews of CIU/CSU patients. Cognitive debriefing and item reduction were performed to finalize the UPDD.

RESULTS: MyHivesDiary is an iOS-based app for use on the iPhone or iPad. Daily, patients can record: itch severity, number of hives, size of largest hive, presence of angioedema, interference with daily activities and sleep, and events pertaining to their urticaria. MyHivesDiary generates reports (graphs and numeric values) for the items listed above and weekly Urticaria Activity Score (UAS7). The reports span last 7 days or one, three, or six months. 7-day report lists daily values. Monthly reports list weekly averages (except for angioedema: it lists the number of angioedema days per week). Patients may choose to email reports in PDF format. All data are stored on patient iOS device only.

CONCLUSIONS: MyHivesDiary is a novel and convenient tool for CIU/CSU patients to track their daily symptoms, the impact CIU/CSU brings to their lives, and events they may associate with CIU/CSU.

423 Weekly Urticaria Activity Score (UAS7) and Dermatology Life Quality Index (DLQI) in Validation of Chronic Spontaneous/Idiopathic Urticaria (CSU/CIU) Health States
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RATIONALE: Urticaria Activity Score (UAS) assesses daily pruritus and number of hives, which summed over a week, gives UAS7. Evidence is scarce on whether the disease health states defined by UAS7 scores can be described with dermatology health-related quality of life (HRQoL) measures such as Dermatology Life Quality Index (DLQI). Here we validate CSU health states defined by categorical UAS7 scores and compare with the DLQI.

METHODS: Pooled patient-level data from baseline and Week-12 for UAS7 and DLQI from 3 randomised phase III trials, evaluating effects of omalizumab on symptoms of refractory CSU, were used. UAS7 score-based health states were defined as follows: urticaria-free = 0; well-controlled urticaria = 1–6; mild = 7–15; moderate = 16–27; and severe urticaria = 28–42. Validated DLQI bands show the impact on patients HRQoL and life (no effect: 0–1; small = 2–5; moderate = 6–10; very large = 11–20; extremely large = 21–30).

RESULTS: UAS7 and DLQI from 3 randomised phase III trials, evaluating effects of omalizumab on symptoms of refractory CSU, were used. UAS7 score-based health states were defined as follows: urticaria-free = 0; well-controlled urticaria = 1–6; mild = 7–15; moderate = 16–27; and severe urticaria = 28–42. Validated DLQI bands show the impact on patients HRQoL and life (no effect: 0–1; small = 2–5; moderate = 6–10; very large = 11–20; extremely large = 21–30).

RESULTS: Baseline UAS7 scores showed that patients had moderate and severe urticaria with mean DLQI scores of 10.9 and 14.3 (p < 0.001) confirming a very large impact on patients. At week 12, mean DLQI scores between adjacent UAS7 health states were statistically different (all p < 0.0001): well-controlled urticaria vs urticaria-free (2.3 vs 0.4); mild vs well-controlled urticaria (4.9 vs 2.3); moderate vs mild urticaria (8.1 vs 4.9); and severe vs moderate urticaria (11.7 vs 8.1). Decrease in urticaria symptoms is associated with improvement in HRQoL.

CONCLUSIONS: CU health states are efficiently described by five UAS7 categories with different levels of impact on patients’ life confirmed by corresponding DLQI scores.
Omalizumab Improves Quality of Life (QoL) in Patients with Chronic Spontaneous/Idiopathic Urticaria (CSU/CIU) As Assessed By the Chronic Urticaria Quality of Life Questionnaire (CU-Q2oL): A Post-Hoc Analysis of Percent Change from Baseline to Week 12

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RATIONALE: Omalizumab, an anti-IgE monoclonal antibody, was evaluated in three Phase III trials (ASTERIA-I/II and GLACIAL); we report CU-QoL data from all three studies.

METHODS: Patients in ASTERIA-I/II and GLACIAL were symptomatic despite approved doses of H1-antihistamines and received omalizumab 75/150mg, or placebo. Patients in GLACIAL received omalizumab 300mg or placebo and were symptomatic despite H1-antihistamines (g4X approved dose) plus H2-antihistamines and/or leukotriene-receptor antagonists. CU-QoL domains were assessed at baseline and Week 12. Omalizumab 75mg data are not presented.

RESULTS: CU-QoL domain scores (%[absolute]) were improved for omalizumab 300mg vs placebo at Week 12:

- Overall: 66[-30.5] vs 42[-19.7],p=0.001[ASTERIA-I]; 69[-31.5] vs 40[-17.7],p<0.001[ASTERIA-II]; 67[-29.3] vs 32[-16.3],p<0.0001[GLACIAL]
- Pruritus: 69[-56.8] vs 34[-28.1],p=0.0001[ASTERIA-I]; 71[-56.5] vs 35[-28.6],p<0.0001[ASTERIA-II]; 65[-51.0] vs 17[-23.0],p<0.0001[GLACIAL]
- Swelling: 72[-16.7] vs 36[-10.7],p=0.1293[ASTERIA-I]; 66[-18.5] vs 36[-8.7],p=0.0134[ASTERIA-II]; 71[-17.6] vs 53[-8.6],p=0.0110[GLACIAL]

Impact on quality of life activities:

- Sleep problems: 53[-30.2] vs 38[-18.8],p=0.0524[ASTERIA-I]; 64[-33.3] vs 33[-18.0],p=0.0007[ASTERIA-II]; 61[-29.4] vs 35[-18.3],p<0.0001[GLACIAL]
- Limits: 64[-19.9] vs 52[-17.9],p=0.2774[ASTERIA-I]; 66[-21.5] vs 37[-11.2],p=0.0166[ASTERIA-II]; 57[-18.8] vs 33[-10.7],p=0.0008[GLACIAL]
- Looks: 61[-27.5] vs 39[-16.6],p=0.0054[ASTERIA-I]; 56[-27.0] vs 39[-16.3],p=0.0061[ASTERIA-II]; 67[-29.6] vs 32[-15.3],p<0.0001[GLACIAL]

CU-QoL domain scores were also improved for omalizumab 150mg:

- Overall: 53[-23.1] vs 42[-19.7],p=0.2891[ASTERIA-I]; 62[-27.0] vs 40[-17.7],p=0.0089[ASTERIA-II]
- Pruritus: 56[-40.6] vs 34[-28.1],p=0.0125[ASTERIA-I]; 57[-45.9] vs 35[-28.6],p=0.0014[ASTERIA-II]
- Swelling: 55[-11.2] vs 36[-10.7],p=0.4157[ASTERIA-I]; 54[-10.6] vs 36[-8.7],p=0.5929[ASTERIA-II]

Impact on quality of life activities:

- Sleep problems: 37[-22.1] vs 38[-18.8],p=0.5399[ASTERIA-I]; 44[-25.3] vs 33[-18.0],p=0.0939[ASTERIA-II]
- Limits: 44[-15.8] vs 52[-17.9],p=0.6854[ASTERIA-I]; 66[-20.0] vs 37[-11.2],p=0.0204[ASTERIA-II]

Looks: 53[-20.6] vs 39[-16.6],p=0.3140[ASTERIA-I]; 63[-27.7] vs 39[-16.3],p=0.0023[ASTERIA-II].

CONCLUSIONS: Omalizumab significantly improved QoL, as measured by CU-QoL, in patients with CSU/CIU refractory to standard of care.
The Urticaria Serial Assessment a Tool for Measuring Clinical Control of Urticaria

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RATIONALE: Prior to a recent publication, no validated, English language tools existed for assessment of control of chronic urticaria in the clinical setting. We aimed to create one to assist in our management.

METHODS: Our Urticaria Serial Assessment (USA) is an 8 item tool designed to measure control of Chronic Urticaria. Each item is scored 1-5 on a Likert type scale with score ranges between 8 and 40. It was developed and tested as part of scheduled patient visits by study subjects in a single clinic at a tertiary care center training in the United States. Content validity was assessed by physician review of questions prior to use. Construct validity was assessed through correlation of USA results with averaged blinded physician assessment (r=0.86, p<0.05). Criterion validity was assessed through correlation of USA results with Urticaria Activity Score (UAS) and Dermatology Quality of Life Instrument (DLQI) as well as tiered clinical intervention assessment based on clinical treatment decisions made at the time to the visit.

RESULTS: Thus far 29 surveys have been collected from 17 subjects. There was significant correlation between blinded physician assessment and USA outcome spearman r=0.82 p=0.00001. Additionally the USA was significantly correlated with 7 day UAS scores r=0.85, p=0.02, same day DQLI score r=0.83, p=0.0005, and clinical intervention r=0.83, p<0.05 with a score of 24 or greater showing 100% sensitivity and specificity for stepping up medical therapy.

CONCLUSIONS: Based upon these preliminary results the USA appears to be a valid tool for use in clinical care of Chronic Urticaria.

Utilization of Screening Laboratory Testing in Chronic Urticaria/Angioedema

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RATIONALE: The prevalence of chronic urticaria/angioedema (CUA) in the general population has been estimated to range from 0.5% to 5%. As part of the initial evaluation, targeted laboratory testing based on history or physical examination findings is appropriate. There have been few studies investigating how often these laboratory tests are abnormal and whether they impact management and patient outcomes.

METHODS: Retrospective analysis of patients with CUA who presented to a metropolitan Chicago allergy/immunology clinic from 2008 to 2013.

RESULTS: A total of 301 cases were included. Of these, 107 had urticaria (35.5%), 56 had angioedema (18.6%), and 138 had urticaria and angioedema (45.8%). Most patients were women (70%), the largest ethnic groups were African-American (43.1%) and Hispanic (39.5%), and the mean age was 34.4 ± 19.6 years. A total of 818 tests were ordered on 230 patients of which 147 tests were abnormal (18%) in 93 patients. The most common abnormalities seen were in complete blood counts (28 abnormal of 141 ordered or 19.9%), serum specific IgE for aeroallergens and foods (24 abnormal of 82 ordered or 29.3%), ESR (20 abnormal of 78 ordered or 25.6%), and comprehensive metabolic panels (16 abnormal of 102 ordered or 15.9%). None of the patients had a specific change in management based on their results. However, 8 patients (2.7%) were lost to follow-up after being referred to another provider for evaluation of their abnormal test results.

CONCLUSIONS: Laboratory testing in CUA patients is unlikely to lead to changes in management that result in improvement in patient outcomes.
The Significance of D-Dimer in Acute Urticaria-Angioedema at the Emergency Room

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RATIONALE: The complexity of the scenario at the Emergency Room (ER), where acute urticaria-angioedema syndrome (UA) represents about 0.7% of admissions, pushes to perform several blood analyses to assess clinical conditions of patients. D-dimer evaluation plays a critical role among these assays, and increased levels suggest the opportunity to rule out lung thromboembolism by expensive and even hazardous diagnostic investigation.

METHODS: 35 patients admitted to the ER of our Hospital with UA underwent physical examination including urticaria/angioedema activity score (UAS/AAS) and a panel of emergency blood analyses. None of them had co-morbidities known to affect fibrinolytic pathway. In all patients with D-dimer levels higher than normal (referral range 0-250 μg/L), a lower limbs venous ultrasonography was performed and they were re-evaluated after 72-96 hours from discharge for physical examination and D-dimer testing.

RESULTS: D-dimer levels were increased in 43% (15/35) of patients, with mean value 662 ± 470 SD μg/L (range 376-4210 μg/L). Although the correlation between D-dimer levels and UAS or AAS was not significant (p=0.5), all the patients with plasma concentrations >500 μg/L had elevated UAS or AAS. Lower limbs venous ultrasonography resulted negative and ruled out deep vein thrombosis in all of them. Paralleling clinical improvement after steroid and antihistamine therapy, D-dimer negative and ruled out deep venous thrombosis in all of them. Paralleling elevated UAS or AAS. Lower limbs venous ultrasonography resulted in all patients with UAS/AAS ≥150. The increase of D-dimer is restricted to the acute phase and is not predictive for deep venous thrombosis in urticaria-angioedema patients.

CONCLUSIONS: The increase of D-dimer is restricted to the acute phase and is not predictive for deep venous thrombosis in urticaria-angioedema patients.
433 Differential Characteristics of MPO-ANCA Positive and Negative Eosinophilic Granulomatosis with Polyangiitis
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RATIONALE: MPO-ANCA is detected in approximately 60% of patients with eosinophilic granulomatosis with polyangiitis (EGPA). We carried out a retrospective cohort study on patients in Tokyo metropolitan area to compare the clinical features between MPO-ANCA positive and negative EGPA.
METHODS: All the patients fulfilled the diagnostic criteria for EGPA of the Japanese research committee of intractable vasculitis. Certificated medical records for application of medical subsidy between 2007 and 2010 were reviewed.
RESULTS: A total of 131 patients were collected, aged 59.3 ± 15.9 years (Mean ± SD) (47 males, 84 females). MPO-ANCA was positive in 53 patients (40.4%). There were no significant differences in ages and gender between ANCA (+) patients and ANCA (-) patients. Arthritis and glomerulonephritis were more prevalent in ANCA (+) patients than in ANCA (-) patients (32.1% vs 17.9% [p=0.0612] and 24.5% vs 2.6% [p=0.0001], respectively). There were no significant differences in frequencies of other manifestations, including purpura, fever, body weight loss, gastrointestinal, heart and pulmonary involvement. Peripheral blood eosinophil counts and serum rheumatoid factors were significantly higher in ANCA (-) patients than in ANCA (+) patients, whereas there were no significant differences in white blood cell counts, platelet counts and serum IgE.
CONCLUSIONS: The results highlight the differential features between MPO-ANCA (+) and MPO-ANCA (-) EGPA, especially the higher prevalence of arthritis and glomerulonephritis in the former as well as the higher eosinophil counts and rheumatoid factors in the latter.

434 Direct and Indirect Economic Burden of Chronic Idiopathic/ Spontaneous Urticaria: An Analysis Based on Adult US Population
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RATIONALE: Economic burden of patients with chronic idiopathic/spontaneous urticaria (CIU/CSU) was assessed using patients currently treated for chronic hives as a proxy.
METHODS: Data were obtained from the US National Health and Wellness Survey (NHWS) in 2010-2012, representative of US adults in terms of age, sex, and ethnicity. Measures included labor force participation, work productivity and activity impairment questionnaire, and self-reported 6-month healthcare use. Cases (current use of a prescription for hives), according to survey year, sex, race, age and Charlson comorbidity index (CCI) and compared using t-test and chi-square.
RESULTS: Cases (n=253) and controls (n=1,012) did not significantly differ in terms of socio-demographic characteristics or mean CCI (all p>0.05). Labor force participation was similar for cases and controls (58.1% vs 62.2%, p=0.24). Cases had higher mean levels of absenteeism (12.64% vs 5.00%), presenteeism (32.71% vs 15.49%), overall work-impairment (37.32% vs 18.26%) and activity-impairment (45.93% vs 26.90%), (p<0.001) relative to controls. The mean number of healthcare provider visits in the prior 6 months was significantly (p=0.01) greater among cases than controls in terms of total visits (8.39 vs 5.03), as well as visits to specific providers, including GPs (1.72 vs 1.25), allergists (0.49 vs 0.07), and dermatologists (0.40 vs 0.16).
CONCLUSIONS: This analysis suggests a considerable economic burden on patients with CIU/CSU in terms of work productivity, activity impairment and use of healthcare resources compared to those without the condition sharing similar socio-demographic characteristics.

435 Experience in the Use of Social Media (whatsapp, e-Mail, facebook, website) By Patients
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RATIONALE: Internet is an opportunity to inform and connect patients and medical professionals. It is uncommon for patients the used of networking services for connect directly with medical professionals.
METHODS: In 2011, 5 allergists, currently 4, of different allergy Offices set up the website: www.alergia-vacunas.es. The website has a page with frequent asked questions (FAQ) on doubts allergen immunotherapy. Since then, all patients that will receive immunotherapy are informed about this website with e-mail and Facebook media contact. In addition, all patients undergoing desensitization to some foodstuffs or severe cases of allergy conditions (anaphylaxis, unstable asthma, venom allergy ...), has the application Watson of their allergist.
RESULTS: In three years, we have reported the existence of this website to about 2,400 patients. At the present time, the Facebook-group “alergia-vacunas” have 441 members. Currently we have a monthly average of 2,205 visits in website www.alergia-vacunas.es. However, only 29 e-mails from 6 patients, 6 chats in Watson application and 5 messages on Facebook monthly. None of these direct contacts have been inappropriate consults or offensive messengers.
CONCLUSIONS: The patients in our region used extensity the information media and only in exceptionally cases direct contact with the allergist. All of them have appropriated causes.

436 Anxiety and Depression in Patients with Allergic Disorders
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RATIONALE: To investigate anxiety and depressive symptoms in allergic patients could be beneficial because both allergic and mood disorders are most common chronic conditions in the world.
METHODS: Patients without known psychiatric disorders were consecutively recruited from out-patient allergy clinic at tertiary level at their first visit. Depression and anxiety symptoms were evaluated using Beck Depression Inventory (BDI) and Beck Anxiety Inventory (BAI) which both are validated in local language. Demographic and clinical features of the patients were recorded from original files.
RESULTS: A hundred and nine patients (mean age: 32.64±1.21, F/M: 83/26) were recruited. The education level was elementary in 44%, high school graduation in 37.9% and college graduation in 43.1%. Among them 32.1% was housewife, 24.8% was student and 43.1% was actively working. Half of them had history of allergic diseases in the first degree relatives and 2/3 of the patients had skin prick test positivity. The mean BDI and BAI scores were 13.23±0.86 and 13.72±1.02. BDI and BAI scores were in normal limits only in 40.4% and 37.6%, respectively. Both scores were significantly higher in housewives and patients with higher education levels. The lowest BDI (10.62±1.27) and BAI (9.42±2.18) scores were observed in patients with drug allergy. The highest BDI (18±11) and BAI (27.11±11) scores were determined in patients with food allergy (27.11±11).
CONCLUSIONS: Regarding high anxiety and depression scores in different allergic disorders, psychological assessment should be a considered as a part of personal management plan.
RATIONALITY: Allergists are consulted for unusual symptomatology. The allergist must understand basic pathophysiology to direct appropriate patient care and avoid potentially harmful interventions. We present a case that highlights this issue.

METHODS: Laboratory analysis and CT abdomen.

RESULTS: 61 year old male with a past medical history of multiple myeloma, hepatitis C with cirrhosis, and cardiovascular disease was admitted to the internal medicine service for infectious colitis and *E. coli* bacteraemia. Allergy/Immunology was consulted for possible acquired angioedema due to the patient’s CT abdomen showing marked bowel wall thickening in the large and small bowel. His labs were significant for low complement 4 (C4) and complement 3(C3). His admission history included abdominal pain that worsened over two days with associated fevers of 102 degrees F. He had complaints of episodic scrotal swelling for 1 year with duration of each episode being 15 minutes. The balance of the ROS was negative. There was no known family history of angioedema.

The primary team requested treatment for suspected acquired angioedema and wanted to administer C1 esterase inhibitor replacement therapy. During consultation, the complete evaluation for acquired angioedema was unremarkable with a C1 esterase inhibitor functional assay being 75%. Education was given to the primary service that C1 esterase inhibitor replacement was not indicated and carried a risk of increased thrombotic events. His complement levels were low because of liver cirrhosis.

CONCLUSIONS: Understanding complement pathophysiology is important for the specialist making recommendations.

440 Youtube Videos for Patient Education on How to Use Nasal Sprays Associated with Insufficient Reliability

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RATIONALIA: Nasal sprays are extremely important for delivery of intranasal medications for allergic rhinitis, however their effectiveness is dependent on proper technique. We hypothesized that there is a paucity of YouTube videos that effectively educate viewers on how to use nasal sprays.

METHODS: The website YouTube.com was queried for the phrase “how to use nasal spray”. The resulting videos were assessed whether they discussed the following 9 steps of nasal spray use: blowing the nose, removing cap, shaking device, priming, properly holding device, tilting head forward, spraying away from septum, gently inhaling or sniffing, exhaling through mouth. Videos were excluded if they lacked English words or text, contained repeated segments of previous videos, addressed non-allergic conditions, were non-educational advertisements, or did not address how to use nasal sprays.

RESULTS: Search phrase returned 7480 videos. The 60 videos on the first three pages of results were analyzed since prior studies have shown that patients are most likely to view these videos. Thirty-four videos were excluded based on criteria above. The remaining 26 videos averaged 133 seconds duration with 10,862 views. Only 2 videos (7.7%) discussed all 9 steps of nasal spray use. On average, most steps were discussed by videos from non-clinical healthcare groups (7.7 steps), followed by clinics and hospitals (3.5 steps), individual healthcare professionals (3.2 steps), and non-healthcare persons (2.5 steps).

CONCLUSIONS: Only 7.7% of videos discussed all steps of correct nasal spray use. Videos from non-clinical healthcare groups were most comprehensive. Creation of patient education videos authored by allergists should be encouraged.
Factors Driving Perceived Health Status Among Patients with Primary Immune Deficiency

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**RATIONALE:** Perceived health status (PHS) is a subjective measure of global health of individuals. While many studies have evaluated outcomes in patients with primary immune deficiency (PID), published literature evaluating PHS among patients with PID is sparse. We evaluated the largest self-reported database of patients with PID to determine the range of factors that may contribute to differences in PHS.

**METHODS:** Data from 2012 National Survey of Patients with Immune Deficiency Diseases conducted by the Immune Deficiency Foundation was studied. Multivariate logistic regression was employed for data analysis.

**RESULTS:** Patients with more than one permanent impairment, limited daily activity (LDA), endocrine problems, infection and emergency room visit history in the past 12 months, less than college degree, and ones who were employed were less likely to report excellent/very good health (EVGH) compared to good health (GH). Patients diagnosed with PID during hospitalization were more likely to report EVGH compared to GH. Patients with LDA, rheumatologic problems, and infection history in the past 12 months were more likely to report fair/poor health (FPH) compared to GH. Patients on immunoglobulin therapy were less likely to report fair/ poor health (FPH) compared to GH.

**CONCLUSIONS:** Our results emphasize the importance of PHS in clinical practice. We suggest that recognizing the factors that drive PHS in patients with PID is important for the development of disease prevention and health promotion programs, and delivery of appropriate health and social services to individuals with PID.

Changes in Health-Related Quality of Life in Patients with Primary Immunodeficiency Disorder (PIDD) Between Time of Diagnosis and 12 Months after Initiation of Immunoglobulin (Ig) Therapy

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**RATIONALE:** PIDD patients often suffer from multiple infections prior to initiation of immunoglobulin (Ig) treatment, which may negatively impact their HRQOL. This study focused on assessing changes in HRQOL among between time of diagnosis and 12 months after initiation of Ig therapy.

**METHODS:** This was a prospective study of newly diagnosed PIDD patients requiring Ig therapy. Patients were recruited from four centers in the US, one in the UK, and one in Brazil. HRQOL was assessed at baseline prior to first infusion, using the SF-36 for adults and the Pediatric Quality of Life Inventory (PedsQL) for children. The same instruments were used to measure HRQOL 12 months after diagnosis.

**RESULTS:** A total of 31 patients enrolled in the study and 25 completed the study. In adult patients (N=14), increases in the mean SF-36 Physical Component Score (PCS) and Mental Component Score (MCS) were observed between baseline and 12-month (36.9 vs. 43.2 and 46.0 vs. 49.0, respectively). Though not statistically significant, both were above or at the Minimally Important Difference (MID=2 for PCS and 3 for MCS). Statistically and clinically meaningful differences were observed in 3 subscales of the SF-36: General Health, Social Functioning, and Role-Physical. Changes in three additional domains (Physical Functioning, Bodily Pain, and Vitality) were above their respective MID. No differences were seen between baseline and 12 months in children.

**CONCLUSIONS:** Study findings demonstrate the value of diagnosis and Ig therapy in improving HRQOL in adult PIDD patients. Further study is warranted to understand the impact of Ig therapy on HRQQL in pediatric patients.

Association of Sports Activities and Rhinitis Symptoms in Schoolchildren Is Influenced By Comorbidities of Eczema

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**RATIONALE:** We have previously reported on the association of sports activities with rhinitis symptoms in schoolchildren. The purpose of this study was to further analyze this association.

**METHODS:** A questionnaire survey regarding histories of sports activities and allergic symptoms based on ISAAC were distributed to the parents of 9-year-old schoolchildren at every primary school in Ohmi-Hachiman City, Shiga Prefecture, Japan. Uni- and multivariate logistic regression models were developed to evaluate associations between sports activities and allergic symptoms.

**RESULTS:** Valid responses were obtained from 590 children (response rate, 78%). Children engaged in any kind of sporting activity showed a significantly higher prevalence of rhinitis symptoms (43.3%) than those not engaged (31.9%; adjusted odds ratio (OR), 1.60; 95% confidence interval (CI), 1.09-2.30, p=0.02), while no such difference was seen for other allergic symptoms. No association was found between the kind of sport (baseball, soccer, swimming, gymnastics, or others), the location of activities (outdoors or indoors), frequencies, and rhinitis symptoms. When background characteristics were compared between children with and without rhinitis symptoms in those with sports activities, children with rhinitis symptoms showed significantly higher comorbidities of eczema (23.5% vs. 7.8%; adjusted OR, 3.8; 95% CI, 2.0-7.2; p<0.0001). No such difference was observed in those without sports activities (18.8% vs. 12.4%; adjusted OR, 1.9; 95% CI, 0.8-4.6; p=0.14).

**CONCLUSIONS:** Children with eczema should be more aware of the occurrence of rhinitis symptoms when participating in sports activities. Enhanced epicutaneous sensitization of inhaled allergens through barrier-disrupted skin might contribute to this association.

Withdrawn
RESULTS: complete an online multiple-choice survey that assessed demographics of Ottawa, Ottawa, ON, Canada, 4Ottawa Hospital Research Institute; University of Ottawa Department of Epidemiology and Community Health, Division of Otolaryngology - Head and Neck Surgery, University of Calgary, Calgary, AB, Canada, 4Division of Otolaryngology-Head and Neck Surgery, The University of Ottawa, The Ottawa Hospital, Ottawa, ON, Canada.

RATIONAL: Chronic rhinosinusitis (CRS) is an inflammatory condition that is reported to affect 2-16% of the United States (US) population. Despite its rising prevalence, there is currently limited data in the literature evaluating the economic burden of this disease. The objective of this study was to determine the US direct healthcare costs for CRS treatment as estimated by the latest medical expenditure panel survey (MEPS).

METHODS: A prevalence-based approach was employed to measure cost of illness for CRS from the latest (2011) MEPS database using a 2 part model: 1) an estimated sum of all health care expenditures; 2) an attribution model for disease-specific estimation of expenditures.

RESULTS: The mean CRS-specific annual expenditure was $5211 [95% Confidence Interval (CI) $4056-$6366] by method 1, compared to $1040 (95% CI $802-$1227) by method 2. The highest contributor to expenditures for CRS patients was hospital inpatient expenses.

CONCLUSIONS: The authors established a range of estimates of the direct medical expenditures associated with CRS. The study demonstrated the economic burden attributable to this disease was an estimated 2.3 to 35 billion dollars in 2011 with a wide variation in the absolute direct expenditures tabulated dependent on the type of estimation model utilized and the prevalence assumed.

Assessment of Food Allergy Knowledge in NYC Elementary School Teachers

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RATIONAL: Pending New York State legislation allows New York City (NYC) schools to stock undesignated self-injectable epinephrine which may be administered by trained volunteer personnel, a role that could be assigned to a teacher.

METHODS: We contacted NYC public school principals to forward a survey link to 1st-5th grade teachers. Teachers could then anonymously complete an online multiple-choice survey that assessed demographics and their knowledge of food allergies and anaphylaxis treatment.

RESULTS: All respondents (n=15) were female with masters degrees. 67% had >11 years teaching experience. Most had at least 1 food allergic child in their classroom (11/15), and had personal experience with food allergies (14/15). 60% incorrectly defined food allergies, and 53% did not know the prevalence of childhood food allergies. Most (11/15) thought allergic reactions occurred in the cafeteria and incorrectly identified the common food allergens. The majority (80%) knew what self-injectable epinephrine was, but only 60% knew about anaphylaxis plans. None had ever administered epinephrine, and 14 of 15 stated that epinephrine was not readily available in the classroom. Most (87%) did not know how to store epinephrine devices, and 47% did not identify the appropriate injection site. While 93% selected the correct hypothetical situation in which to give epinephrine and 87% knew to call 911 after administering epinephrine, only 33% felt confident in using self-injectable epinephrine.

CONCLUSIONS: NYC teachers have deficient knowledge regarding food allergies and are not comfortable managing anaphylaxis. Further needs assessment would be useful in developing learning modules to educate teachers about food allergies and anaphylaxis.

Cooking Community Websites: A Possible Edudriver for Patients with Egg/Wheat Allergy

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RATIONAL: Cooking community webpage (CCW) is a social network for individual who has (not) suffered from food allergy. However, accessibility, readability, usability, and reliability 1,2) of CCW has never evaluated.

METHODS: 12 CCWs were stratified into three groups by their accessibility level-80, 81-90, 91-with the LIDA tool (Minervation Ltd., Oxford, UK) 1). Among intermediate accessibility group, a CCW was chosen to abstract recipes with search term ‘NO EGG’ and ‘NO WHEAT’. The first-50 recipes each were subjected to analyze the Robert Gunning Fog Index (GFI) to assess readability 1,2), Usability and Reliability were with LIDA tool 1). Average index were tested statistical difference with t-test as significant in p 0.05.

RESULTS: Allthecooks (R) 3), accessibility 83, was investigated in this study. GFIs in ‘NO EGG’ and ‘NO WHEAT’ were 7.12±1.12 and 7.08±1.03, respectively, both were within ideal level and had not significant difference. Usability could not integrate interrater difference. Reliability could not assess because all browsed CCWs except Cookpad (R) 4) did not 1) report a robust quality control procedure, 2) receive any expert opinion, 3) update regularly, and 4) cite relevant sources where appropriate.


The Efficacy of the Tokyo Metropolitan Food Allergy Emergency Manual

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RATIONAL: The death of a 11 year old student related to an anaphylactic reaction by school lunch in 2012 prompted the need for a practical and effective action plan for food allergy emergency at schools in Japan. The Tokyo Metropolitan Food Allergy Emergency Manual was developed in 2013 with this purpose, and is currently accepted in many areas. Our aim was to evaluate the efficacy of this manual by a scenario-based questionnaire.

METHODS: We conducted a survey among nursery and school personnel attending a food allergy education program held by the board of education of three cities in Tokyo. The questionnaire included three scenarios of children showing mild to severe allergic symptoms. We compared whether the action for each scenario taken by the respondents changed with the guide of the Tokyo Metropolitan Food Allergy Emergency Manual.

RESULTS: The questionnaire was answered by 241 attendants. For the scenario of a severe allergic reaction, the proportion of respondents choosing to use the epinephrine auto-injector changed from 66.5% with the guide of the manual (p=0.01). Taking oral medication for mild symptoms was chosen by 71.6% of the respondents without the manual, and 90.1% with the use of the manual (p<0.01). The skills of using the epinephrine auto-injector were correctly answered by 70.3% when using the explanation with photos as a reference.

CONCLUSIONS: The Tokyo Metropolitan Food Allergy Emergency Manual showed to be effective in guiding school personnel to choose the correct action for severe food allergic reactions in a scenario-based questionnaire.
CONCLUSIONS: Of 50 completed surveys, 94% of patients were referred to the allergy clinic by pediatricians for concern of food allergy. Self-injectable epinephrine was prescribed by that referring provider for 64% of patients. Of those prescribed epinephrine, 94% filled the prescription, and 69% were trained in its use. 18% of all referred patients were provided with an emergency action plan. The majority of patients who had undergone prior testing had been prescribed epinephrine. Of those prescribed epinephrine, 94% filled the prescription, and 69% were trained in its use. 18% of all referred patients were provided with an emergency action plan. The majority of patients who had undergone prior testing had been prescribed epinephrine. 18% of patients referred with testing had prior positive results. Of patients who did not have testing by the referring provider, 77% were prescribed epinephrine. RATIONALE: Food allergy management and diagnosis of food allergy have previously been evaluated by a physician and received management advice. However, the prior work-up as well as degree of education and instruction to family members varies widely.

METHODS: An anonymous survey was distributed to parents of children presenting to the pediatric allergy clinic for their initial specialist evaluation of suspected food allergy. The data was analyzed for trends in avoidance advice, epinephrine prescriptions, and allergy testing. Because the survey was anonymous without identifying information, it was exempt from IRB approval.

RESULTS: Of 50 completed surveys, 94% of patients were referred to the allergy clinic by pediatricians for concern of food allergy. Self-injectable epinephrine was prescribed by that referring provider for 64% of patients. Of those prescribed epinephrine, 94% filled the prescription, and 69% were trained in its use. 18% of all referred patients were provided with an emergency action plan, outlining steps for treatment in the case of an allergic reaction. Approximately 34% of patients had already undergone some form of allergy testing, the majority food-specific IgE levels, and all those referred with testing had prior positive results. Of patients who did not have testing by the referring provider, 77% were prescribed epinephrine.

CONCLUSIONS: The majority of referring physicians prescribed epinephrine for patients with suspected food allergy, but only a minority was concurrently provided with an emergency action plan. The majority of patients who had underwent prior testing had been prescribed epinephrine.

Understanding Risk-Taking Behavior in Adolescents with Food Allergy

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RATIONALE: The risk of a food allergy fatality from anaphylaxis is disproportionate among adolescents and young adults. Although there is an urgent need to understand food allergy risk-taking behavior in adolescents given an increase in both prevalence and severity of food allergy, limited research of risk-taking behaviors among food allergic adolescents exists.

METHODS: A short web-based survey regarding risk-taking behaviors is currently being administered to adolescents (14-22 years old) with food allergy. Participants are being recruited electronically through food allergy support groups and in-person at food allergy conferences and teen summits. The data collection period is from June to December 2014. Survey questions assess food allergy history, food allergy reactions, food allergy risks, food allergy support, general risk assessment, and demographics. No protected health or identifying information is being collected.

RESULTS: Data collection is currently under way. Preliminary results (N=80) indicate respondents are mostly female (70%), white (88%), with an average age of 16.6 years (SD=2.1). Regarding selected risk-taking behaviors analyzed, 1% of respondents do not believe their allergy is life-threatening. Additionally, 14% consume homemade foods not knowing what they contain. Furthermore, 8% of respondents do not carry injectable epinephrine, and 44% do not wear medical jewelry. Future analyses will include regression models to examine the association between risk-taking behavior and social support.

CONCLUSIONS: It is important to understand the risk-taking behavior of adolescents with food allergy in order to better support this critical population. Strategies aimed at identifying best practices for adolescents will be determined and formulated in recommendations for risk reduction and continued research.
Obesity has been associated with increased risk of asthma. However, little is known about the association of obesity to another atopic disease, allergic rhinitis (AR). We examined such link in U.S. children and adults.

**METHODS:** Cross-sectional study of obesity indicators and AR in 8,165 participants from the 2005–2006 National Health and Nutrition Examination Survey (NHANES), a representative sample of the non-institutionalized U.S. population. Measures of obesity included body mass index (BMI) and waist circumference (WC) z-scores. AR was defined as physician diagnosis of hay fever or allergies, plus nasal-ocular symptoms in the past year. Regression models were adjusted by age, sex, race and ethnicity, and household income.

**RESULTS:** 5,218 adults (51.8% female, mean age 45.5 yrs, range = 18-85 yrs) and 2,947 children (48.8% female, mean age 11.2 yrs, range = 5-17 yrs) were included in the analysis. The prevalence of AR was 21.3% in adults and 16.9% in children. Among adults, being overweight or obese (BMI > 30th percentile) was associated with increased odds of AR (odds ratio = 1.21 [95% confidence interval = 1.02-1.43], p = 0.03), as was central obesity defined by increased WC (OR = 1.31 [1.08-1.58], p = 0.005). Each 1-Kg/m2 increase in BMI was associated with -1.4% [0.6-2.1%] increase in the odds of AR (p < 0.001), and each 1.0 z-score increase in WC was associated with -9.6% [2.5-17.1%] increase in the odds of AR (p = 0.007). No significant association between obesity and AR was found in children.

**CONCLUSIONS:** In adults, being overweight or obese was associated with increased risk of AR. No such evidence was found among children in our study.

**Inadequate Recognition of Allergic Rhinitis (AR) By Resident Physicians in Children Hospitalized for Asthma**

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**Rationale:** The prevalence of AR among asthmatic children is estimated at 60% and uncontrolled AR is associated with poor asthma control. We hypothesized that this relationship is underappreciated by pediatric residents caring for hospitalized children with asthma.

**Methods:** A retrospective chart review of 450 patients, aged 5-16 years, discharged (2011-2013) from an inner city teaching hospital with a diagnosis of asthma. Data collected specific to AR included medical history, physical exam, admission/discharge diagnosis, and treatment.

**Results:** Of 450 study participants, 365 (81.1%) were 5-11 years, 85 (18.9%) were 12-16 years; 41.6% were female. 119 (26.4%) were diagnosed with AR on admission, 80 (17.8%) diagnosed at discharge (p = 0.003). 164/450 (36.4%) had asthma severity classification noted at admission. 434 (96.4%) had documented number of prior hospitalizations, a measure of asthma severity. AR diagnosis on admission was reported in 59/210 (28.1%), 44/173 (25.4%) and 14/51 (27.5%) with 0, 1-4 and ≥5 prior hospitalizations, respectively. AR diagnosis on discharge was reported in 46/210 (21.9%), 26/173 (15%) and 8/51 (15.7%) with 0, 1-4, and ≥5 prior hospitalizations, respectively. Asthma severity did not influence admission or discharge AR recognition (p = 0.619). 8.4% received intranasal corticosteroids.

**Conclusions:** The diagnosis of AR was unexpectedly lower than published norms for children with asthma, and there was statistically significant decrease in recognition from admission to discharge. Our study demonstrates that residents underestimate the relationship between AR and asthma and inadequately diagnose and treat AR in hospitalized children with asthma. If confirmed, these findings suggest the need for alternative approaches to integrate AR management into care of hospitalized asthmatics.

**Study for Assessing Prevalence of Local Allergic Rhinitis Among Rhinitis Patients**

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**Rationale:** Diagnosis of allergic rhinitis is based on clinical manifestations and a positive result for skin prick test or serum immunoglobulin E to aeroallergens. A condition involves a localized nasal allergic response in the absence of systemic atopy was identified as local allergic rhinitis (LAR). Our objective was to investigate the prevalence of local allergic rhinitis in patients comes with clinical manifestations of rhinitis.

**Methods:** A cross-sectional study was conducted on 200 rhinitis patients recruited from Allergy and ENT clinics at Ain Shams University Hospitals. Allergic history, clinical examination, skin Prick test and serum total IgE all were done. Allergic rhinitis was diagnosed and classified according to the criteria set out by (ARIA) guidelines 2001. Nasal allergen provocation test (NAPT) and nasal specific IgE (sIgE) were done in patients with negative skin prick test and normal serum total IgE.

**Results:** Eighty (n = 160) of the rhinitis patients showed positive skin prick test and high serum total IgE, and these were considered allergic rhinitis patients (AR) with systemic atopy. For the remaining 20% (n = 40); positive nasal allergen provocation (NAPT) test was obtained in 62.5% (25/40) of them, which represent local allergic rhinitis (LAR) patients while 37.5% (15/40) were negative to NAPT which represent non allergic rhinitis (NAR) patients. Also nasal specific IgE was positive in 16/40 patients.

**Conclusions:** LAR is a newly described type of rhinitis, a common among rhinitis patients in Egypt but still misdiagnosed. It can affect patients previously diagnosed as non-allergic rhinitis.

**Coexistence of Dual Systemic Allergic Rhinitis and Local Allergic Rhinitis**

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**Rationale:** In this study we investigated if seasonal systemic allergic rhinitis (SAR) induced by pollens and local allergic rhinitis (LAR) induced by perennial allergens may occur in the same patient.

**Methods:** Twenty-nine patients with seasonal SAR and positive SPT to grass and/or olive pollen with well-defined symptoms during pollen season in addition to nasal symptoms throughout the year were evaluated. Clinical questionnaires, skin tests, serum specific IgE, nasal levels of tryptase and eosinophil cationic protein, and nasal allergen provocation test (NAPT) with grass, olive, *D. pteronyssinus* (DP), and *Alternaria alternata* (AA) were performed.

**Results:** The coexistence of dual SAR-LAR was confirmed in 23 patients by NAPT (82.1%) with positive response to AA in 9 patients (22%), to DP in 19 (46.3%), and to both AA and DP in 13 (31.7%). A total concordance between SPT and NAPT results was obtained. The SPT and NAPT with seasonal pollens were positive to grass in 11 patients (26.8%), to olive in 20 (48.8%), and to both grass and olive in 11 (26.8%). No significant differences between seasonal and perennial allergens threshold concentrations were observed. The 56.5% of patients reported a seasonal onset of nasal symptom followed by perennial symptoms in the next years. The 30.4% a perennial onset of symptoms with a clear spring worsening, and the 13% could not remember the onset of the disease.

**Conclusions:** These results demonstrate the coexistence of perennial LAR in patients with seasonal SAR who developing symptoms throughout the year has negative SPT should be explored.
A Multicentre Cross-Sectional Survey of Allergic Sensitisation to Subtropical and Temperate Grass Pollens
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RATIONALE: Grass pollens (GP) are major triggers of allergic rhinitis and asthma but allergic sensitisation to pollen of subtropical (Panicoideae and Chloridoideae) and temperate (Pooidaeae) species in patients from diverse biogeographical regions is not well understood.

METHODS: Subjects (non-atopic, n = 31, other allergies; n = 42) and patients with allergic rhinitis (n = 321) were recruited at specialist centres in Queensland (subtropical), Adelaide, Perth and Sydney (temperate). Clinical history and skin prick test (SPT) to GP extracts were assessed. Serum total and specific IgE to GP extracts and biotinylated allergen component-streptavidin ImmunoCAPs, were measured. Subjects with prior GP immunotherapy were excluded. Data was analysed by non-parametric tests.

RESULTS: GP-allergic patients from Queensland showed higher SPT and IgE to Bahia and Bermuda GP as well as Paspalum and Cyperus than Ryegrass pollen and Lol p 1. In contrast, patients from Adelaide and Sydney showed higher SPT and IgE to Ryegrass than Bermuda and Johnson GP. In Perth, SPT to Ryegrass was higher than Johnson GP but IgE with Ryegrass was higher than both Johnson and Bermuda GP. Sensitivity to Bahia GP did not differ from Ryegrass in patients from Adelaide, Sydney or Perth. However, IgE to Lol p 1 was higher than IgE to subtropical group 1 allergens in patients from Adelaide, Sydney and Perth.

CONCLUSIONS: Patients with GP allergy show significant differences in levels of allergic sensitivity with subtropical and temperate GP depending on biogeographical region. Primary sensitisation to different types of grasses should be considered in choice of GP immunotherapy.

Distinctive Prevalence of Allergic Rhinitis Among Adults in Urban and Rural Areas of China: A Population-Based Cross-Sectional Survey
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RATIONALE: The aim was to compare the prevalence of self-reported and confirmable allergic rhinitis (AR) with both positive in questionnaires and skin prick tests (SPTs) among adults living in urban and rural areas of China.

METHODS: Adults from a community in Beijing and a village in Baoding were selected as representative urban and rural dwellers, respectively. All eligible residents were enrolled from the population register and received a face-to-face interview using modified validated questionnaires. The AR-positive and AR-negative participants who responded to the questionnaires were randomly selected to be investigated using SPTs.

RESULTS: 803 participants in rural area and 1499 participants in urban area completed the questionnaires, whose response rates were 75.9% and 81.5%, respectively. The prevalence of self-reported AR of rural area (19.1%) was significantly higher than that of urban area (13.5%). The elementary school of education level increased the risk of having AR (adjusted OR = 2.198, 95% CI = 1.072-2.236), whereas the low, below-moderate and above-moderate yearly income were the significant protecting factors for AR (adjusted OR = 0.551, 95% CI = 0.377-0.805; adjusted OR = 0.495, 95% CI = 0.306-0.799; adjusted OR = 0.489, 95% CI = 0.275-0.868, respectively). The positive SPT rates among the subjects with self-reported AR between rural and urban areas were 32.5% and 53.3%, which led to the prevalence of confirmable AR being 6.2% and 7.2% in the rural and urban adults, respectively.

CONCLUSIONS: the prevalence of confirmable AR is similar between rural and urban areas in China, although there is higher prevalence of self-reported AR in the former.

The Causes and Clinical Features of Chronic Cough in School-Age Children in China
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RATIONALE: To investigate causes and clinical features of chronic cough of school-age children in China.

METHODS: The outpatients in Children’s Hospital Affiliated to Soochow University from March 2012 to December 2013 newly diagnosed with chronic cough who cough >4 weeks, have no obvious signs of lung and no abnormal chest radiograph are enrolled in this study. The sputum EOS count, airway provocation test (BPT), skin allergy testing (SPT) , sinus CT scan and 24 hours esophageal pH measurement. were done at beginning in all patients. The cough symptom score and visual analog integrator (VAS) also were done ,then made the preliminary diagnosis and gave appropriate treatment to patients. The efficacy of treatments were evaluated after 2 week and the induced sputum cell counts, pulmonary function, airway challenge test were done after 4 weeks and made final causes diagnosis. of children with chronic cough in China.

RESULTS: The 118 children (aged 6 to16 years old) with chronic cough, who were enrolled. Single cause: Upper Airway Cough Syndrome (UACS), Cough Variant Asthma (CVA), Post Infection Cough (PIC) , Tourette (Tic) ,Gastroesophageal Reflux Cough (GERC) and Eosinophilic Bronchitis(EB) accounts for 31.35% (37/118), 14.41%(17/118), 10.17% (12/118), 2.54%(3/118), 1.70%(2/118), 1.70%(2/118). Multiple causes:38.18%(45/118 ),among them, CVA + UACS in 37 cases , others in 8 cases.

CONCLUSIONS: The commonest cause of chronic cough in School-age children were UACS, CVA ,PIC . The GERC and EB in children are less than them in adult. Multiple causes are common in chronic cough of children in China.
460 Initial Evidence of Sustained Efficacy of House Dust Mite Synthetic Peptide Immuno-Regulatory Epitopes 2 Years after a Short Course of Treatment in House Dust Mite (HDM) Allergic Subjects

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RATIONALE: House Dust Mite Synthetic Peptide Immuno-Regulatory Epitopes (HDM-SPIRE) has previously been shown to significantly reduce rhinoconjunctivitis symptom scores in house dust mite (HDM) allergic individuals one year after a short course (4 doses in 12 weeks) of treatment. In this study, subjects returned to evaluate continued efficacy two years after the start of treatment.

METHODS: 72 of the 116 subjects who had previously participated in the one year randomised, double-blind, placebo-controlled study underwent exposure to HDM allergen in an exposure chamber ~2 years after starting a short course (4 or 11 doses) of HDM-SPIRE or placebo. No further drug was administered. Symptom scores were recorded during the 4-hour exposure period on three consecutive days and were compared to time-matched symptom scores from a baseline (pre-dosing) assessment.

RESULTS: The mean reduction in symptom scores over the 3 days (pre-specified endpoint) in subjects receiving 4 doses of HDM-SPIRE (-6.49 ±4.28) was greater than the reduction in placebo subjects (-4.70 ±3.71). The 4 dose regimen out-performed the 11 dose regimen in this analysis. Subjects with more severe symptoms at baseline had an even greater reduction in symptom scores in the 4 dose HDM-SPIRE group (-7.59 ±4.10) than the placebo group (-4.92 ±4.09).

CONCLUSIONS: Despite a marked placebo effect, 4 doses of HDM-SPIRE 12 nmol was associated with a clear trend towards persistence of efficacy 2 years after dosing. This persistence of effect should be confirmed in larger studies. HDM-SPIRE is a potentially exciting new treatment for HDM allergy.

461 Safety of House Dust Mite Synthetic Peptide Immuno-Regulatory Epitopes in Patients with House Dust Mite Allergy and Controlled Asthma

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RATIONALE: House Dust Mite Synthetic Peptide Immuno-Regulatory Epitopes (HDM-SPIRE) has previously been shown to significantly reduce rhinoconjunctivitis symptom scores one year after a short course (4 doses) of treatment. In this study, a preliminary assessment of the safety of HDM-SPIRE was made in subjects with controlled asthma.

METHODS: Patients with House Dust Mite (HDM) allergic rhinoconjunctivitis and GINA step 1 (N=17) or GINA 2 (N=13) controlled asthma were randomised to receive 4 doses of HDM-SPIRE 12-nmol or matching placebo by intradermal injection at 4-weekly intervals. Lung function was assessed for 8-hours after the first dose and 1-hour after subsequent doses. Other assessments included the Asthma Control Questionnaire (ACQ) adverse events, vital signs, safety laboratory tests and breathlessness scores.

RESULTS: HDM-SPIRE was well tolerated with no treatment related adverse events (AE). Mean FEV1 values were similar in the two treatment groups both before and after dosing. No subject experienced a reduction of 20% or greater in either FEV1 or PEFR. ACQ scores were similar in the HDM-SPIRE and placebo groups. One placebo-treated subject experienced an AE of asthma exacerbation and one HDM-SPIRE subject had a change in ACQ status from controlled to partially controlled. Breathlessness scores were low in both treatment groups before dosing and were lower still after dosing. There were no other safety findings.

CONCLUSIONS: In this preliminary assessment in subjects with controlled asthma, HDM-SPIRE was found to be well tolerated with no deleterious effect on asthma symptoms or control. The safety and efficacy of HDM-SPIRE should be further evaluated in asthma subjects.

462 The Nasal Allergen Challenge Protocol of the Allergic Rhinitis Clinical Investigator Collaborative (AR-CIC): Validation in a Clinical Trial of Cat Synthetic Peptide Immunoregulatory Epitopes (Cat-SPIRE)

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RATIONALE: Cat-PAD is the first in a new class of synthetic peptide immunoregulatory epitopes (SPIREs), derived from Fel d 1, being developed for the treatment of cat allergy. We aimed to clinically validate the nasal allergen challenge (NAC) protocol utilized in the Allergic Rhinitis Clinical Investigator Collaborative (AR-CIC) in a study evaluating biomarkers of efficacy for Cat-PAD.

METHODS: An open-label study of 20 cat-allergic participants across 2 centres in Canada, with regular exposure to cats. Participants were challenged intra-nasally with escalating (4-fold) increments of cat allergen until a Total Nasal Symptom Score (TNSS) of 8/12 and a Peak Nasal Inspiratory Flow (PNIF) reduction of ≥50% were reached. This allergen concentration was then re-administered in a pre-treatment NAC visit, with TNSS and PNIF recorded at baseline, 15min, 30min, 1hr, and hourly up to 12hrs post-NAC. 4 x 6nmol injections of Cat-PAD were administered q1wk. Follow-up NAC was conducted 1 month post-treatment.

RESULTS: Following treatment, post-NAC TNSS was significantly reduced compared to pre-treatment at 15min (p<0.01), 30min (p<0.05), 1hr (p<0.01), 2hrs (p<0.05), and 4hrs (p<0.05). Maximal PNIF values following NAC were significantly higher (i.e. better) at 2hrs (p<0.01) and 4hrs (p<0.05) compared post to pre-treatment NAC visits. The increase in TNSS compared to baseline following NAC was significantly less post treatment than pre-treatment from 2hrs to 4hrs post-NAC.

CONCLUSIONS: This study supports the validity of the AR-CIC protocol for testing the efficacy of novel therapeutics. 1 Cat-PAD, the first in a new class of SPIREs, is effective in reducing allergic rhinitis symptoms following NAC with cat allergen.
643 Sustained Efficacy of Allert Allergy Vaccine after a Second Birch Pollen Season: A Phase IIb
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RATIONALE: Allert™ (Anergis SA, Switzerland), based on three contiguous overlapping peptides (COPs) derived from Betv1, was successfully administered according to an 2-month immunotherapy regimen to patients with birch pollen allergic rhinoconjunctivitis in a placebo-controlled, double-blind, randomized, multicenter, phase IIb study (AN004T, 2013), and reached efficacy and safety endpoints. The aim of the current study (AN005T) was to assess the efficacy of Allert™ during a second follow up seasonal exposure without additional treatment.

METHODS: 196 patients out of the 239 patients from AN004T study, including 3 arms (placebo, 50µg and 100µg COPs in Aluminum Hydroxide), were enrolled into the follow up study AN005T during the 2014 birch pollen season. Efficacy was evaluated using the combined Rhinoconjunctivitis Symptom and Medication Score (RSMS) as primary endpoint as well as quality of life assessment and other secondary endpoints.

RESULTS: According to per protocol analysis, LS Mean RSMS was improved by 21% with Allert 50µg and 18% with Allert 100µg (Wilcoxon: p=0.02 and p=0.07, respectively). Both Allert 50µg and 100µg doses were associated with similar improvements in quality of life (Mini-RQLQ: 21% and 20%; p=0.03 and p=0.05, respectively). Night-time Nasal Symptom Score (NNSS) was improved by 30 and 39% (p=0.014 and p=0.003, respectively).

CONCLUSIONS: Allert™ was previously found to be safe, well tolerated and efficacious during the first birch pollen season. This follow up study during a second seasonal exposure shows sustained efficacy in improving RSMS, Mini-RQLQ and NNSS, supporting a long term effect of an ultra-fast immunotherapy formulation based on a mixture of COPs derived from Betv1.

644 Lamp-Based DNA Vaccine for Japanese Red Cedar Allergy
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RATIONALE: Allergies caused by Japanese Red Cedar (JRC) pollen affect up to 45% of Japanese and effective therapeutics is desired. ITTs JRC-LAMP-Vax technology utilizes the lysosomal targeting property of Lysosomal-associated membrane protein (LAMP) to enhance the MHC class II presentation and CD4 T cell responses to the target proteins, such as the immuno-dominant allergens Cry J1 or J2 from the JRC pollen.

METHODS: Cry J1 or J2 DNA sequence were fused with the LAMP sequence. Plasmid DNAs were administered to Balb/c mice by intramuscular, intradermal, or by Biovector ID delivery. We first compared the efficacy of such administration routes by testing Cry J1 or J2 specific IgG and IgE antibodies. Then, we isolated CD4+ or CD8+ T cells from immunized mice and adoptively transferred them into naive mice followed by Cry J1/J2 challenges.

RESULTS: First, we demonstrated that Cry J1-LAMP and Cry J2-LAMP immunized mice produced significantly higher titers of anti-Cry J1/J2 IgG2a antibody (Th1 type) than the Th2 type IgG1 (P < 0.05). Cry J1/J2 specific IgE levels in vaccinated mice were lower than those of the controls (P<0.05). In addition, we showed that the Bioject delivery is the best route in terms of maintenance and degree of IgG2a antibody production. Finally, we demonstrated that transfer of CD4+, but not CD8+, T cells from vaccinated mice protected recipients upon Cry J1/J2 challenge.

CONCLUSIONS: Our results suggest that the JRC-LAMP-Vax vaccine is an effective therapeutics for JRC induced allergy in our mouse model. The DNA vaccine protects animals through the mechanism of Th1/Th2 skewing.

645 Persistence of Elevated Anti-Bet v 1 IgG4 Prior and during the Second Pollen Season after Allert Ultra-Fast Immunotherapy; Results from a Phase IIb Study Follow up
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RATIONALE: Allert™, a mix of three Contiguous Overlapping Peptides (COPs) derived from the major birch pollen allergen Bet v 1, was administered to allergic volunteers in a phase IIb study. Subjects received 5 subcutaneous injections within 2 months and showed an improvement in rhinoconjunctivitis symptoms and medication scores during the first and second birch pollen seasons. The present study shows the levels of allergen specific immunoglobulins in patients followed through the second pollen season without treatment.

METHODS: Blood was collected before, at peak and after the 2014 birch pollen season. Anti-Bet v 1 IgG4 and IgE were quantified by ELISA.

RESULTS: AllertT administration had been previously shown to significantly increase Bet v 1 specific IgG4 by around 20 fold compared to placebo in 2013. Prior to the second pollen season, anti-Bet v 1 IgG4 levels remained significantly elevated compared to placebo. Specific IgG4 further rose during the pollen season reaching about 4 fold pretreatment level. No difference was observed in anti-Bet v 1 IgE levels between placebo and treated groups.

CONCLUSIONS: Ultra fast immunotherapy with Allert, shown to be efficacious in both first and second pollen seasons, induces a persistent elevated anti-Bet v 1 IgG4 response even one year after administration. No major changes in anti-Bet v 1 IgE levels were observed in the treated groups except for a slight seasonal increase also observed in the placebo group. Persistent IgG4 response indicates a potential long term effect of Allert treatment, coherent with previous phase I/IIa immunological results.

646 Epigenetic Changes Following Epicutaneous Immunotherapy in Peanut Sensitized Mice
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RATIONALE: Epicutaneous immunotherapy (EPIT) is a promising route for treating food allergies and animal models show the sustainability of the protection. We investigated in peanut-sensitized mice the implementation of epigenetic mechanisms underlying this long-lasting therapeutic effect.

METHODS: Mice were orally sensitized to peanut and then treated by EPIT or sham. Mice were sacrificed every 2 weeks during EPIT and also 8 weeks after the end of EPIT. DNA methylation was analysed in spleen and blood samples by restrictive enzyme digestion and quantitative-PCR and in sorted CD4, CD8 and CD19 cells from spleen and blood by pyrosequencing.

RESULTS: In splenocytes, a significant hypermethylation of the Gata3 CpG islands was induced by EPIT versus Sham, starting from the 4th of treatment (p<0.05). This hypermethylation was sustained after the end of EPIT. In circulating blood cells, the hypermethylation in the Gata3 CpG islands was observed only at the 8th week of EPIT (vs Sham, p<0.05). In spleen and blood CD4 cells, a significant hypermethylation for CpG island of Gata3 was observed from the 4th week of EPIT and persisted following the end of treatment. In parallel, a significant hypomethylation was obtained in the Foxp3 CpG island in spleen and blood CD4 cells from the 4th week of EPIT compared to Sham, persisting after the end of treatment. No modification was observed for the Thet transcription factor in whole or in sorted T and B cells sorted from spleen and blood.

CONCLUSIONS: Epigenetic modifications of the DNA methylation of Th2 and Treg transcription factor appears a major trait of EPIT induced immunomodulation.
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467 Epicutaneous Immunotherapy Prevents from Induction of Anaphylaxis to Further Allergens
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RATIONALE: We have previously established in sensitized mice that epicutaneous immunotherapy (EPIT) protects against sensitizations to other allergens. The aim of this study was to evaluate this protection against anaphylaxis.

METHODS: After sensitization to milk, mice were treated by EPIT or Sham, and 2 weeks later, submitted to a peanut-sensitization procedure. Mice were then intravenously challenged with peanut. In a second experiment, CD4+CD25+T cells were isolated from spleen of milk-sensitized mice after EPIT or Sham, and transferred into naive mice. Recipient mice were submitted to peanut sensitization and intravenously challenged with peanut. In a third experiment, Tregs were obtained from Foxp3+gfp mice sensitized to peanut and EPIT-treated, then adoptively transferred. Recipient mice were submitted or not to peanut sensitization before transfer, then all sensitized and IV challenged to ovalbumin. Outcome tests were rectal temperature, hypersensitivity reactions and blood mouse mast cell protease-1 (mMCP1).

RESULTS: After IV administration of peanut, the Sham group exhibited a significant drop in temperature (p<0.001), severe systemic score (grades 2 to 4), and a significant increase in blood mMCP1. Milk-EPIT treated mice did not developed drop in temperature after peanut sensitization and IV challenge. Blood mMCP1 was decreased compared to Sham (p<0.05). The adoptive transfer of milk EPIT-induced Tregs to naive mice protected from sensitization to peanuts and prevented from the induction of anaphylaxis (p<0.05). A partial protection was obtained by the transfer of Foxp3-Tregs and IV challenge to ovalbumin either in naive and previously peanut-sensitized recipient mice.

CONCLUSIONS: Allergen-specific EPIT protects against sensitization to other allergens via a Treg mechanism.

468 Virus Detection and Cytokine Profile in Relation to Age Among Acute Exacerbations of Childhood Wheezing
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RATIONALE: Little information is available regarding eosinophil activation and cytokine profiles in relation to age in virus-induced bronchial asthma. We therefore explored the association between age, respiratory viruses, serum eosinophil cationic protein (ECP), and cytokines/chemokines in acute exacerbations of childhood wheezing/asthma.

METHODS: We investigated viruses in nasal secretions from 88 patients with acute exacerbation of childhood wheezing by using antigen detection kits and/or RT-PCR, followed by direct DNA sequencing analysis. We also measured peripheral eosinophil counts, and the serum levels of ECP and 27 types of cytokines/chemokines in 71 virus-induced acute wheezing cases and 13 controls.

RESULTS: Viruses were detected in 71 (80.7%) of the 88 samples. The three major viruses detected were rhinoviruses, RS viruses, and enteroviruses; enteroviruses were found to be dominant in patients aged ≥3 years. There was no change in the levels of rhinoviruses and RS viruses between the two age groups, defined as children aged <3 years and children aged ≥3 years. Serum concentrations of ECP, IL-5, and IP-10 were significantly elevated in virus-induced acute wheezing cases compared with controls. Serum ECP values were significantly higher in patients with virus-induced wheezing at age ≥3 years compared with those aged <3 years. Among the 27 cytokines/chemokines, serum IP-10 was significantly higher in virus-induced wheezing in patients <3 years than in those ≥3 years. Serum ECP and IL-5 production correlated significantly with age, whereas serum IP-10 showed an inverse correlation with age.

CONCLUSIONS: Age-related differences in cytokine profiles and eosinophil activation may be related to virus-induced acute exacerbations of childhood wheezing/asthma.

469 (1) Production of CCR4-Binding Chemokines in Response to Rhinovirus Infections in Asthmatic Children
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RATIONALE: CC Chemokine Receptor 4 (CCR4) is expressed by Th2 cells, regulatory T cells, eosinophils and mast cells and acts to direct their migration to inflammatory sites along MDC (CCL22) and TARC (CCL17) gradients, which facilitate Th2-mediated inflammation. The relationship between MDC and TARC produced during rhinovirus (RV)-induced asthma attacks has yet to be determined.

METHODS: Nasal washes (NWs) from 287 children (ages 7-12) enrolled in the Emergency Room at the Hospital Nacional de Niños in Costa Rica were evaluated using single-plex magnetic bead assays for MDC, and TARC in a subset of 87 children (EMD Millipore Corp.). The subjects included wheezing children (W) with or without positive tests for RV (RV+ or RV-; n = 58 and 38, respectively), stable asthmatics (SA; n = 65) and non-asthmatic controls (C; n = 126).

RESULTS: MDC levels in NWs were significantly higher in asthmatic children than controls: W (77 pg/mL) vs C (25 pg/mL), p<0.0001, and SA (38 pg/mL) vs C (25 pg/mL), p = 0.01, but were highest among the wheezing children infected with RV (W RV+ 96 pg/mL vs. W RV- 54 pg/mL, p = 0.003). Although TARC levels were also higher in NWs from the asthmatic children than controls (W 3.3 pg/mL vs C 1.8 pg/mL, p = 0.02; SA 3.4 pg/mL vs C 1.8 pg/mL, p = 0.01), there was no statistical difference between W RV+ and W RV- (3.4 pg/mL and 3.1 pg/mL, respectively, p = 0.64).

CONCLUSIONS: While they share the CCR4 receptor and both chemokines correlate with Th2-mediated disease processes, MDC appears to be differentially affected by RV whereas TARC does not.
CD4 T Cell Chemotaxis to CCL28 Requires Proper Chemokine Tertiary Structure, but Is Not Species Restricted

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RATIONALE: We previously demonstrated that post-viral atopic disease depends upon production of CCL28, a chemokine that is chemotactic for CD4 T cells via CCR3 and CCR10. Chemokines generally demonstrate cross-species conservation, particularly those of mouse and human origin, and usually require proper conformation to have biologic function. We hypothesized that the native form of human CCL28 would be capable of driving mouse Th2 cell chemotaxis, but that the unfolded form of the chemokine would be unable to do so.

METHODS: CD4 T cells isolated by positive immunomagnetic selection from C57BL6 mouse spleens were placed in the upper chamber of a 24 well Transwell system, with folded or unfolded recombinant human CCL28 (0, 1, 3, 10, or 30µg/ml) in the upper or lower chamber (checkerboard assay). After 3 hours, the number of cells that migrated to the lower chamber in each well was determined by flow cytometry. The effect of chemokinesis, or random cell movement, was subtracted from movement by chemotaxis to isolate cells moving in response to the chemokine.

RESULTS: Recombinant human CCL28 induced significant migration of mouse CD4 T cells at 3 and 10µg/ml (each p<0.05; n=3-4). Unfolded recombinant human CCL28 was not able to cause directed cell CD4 T cell movement at any of the tested concentrations (n=3-4).

CONCLUSIONS: Human CCL28 is capable of driving mouse CD4 T cell chemotaxis, and this effect requires proper chemokine conformation. These data suggest that a mouse migration assay could be used as a screening test for compounds that have anti-CCL28 activity as potential human therapeutics.

472 Double-Stranded RNA Stimulates TLR3-Dependent Upregulation of IL-33 Transcript and Protein in Pulmonary Microvascular Endothelial Cells

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RATIONALE: IL-33, an IL-1 family cytokine, has attracted attention as a critical cytokine in the development of allergic diseases because of its potent ability to induce type 2 immunity and its strong genetic association with asthma. IL-33 has been reported to be preferentially and constitutively expressed in the nucleus of such tissue structural cells as epithelial and endothelial cells, and it is released by necrotic tissue cells after tissue injury and/or trauma as a damage-associated molecular pattern. However, the precise mechanisms of IL-33 synthesis in tissue cells are still largely unknown. To clarify the molecular mechanisms of IL-33 synthesis in tissue cells.

METHODS: Human microvascular endothelial cells from the lung (HMVEC-L) and normal human bronchial epithelial cells (NHBE) were stimulated with various proinflammatory cytokines and toll-like receptor (TLR) agonists. IL-33 mRNA and protein were determined by qPCR, ELISA and Western blotting. Gene silencing of TLR3 was performed using specific siRNA.

RESULTS: Among the stimulants tested, only polynosinic-polycytidylic acid (poly I:C), a synthetic analog of viral double-stranded RNA, dramatically increased the levels of mRNA, release and nuclear expression of IL-33 in HMVEC-L. None of the stimulants increased IL-33 expression in NHBE. Gene silencing of TLR3 significantly inhibited poly I:C-induced IL-33 expression in HMVEC-L.

CONCLUSIONS: This study showed that IL-33 synthesis and release from pulmonary microvascular endothelial cells can be regulated by TLR3 activation. Our findings suggest that severe respiratory tract viral infections reaching to the microvascularity may cause robust augmentation of endothelial-derived IL-33, leading to development of asthma.
**474 The RNA-Binding Protein HuR Regulates CD4+ T Cell Differentiation and Is Required for Normal IL-2 Homeostasis and Allergic Airway Inflammation**  
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**RATIONALE:** The RNA binding protein (RBP), HuR, (elavl1) has been shown to posttranscriptionally regulate many cytokines and chemokines including Th2/Th17 differentiation via increased stabilization of key mRNA targets. HuR CD4+ T cells from over-expressing transgenic mice had significant increases in Th2 cytokines. We hypothesized HuR regulates CD4+ T cell differentiation and is required for normal IL-2 homeostasis.  
**METHODS:** We made novel HuR KO models to study HuR during CD4+ T cell activation. We used distal lck-cre-HuRKO mice to investigate HuR KO in T cell differentiation and allergic airway inflammation. YFP identifies cells which have ablated HuR.  
**RESULTS:** Activated YFP+ CD4+ T HuR KO cells had decreased Gata-3 and Th2 cytokines and could not shut off IL-2 (increased 30-fold mRNA and 700% protein). YFP+ HuR KO T cells had defects in proliferation, mRNA targets. HuR CD4+ T cells from over-expressing transgenic mice including Th2/Th17 differentiation via increased stabilization of key mRNA targets. HuR CD4+ T cells from over-expressing transgenic mice had significant increases in Th2 cytokines. We hypothesized HuR regulates CD4+ T cell differentiation and is required for normal IL-2 homeostasis.  
**CONCLUSIONS:** These data suggest that HuR plays a major role in CD4+ T cell differentiation and normal IL-2 homeostasis by controlling expression of CD25 and other key regulators of T cell differentiation, particularly during the allergy season.

**475 A Critical Role for IL4 in the Neonate after Exposure to Aerosolized Ovalbumin in a Murine Model of Allergen Sensitization**  
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**RATIONALE:** Epidemiological studies have suggested that childhood exposure to allergens can lead to a Th2 response culminating in allergic asthma. We hypothesized that blocking the activity of IL4 with antibodies during the time of neonatal exposure to allergens can significantly reduce the risk of allergic sensitization at adulthood.  
**METHODS:** On day 0, neonatal BALB/c mice were exposed to a 1% aerosolized ovalbumin (OVA) for 20 mins while the controls received aerosolized PBS. One group from the OVA exposed mice received 0.5 mg of anti-IL4 also on day 0. At adulthood, all groups received additional doses of aerosolized OVA. Blood samples were obtained for eosinophil and antibody analyses. In-vitro re-stimulation of lung cells for cytokine analysis was also carried out.  
**RESULTS:** Mice exposed to aerosolized OVA as neonates were primed for a Th2 response with elevated levels of eosinophils, OVA specific IgE and Th2 cytokines (IL4, 5, 10, 13) but no detectable levels of IFN-gamma. The PBS controls that received aerosolized OVA at adulthood (8 weeks old) were unresponsive with respect to OVA specific IgE, Th2 cytokines and IFN-gamma. A single dose of anti-IL4 when given on day 0 to neonates concurrently with aerosolized OVA prevented the dysregulated allergic response to OVA at adulthood. Furthermore, these anti-IL4 treated neonatal mice, made a normal Th2 response when primed with an irrelevant antigen such as TNP-KLH while remaining unresponsive to aerosolized doses of OVA.  
**CONCLUSIONS:** Treatment with anti-IL4 at infancy may have therapeutic benefits against common allergens when administered in individuals particularly, during the allergy season.
Platelets Constitutively Express Interleukin-33 Protein and Modulate Eosinophilic Airway Inflammation

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RATIONALE: Although platelets play a key role in allergic inflammation in addition to their well-established role in hemostasis, the precise mechanisms of how platelets modulate allergic inflammation are not fully understood. Interleukin-33 (IL-33) is an essential regulator of allergic inflammation.

METHODS: IL-33 protein in human platelets, in a megakaryocyte cell line, MEG-01, and in bone-marrow-derived mouse megakaryocytes was detected by Western blot analysis and fluorescent immunostaining. We examined the functional relevance of IL-33 protein in platelets by comparing platelet-intact and platelet-depleted groups in a murine model of IL-33-dependent airway eosinophilia elicited by intranasal administration of papain. We further compared the additive effect of administration of platelets derived from wild-type versus IL-33-deficient mice on the papain-induced eosinophilia.

RESULTS: Platelets and their progenitor cells, megakaryocytes, constitutively expressed IL-33 protein (31 kDa). Papain-induced IL-33-dependent airway eosinophilia in mice was significantly attenuated by depletion of platelets. Conversely, concomitant administration of platelets derived from wild-type mice, but not from IL-33-deficient mice, enhanced the papain-induced airway eosinophilia.

CONCLUSIONS: Our novel findings strongly suggest that platelets may be important cellular sources of IL-33 protein in vivo, and that platelet-derived IL-33 may play a pivotal role in airway inflammation. Therefore, platelets might become an attractive novel therapeutic target for asthma.

The Role of TSLP in Experimental Allergic Rhinitis

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RATIONALE: Thymic stromal lymphopoietin (TSLP) is an epithelial derived cytokine, which plays a pivotal role in initiation and activation of allergic inflammation. Although genetic epidemiological studies predicted the involvement of TSLP-TSLPR signal in allergic rhinitis (AR) pathogenesis, the precise role of the cytokine in the disease pathogenesis is poorly understood. This study investigated the function of TSLP on AR by sensitizing BALB/c wild-type (WT) and TSLP receptor (TSLPR)-deficient mice with an allergen by the nasal route.

METHODS: WT and TSLPR-deficient mice were nasally administered ragweed pollen over consecutive days. Rhinitis symptoms were evaluated by examining the frequency of sneezing for 10 min immediately after every nasal challenge. Eosinophilic infiltration in nasal mucosa, Th2 cytokine production, and serum ragweed-specific IgE level were examined 24 hr after the final nasal challenge.

RESULTS: Serial nasal sensitization of ragweed induced an allergen-specific increase in sneezing, nasal eosinophilic infiltration, and Th2 cells accumulation in cervical lymph nodes in WT mice. TSLPR-deficient mice were defective in sneezing but developed normal eosinophilic infiltration and Th2 cell activation. Furthermore, both WT and TSLPR-deficient mice produced comparable levels of ragweed-specific IgE in the sera.

CONCLUSIONS: TSLPR signaling is crucial for inducing sneezing in AR, but is dispensable for Th2 inflammation induced by nasal challenge with ragweed. Because ragweed-specific IgE production is not defective in TSLPR-deficient mice, TSLP may act on the parallel or downstream of the activation of FcεRI on mast cells.

TSLP Signaling Pathway Is Required for COX Inhibition-Induced Augmentation of Allergic Airway Inflammation

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RATIONALE: We have previously published that the cyclooxygenase (COX) inhibitor indomethacin markedly increased allergic airway inflammation in mice when administered during allergic sensitization. In this study, we tested the hypothesis that the augmentation of allergic inflammation caused by COX inhibition requires the TSLP and IL-33 signaling pathways.

METHODS: We used WT, TSLP receptor (TSLPR) knockout (KO) and IL-33 receptor (ST2) KO mice in the OVA model of allergic airway inflammation. The mice were sensitized with an intraperitoneal injection of OVA protein conjugated with aluminum hydroxide and treated with indomethacin or vehicle in drinking water for 5 days in the peri-sensitization period. Two weeks after sensitization, the mice were challenged with aerosolized OVA for 4 consecutive days and the following day BAL fluid was harvested for inflammatory cell counts and IL-5 and IL-13 measurement.

RESULTS: Indomethacin treatment during allergic sensitization increased numbers of total cells and eosinophils and significantly increased IL-5 and IL-13 in BAL fluid in WT and ST2 KO mice, but not in TSLPR KO mice, compared to vehicle treatment. These results indicate that TSLPR signaling pathway, but not IL-33 signaling pathway, was required for indomethacin-induced augmentation of allergic airway inflammation.

CONCLUSIONS: These findings suggest that COX inhibition may affect the expression and function of the epithelial-derived cytokine TSLP, which in turn enhances Th2 immune responses and allergic inflammation.
Human Bronchial Epithelial Cell-Derived Factors from Severe Asthmatics Can Stimulate Local Eosinophilopoietic Responses
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RATIONALE: Bronchial epithelial cells activated by allergens, viruses or environmental pollutants produce cytokines including thymic stromal lymphopoietin (TSLP) and IL-33 that can initiate Th2 inflammatory processes. We hypothesize that bronchial epithelial cell-derived factors from asthmatics can stimulate the local maturation of eosinophil progenitors in the airways.

METHODS: Bronchial epithelial cells supernatants (BECSN) were obtained from cultures of isolated bronchial epithelial cells from normal non-atopic controls (NC; n=8), mild atopic asthmatics (AA; n=9) and severe eosinophilic asthmatics (SEA; n=5). Non-adherent mononuclear cells (NAMCs) and enriched CD34+ cells from the blood of mild asthmatics were co-cultured in methylcellulose to assess eosinophil/basophil colony (Eo/B-CFU) formation after 14 days. Cultures were set up with BECSN alone or in the presence IL-5 (1 or 10 ng/ml).

RESULTS: Compared to media control, BECSN from SEA and AA but not NC stimulated significant Eo/B-CFU (SEA>AAG<NC). In the presence of exogenous IL-5 (10 ng/ml), there was a significant increase in the number of Eo/B-CFUs grown in co-cultures of BECSN from SEA (p<0.05) but not AA (P>0.05) or NC (5±0.5; 4±1±3; 9±2±3; 7±2; 3±Eo/ B-CFU respectively). Colony growth stimulated by BECSN from SEA was significantly attenuated by a neutralizing TSLPR antibody. Cultures of blood NAMNC with rTSLP (1-100 pg/ml) alone stimulated significant Eo/B-ESU-CFU and this was significantly enhanced in combination with IL-5 (1 ng/ml).

CONCLUSIONS: Our results show that through the production of TSLP, bronchial epithelial cells can direct eosinophil differentiation and maturation from progenitor cells which in turn may perpetuate eosinophilic inflammation in patients with chronic eosinophilic asthma.

Vitamin D Deficiency in a Young, Atopic Pediatric Population
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RATIONALE: Studies have demonstrated that low serum 25-hydroxyvitamin D levels are associated with increased severity of asthma and allergy in children, elevated IgE and eosinophil count, increased asthma-related hospitalizations, and greater use of anti-inflammatory medications. The prevalence of vitamin D deficiency is not well-established, especially in young children. Varying amounts of vitamin D supplementation are commonly used, and there is no consensus of the exact dose of vitamin D supplementation required for normalization.

METHODS: 47 atopic children with inadequate vitamin D levels were randomized to a control group receiving 400 IU/day vitamin D supplementation or treatment group receiving 1000 or 2000 IU/day, depending on levels (20-30 ng/mL or <20 ng/mL respectively). 25-hydroxyvitamin D, total IgE, immunoCAP for environmental allergens, and CBC with differential were measured. Vitamin D was supplemented for 3 months.

RESULTS: At baseline, 15% of patients had sufficient vitamin D levels (>30ng/mL), 68% were insufficient (20-29ng/mL), and 17% deficient (<19ng/mL). After 3 months of supplementation, 41% achieved normal vitamin D levels, but 53% remained insufficient and 6% deficient. 38% of patients receiving 400IU normalized, 43% receiving 1000IU normalized, and 50% of those receiving 2000IU normalized. No significant trends were found in IgE, immunoCAP or eosinophil count.

CONCLUSIONS: This study suggests the prevalence of vitamin D deficiency and insufficiency in young atopic patients is high, and current practices of vitamin D supplementation are insufficient in normalizing vitamin D levels in many children. Long-term studies are needed to establish recommended treatment dosing and to examine the effect of vitamin D normalization on allergic inflammation.

Cat Dander Extract Require TLR4/MD2 to Induce Both ROS Generation and Neutrophil Recruitment
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RATIONALE: Airway neutrophilia is a hallmark of severe and sudden-onset asthma. Cat dander is a major indoor antigen that induces an early wave of neutrophil recruitment in asthma subjects (PMID16815145). However, the innate immune mechanisms that contribute to this early neutrophil recruitment induced by cat dander are unknown.

METHODS: WT mice, Tlr4 knockout (KO) mice and Cdl4KO mice were intranasally challenged with cat dander extract. BALF levels of neutrophils were quantified 16 h later. Three HEK 293 cell lines (cells that do not express TLR4, CD14 or MD2 (TLR4null), cells that overexpress TLR4, but not CD14 and MD2 (TLR4null), and cells that overexpress TLR4, CD14 and MD2 (TCR4null) were stimulated with cat dander extract, and intracellular ROS generation and IL-8 secretion were quantified.

RESULTS: Intranasal cat dander extract challenge in WT mice increased recruitment of neutrophils. These effects were attenuated in Tlr4KO mice, but not in Cdl4KO. Stimulation with cat dander extract induced CXCL secretion in TCMHi cells but not TLR4null cells or TLR4null cells. TLR4null cells transfected with a plasmid to overexpress MD2 secreted IL-8 by stimulation with cat dander extract. Suppression of M2 in lungs by intravenous siRNA administration prior to allergen challenge attenuated cat dander extract-induced neutrophil recruitment. Cat dander extract also increased intracellular ROS in TCMHi cells but not TLR4null cells or TLR4null cells.

CONCLUSIONS: Cat dander extracts utilizes TLR4/MD2 to induce IL-8 secretion, neutrophil recruitment and induce oxidative stress in the airways.

Chronic LPS Exposure Reduces Accumulation of Pro-Atopic CD49d+ Neutrophils in the Airways Post-Paramyxoviral Respiratory Infection
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RATIONALE: Viral upper respiratory infections increase the risk of developing atopic disease. Using Sendai virus (SeV, a paramyxovirus), we previously showed that post-viral atopic disease is dependent upon accumulation of CD49d+ neutrophils in the airway. A single exposure to high dose endotoxin (LPS, 3 μg) prevents this accumulation, suggesting an interaction of the hygiene and viral hypotheses. The hygiene hypothesis posits that chronic LPS exposure decreases risk of atopy. We thus sought to determine the effect of chronic LPS exposure on SeV-mediated accumulation of CD49d+neutrophils in the airways.

METHODS: C57BL6 mice were given 0.1 μg LPS, 0.3 μg LPS, 3.0 μg LPS or PBS intranasally daily. On day 8, all mice were infected with 2x105 pfu SeV. On day 11, bronchoalveolar lavage (BAL) fluid was analyzed for CD49d+neutrophils by flow cytometry.

RESULTS: The frequency of CD49d+ neutrophils in the airways of PBS-treated and SeV-infected mice was 31.0%±4.2% (n=4). There was a trend for chronic LPS exposure to reduce the frequency of these cells (20.7%±6.9%, p=0.119; 18.6%±5.9%, p=0.069; and 23.8%±1.3%, p=0.077, for 0.1 μg, 0.3 μg, and 3.0 μg LPS, respectively, n=2).

CONCLUSIONS: Chronic low dose exposure to LPS appears able to reduce SeV-mediated accumulation of CD49d+neutrophils in the airways. These data support the hygiene hypothesis may mitigate virus-induced risk of atopic disease. Future studies will examine whether chronic exposure to LPS reduces development of atopic disease and whether longer duration LPS exposure has a similar effect.
Use of Multi-Parameter Flow Cytometry to Determine Cord Blood Innate Immune Function Associated with Prenatal Farming Exposure

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RATIONALE: There is accumulating evidence that immune maturation may be affected by farming exposures in utero. To date, studies have not characterized these differences at the single cell level.

METHODS: Pregnant women, recruited from rural Wisconsin, were grouped according to farm exposure (farm exposure is defined as living on a farm with direct animal contact 4 days per week). A multi-parameter flow cytometry assay was developed to define cord blood innate cell responses to varied TLR agonists and rhinovirus for sample processing and activation at a single study site. Sample staining and acquisition was performed at a second study site using LSRII (BD Biosciences) and FlowJo for analysis. Rank-transformed ANOVA was used for statistical analysis and p values were adjusted for gender.

RESULTS: The assay coefficient of variation was, on average, <15% (range for various agonists: 6.5-27%). An inter-assay analysis of the first 22 study subjects (13 farm and 9 non-farm) revealed potential differences in TNF responses between the farm vs. non-farm groups. For example, R848 (TLR 7/8) induced TNF expression in 44% vs 34% of plasmacytoid DCs (p = 0.05); and LPS (TLR4) induced TNF expression in 19% vs 25% myeloid DCs (p = 0.08).

CONCLUSIONS: Using a single center for sample processing, we have optimized a multi-parameter flow cytometry assay to analyze at the single cell level innate immune function at birth. Our preliminary data demonstrate the validity of this assay for interrogating the impact of in utero farming exposure on immune maturation and subsequent protection from allergic and respiratory diseases.

Effect of TREM1 Deficiency in Post-Viral Induced Asthma Disease

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RATIONALE: Using the Sendai virus (SeV) post-viral asthma disease model, we have shown that a subset of CD49d expressing neutrophils (CD49d+ PMN) is critical for post-viral asthmatic disease development through their induction of FcεRI expression on lung conventional dendritic cells (cDC). Transcriptome analysis of CD49d+ PMN revealed significantly lower expression of the Triggering Receptor Expressed by Myeloid cells 1 (Trem1) gene. As TREM1 triggers pro-inflammatory responses in phagocytes, we undertook this study to examine how Trem1 deficiency affected the CD49d+ PMN response during SeV infection.

METHODS: Wild type C57BL/6 or Trem1 deficient (Trem1−/−) mice were inoculated intranasally with 2x10⁵ pfu SeV. On day 3 post-inoculation (PI), single cell preparations from the BAL were stained for Gr1 and CD49d expression to enumerate CD49d+ PMN. On day 7 PI, lung cDC were examined for FcεRI expression by flow cytometry.

RESULTS: Trem1−/− mice had significantly higher frequency of CD49d+ PMN in the BAL on day 3 PI SeV compared with WT mice (19.0±6.6% Vs. 16.3±1.0, mean±SEM %, p = 0.03, n = 7). However, lung cDCs from Trem1−/− mice showed marginally lower expression of FcεRI at day 7 PI (1.74±0.06 Vs. 1.91±0.02, mean±SEM Fold MFI FcεRI, p = 0.048, n = 3).

CONCLUSIONS: Although Trem1 deficiency led to increased CD49d+ PMN accumulation in the BAL during SeV infection, this increase did not translate into higher FcεRI expression on cDC. TREM1 may have separate effects on PMN and cDC and the summ effect on the SeV post-viral induced asthma model is under current investigation.

Prior Allergen Sensitization Improves Outcome in a Murine Pseudomonas Aeruginosa Pneumonia Model. Bethany Lussier, Terry Hsieh and Daniel G. Remick

Bethany L. Lussier, MD, Terry Hsieh, Daniel Remick; Boston University.

RATIONALE: Prior publications suggest that pretreatment with heat-killed bacteria may induce lung defenses. Additionally, evidence supports a critical role for activated mast cells in protective innate immune responses to bacterial infections.

METHODS: Adult female ICR mice were sensitized according to protocol with low dose (100 ng) intratracheal cockroach allergen (CRA) on days 0, 14 and 21. A second group of mice were given the CRA on days 0,14 with allergen challenge withheld on day 21. Twelve hours after third allergen challenge on day 21, Pseudomonas aeruginosa (1.25-5x10⁶ CFU) was instilled intratracheally. Respiratory parameters were measured by unrestrained whole body barometric plethysmography to allow for longitudinal comparison. On day 28, plasma and BAL were collected for analysis.

RESULTS: Mice without third allergen challenge exhibit increased obstruction based on respiratory time and a 500% increase in airway hyper-reactivity, as well as a reduced tidal volume persisting up to 7 days of infection. Mice receiving a third allergen challenge prior to infection had no significant changes from baseline respiratory parameters (p<0.05). There is no significant difference between BAL cell counts of normal mice and mice receiving a third allergen challenge prior to infection. There is a two-fold increase in BAL total cellularity (5.6x10⁸ vs. 2.2x10⁸, p<0.0001), neutrophils (p = 0.03), and macrophages (p = 0.0011) and a three-fold increase in eosinophils (p = 0.0033) in mice not exposed to allergen challenge prior to infection.

CONCLUSIONS: In mice, acute exposure to allergen prior to infection confers a potential benefit. Further investigation is necessary to confirm changes in inflammatory response.

Rhinovirus-Induced Immune Response in Nasal Epithelial Cells

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RATIONALE: We aimed to compare immune response to rhinovirus (RV) infection and TLR stimulation in human nasal epithelial cells (HNECs) from patients with allergic rhinitis and healthy subjects.

METHODS: Primary HNECs obtained by nasal brushing from 7 patients with allergic rhinitis (AR) and 9 non-atopic healthy controls (HCs) were grown to confluence and infected with rhinovirus RV-1B (MOI 0.5) or stimulated with TLR agonists (TLR3,TLR7/8 and TLR 9). IFN-α, IFN-γ, IFN-β and RANTES proteins were measured in cell supernatants at 8, 24 and 48h. mRNA expression for interferons , RANTES, but also for IFR3 and IFRF was determined using Real-Time PCR.

RESULTS: In both groups RV infection induced IFN-α1 and RANTES mRNA expression and protein release and resulted in parallel increase in IFRF mRNA expression . Significant increase in IFN-α, IFRF and RANTES mRNA expression was observed at 24h as compared to 8h post infection in AR (p<0.05) and in HCs (p<0.05). Expression of IFN-ε1, IFN-β and IFRF were not affected by RV infections Among the three TLR agonist tested, only Poly I:C (TLR3 agonist) induced IFN-α and RANTES mRNA expression as well as protein release. There were no significant differences in interferons type I, type III and RANTES gene expression or protein release between AR patients and HCs. RV replication assessed in cell supernatants was similar in both group of subjects.

CONCLUSIONS: Antiviral response in the upper airway epithelial cells from patients with allergic rhinitis is not different from healthy, non-atopic subjects.
**Exosomal Mir-155 Secretion during Rhinovirus Infection in EARLY Childhood**

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**Rationale:** Innate immune responses are fine-tuned by small non-coding RNA molecules termed microRNAs (miRs) that modify gene expression in response to the environment. During acute infections miRs can be secreted in extracellular vesicles (exosomes) to facilitate cell-to-cell genetic communication. In this study we investigated the potential role of exosomal miRs in modulating airway antiviral immunity during rhinovirus (RV) infection, the most common cause of asthma exacerbations in children.

**Methods:** Nasal airway secretions were obtained from children (<3 yrs old) during PCR-confirmed RV infections and age-matched controls (n=20). Nasal exosomes were isolated with polymer-based precipitation (exoquick method) and miRs profiled using NanoString microarrays. Exosomal miRNA results were contrasted with *in vitro* data from air-liquid interface (ALI)-differentiated human bronchial epithelium (HBE). RESULTS: Nasal exosomal miR profiling identified miR-155 as the top miR present in children with RV (n=10) but not in control subjects (n=10). Nasal exosomal miR profiling overlapped significantly with exosomal miRs isolated from *in vitro* HBE secretions indicating an epithelial secretory origin of the isolated nasal exosomes. Through the use of bioinformatics tools, we identified that miR-155 predicted target genes regulate toll-like receptor (TLR)-mediated signaling.

**Conclusions:** Our data indicate that acute RV infection in young children is associated with airway secretion of exosomes containing miR-155, which is predicted in *silo* to regulate antiviral immunity. Further characterization of the potential immune regulatory role of virally-induced exosomal miR secretion will enhance our knowledge about the origins of virally-induced asthma and may identify new strategies to treat and monitor this condition in children.

**Different Inflammatory Mechanisms of Human Metapneumovirus and Respiratory Syncytial Virus**

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**Rationale:** Human metapneumovirus (HMPV) and respiratory syncytial virus (RSV) share some epidemiological and clinical characteristics; however, few studies have examined whether these viruses induce similar cytokine responses. This study compared cytokine profiles in HMPV and RSV patients to investigate their inflammatory pathways.

**Methods:** 128 nasopharyngeal aspirate specimens were collected from 128 pediatric patients hospitalized with acute respiratory infection including wheezing and tested for 7 common respiratory viruses. They were divided into HMPV (n=27) and RSV groups (n=101). Th1(IFN-γ), Th2(IL-4, IL-13) and Th17(IL-1β) cytokine profiles were analyzed.

**Results:** IFN-γ levels in the 2 groups were statistically similar (P=0.08). IL-4 levels were significantly higher in the RSV compared to HMPV group (P<0.0001). IL-13 levels in both groups were under detection level. IL-1β levels were significantly higher in the HMPV compared to the RSV group (P<0.0001).

**Conclusions:** Our results suggest that HMPV and RSV have different inflammatory mechanisms. HMPV induces airway inflammation by the Th17 pathway through release of IL-1β, whereas RSV acts through the Th2 pathway.

**Restoration of Respiratory Syncytial Virus-Induced Airway Barrier Dysfunction By Cyclic AMP Activation**

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**Rationale:** Respiratory Syncytial Virus (RSV) infects airway epithelial cells leading to bronchiolitis and susceptibility to long-term wheezing. It has been shown that viral load has positive association with disease severity. We previously showed that RSV induces dysfunction of epithelial apical junctional complexes (AIC), the major component of airway barrier. Disruption of barrier function can stimulate airway inflammation by enhancing permeability to inhaled allergens. Cyclic adenosine monophosphate (cAMP) is an important second messenger regulating many intracellular functions. In this study we examined whether activation of cAMP signaling restore RSV-induced AIC dysfunction.

**Methods:** Differentiated human bronchial epithelial cells were infected with RSV strain-A2 multiplicity of infection (MOI) of 0.5-2.0. Cells were pre-treated with analogs of cAMP including Forskolin and 8-Bromo-cAMP. AIC function was evaluated using transepithelial electrical resistance (TEER) and paracellular permeability to fluorescein isothiocyanate (FITC)-dextran (3 kDa). AIC structure was studied using immunofluorescence staining and confocal microscopy. RSV replication was determined using real-time-PCR and plaque assay.

**Results:** Pre-treatment with cAMP analogs significantly attenuated RSV-induced decreases in TEER, and increases in permeability. These analogs also restored cellular localization and distribution of AIC proteins. Notably, cAMP analogs significantly decreased RSV replication even when administered 24h after the initiation of infection.

**Conclusions:** Our findings provide new insights into the regulation of the epithelial barrier by a clinically significant virus with poorly understood pathogenesis, and implicate a novel protective role of cAMP signaling on RSV-induced airway AIC disruption. Future studies to determine the therapeutic potential of elevating intra-epithelial cAMP levels in RSV bronchiolitis and respiratory infection should be worthwhile.

**Exhaled Nitric Oxide Performance Compared to Methacholine Challenge in Pediatric Patients**

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**Rationale:** Exhaled nitric oxide (FeNO) and Methacholine challenge (MCH) are both utilized in the diagnostic approach to asthma in the pediatric population. We hypothesize that FeNO can decrease the need for MCH testing.

**Methods:** Retrospective chart review of pediatric patients 4-18 years old seen at a tertiary referral center between 11/01/2009 - 8/31/2013 receiving FeNO and MCH within 2 weeks.

**Results:** 259 patients were identified. Demographics: Average age was 11.5 years (SD +/- 3.96 years). 134 (51.7%) females and 125 (48.3%) males; 232 (89.6%) Caucasian, 12 (4.6%) Black, 15 (5.8%) Asian. Plotting the ROC revealed an AUC of 0.55 and optimal intercept point of 30 ppb. Using this cutoff, 38 patients were positive for both MCH and FeNO, 102 patients had a positive MCH but negative FeNO, 12 patients had a negative MCH but positive FeNO, and 107 patients had both negative (p<0.01). Diagnostic profile of an FeNO for positive MCH: sensitivity 27.14% (95% CI: 19.9%-35.30%), specificity 89.92% (95% CI: 83.04%-94.67%), positive likelihood ratio 2.69 (95% CI: 1.48-4.91), negative likelihood ratio 0.81 (95% CI: 0.72-0.91), positive predictive value 76.00 (95% CI: 61.83-86.93%), and negative predictive value 51.60% (95% CI: 44.21-58.15%).

**Conclusions:** In the pediatric population, using a cutoff of 30 ppb, a positive FeNO increases the likelihood of MCH positivity. In pediatric patients with respiratory complaints, point of care FeNO may be a reasonable diagnostic option prior to obtaining MCH.
Saliva-SP-D Is a Practical Marker to Identify the Peripheral Airway Inflammation
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RATIONALE: Forced oscillation technique (FOT) is a utilitarian tool to verify the localization of airway structural changes, i.e. airway remodeling by measuring airway resistance and reactance. However, the interpretation of children’s data obtained by FOT require cautions, since the airway resistance is relatively higher than that of adults and changes with age. Meanwhile, serum levels of surfactant protein D (SP-D) have been shown a biomarker of bronchial asthma.
METHODS: 22 asthmatic children and 11 healthy controls were recruited to the study. After taking informed consent from guardians, airway resistance (R5 and R20) and reactance (X5) were measured by forced oscillation technique using Mostgraph 01. Blood and saliva were then collected from the patients, and levels of SP-D were evaluated by ELISA. The relationship between the SP-D levels and the airway resistance adjusted by age and height were assessed.
RESULTS: The levels of serum SP-D were not correlated with that of saliva SP-D (P = 0.78, r = 0.02). Saliva SP-D was elevated during asthma attacks compared with stable status (P = 0.01). The R5-R20 value, which represented peripheral airway resistance showed a positive correlation with saliva SP-D levels (P = 0.0321, r = 0.4579), whereas the R20 corresponding to central airway resistance did not.
CONCLUSIONS: Saliva SP-D may be a practical marker to identify the peripheral airway inflammation of bronchial asthma. Further studies will be needed to clarify the underlying mechanisms of SP-D secretion in asthmatic patients.

Hospital Admission Associated with Higher Total IgE Level in Pediatric Patients with Asthma
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RATIONALE: Serum IgE is implicated as a significant mediator of seasonal asthma exacerbation in pediatric patients and correlates with disease severity in younger patients with asthma. While measurement of total serum IgE is important in the assessment of a patient with severe asthma, it’s unknown whether serum IgE levels increase during an asthma exacerbation. Our objective was to determine whether there was an association between elevated total serum IgE level and hospitalization for asthma exacerbation in pediatric patients.
METHODS: A retrospective analysis of de-identified clinical data from pediatric patients (ages 3–26) with the diagnosis of asthma and a total IgE level drawn during a clinic visit (Outpatient cohort) or during a hospital admission (Inpatient cohort) at Cincinnati Children’s Hospital Medical Center were included.
RESULTS: The Inpatient cohort had a significantly higher (four-fold) total IgE level than the Outpatient cohort (P < 0.001). When matched for the season when IgE levels were drawn, the Inpatient cohort had significantly higher total IgE levels than the Outpatient cohort regardless of season. Regression analysis revealed that age and race were also significantly associated with higher total IgE levels. Every one-year age increase was associated with a 1.08-fold increase in total IgE levels (P = 0.004). African Americans had 2.81-fold higher total IgE levels than Caucasians (P = 0.017).
CONCLUSIONS: Abnormally high total serum IgE levels were significantly associated with hospitalization for asthma exacerbation regardless of the season. Further investigation is needed to investigate the utility of elevations in total serum IgE in an individual as a biomarker for exacerbations.

Polyunsaturated Lysophosphatidic Acid As a Potential Asthma Biomarker
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RATIONALE: Lysophosphatidic acid (LPA), a lipid mediator present in biological fluids, is generated by the enzymatic action of autotaxin (ATX) that hydrolyzes lysophosphatidylcholine (LPC) to LPA. We previously demonstrated increases in total LPA levels after bronchoprovocation with allergen in asthma subjects. The purpose of this study was to determine the profile of LPA molecular species in exhaled breath condensate (EBC) of asthma subjects.
METHODS: Five subjects with intermittent asthma were recruited. Asthma diagnosis was confirmed by spirometry with bronchodilator reversibility or methacholine challenge. EBC and BALF were collected before subsegmental broncho-provocation with allergen and 48 hours post-provocation. Samples were frozen at -80°C until analysis for LPA by mass spectrometry.
RESULTS: Three of 5 subjects provided adequate EBC sample for analysis. Of the 3 subjects, 2 demonstrated a strong allergic inflammatory response to allergen challenge and 1 had an attenuated response. Lipodomic analysis in the 2 subjects with a strong allergic response revealed a selective increase in polyunsaturated LPA 22:2.5 and LPA 22:2.6 species in BALF. The LPA composition in EBC did not correspond to that in BALF. EBC LPA molecular species were predominantly saturated LPA 16:0 and 18:0. The non-responding subject had no increase in total LPA level or LPA 22:5 in BALF or EBC.
CONCLUSIONS: Our findings suggest that LPA 22:5 is selectively increased in the airway of allergic asthmatics, and may translate into a novel biomarker and target for asthma treatment. However, the increase in LPA 22:5 in BALF was not reflected in the EBC, suggesting poor correlation between BALF and EBC.

Serum Periostin Levels Correlates with Exercise-Induced Bronchoconstriction in Asthmatic Children
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RATIONALE: The significance of periostin as a biomarker of TH2-induced airway inflammation, and as a measure of the response to 

All abstracts are strictly embargoed until the date of presentation at the 2015 Annual Meeting.
Lipopolysaccharide-Responsive Beige-like Anchor Is Required for Both Activation and Deactivation of NFkB
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RATIONALE: The molecular mechanisms why mutations of the lipopolysaccharide-responsive beige-like anchor (LRBA) paradoxically cause both autoimmunity and common variable immunodeficiency (CVID) are unknown. LRBA regulates vesicle trafficking and signal transduction required for the regulation and function of many immune molecules. It is hypothesized that LRBA deficiency attenuates both activation and deactivation of nuclear factor kappa beta (NFkB) resulting in immunodeficiency and autoimmunity.

METHODS: LRBA was knocked down or repressed in Raji lymphoma cells by the short hairpin RNA (shRNA) or dominant negative mutant (DNM) techniques. The transcription activity and phosphorylated levels of NFkB was analyzed using the luciferase reporter assay, Western blot and flow cytometry.

RESULTS: LRBA repression attenuates both NFkB activation and deactivation, inhibits NFkB nuclear translocation and increases tumor necrosis factor alpha (TNFa) and cell survival. The attenuated NFkB activation may result from inhibited NFkB nuclear translocation, while the attenuated NFkB deactivation may result in the increased TNFas and cell survival.

CONCLUSIONS: These results suggest that the attenuation of NFkB activation may explain LRBA deficient immunodeficiency, while the prolonged NFkB activity and the increased proinflammatory cytokine TNFas and cell survival may explain LRBA deficient autoimmunity. This activation and deactivation (AnDA) model reveals a novel regulation mode for NFkB. It also provides a molecular mechanism for the paradoxical association of immunodeficiency and autoimmunity, which is a fundamental question in the immune system.

Convergence of Clinical and Cellular Phenotypes Among Patients with STAT3 and ERBB2IP Mutations
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RATIONALE: Recent candidate gene and genome-wide association studies have identified a single nucleotide polymorphism (SNP) rs1837253 of thymic stromal lymphopoietin (TSLP) to be inversely associated with asthma and related-traits. Rs1837253 is suggested to have functional consequences on TSLP expression due to the absence of linkage disequilibrium to other SNPs. The objective of this study was to evaluate the expression and secretion of TSLP as a function of rs1837253 genotype using nasal epithelial cells (NEC) cultured from non-atopic and atopic individuals.

METHODS: Genomic DNA was isolated from mouthwash samples and genotyped for rs1837253 using TaqMan® genotyping assay. NEC from the nasal turbinate were collected, expanded and cultured, and the induction of TSLP by polylC was examined.

RESULTS: The data were in Hardy Weinberg equilibrium. Atopic sensitization itself did not affect basal or polylC-mediated secretion of TSLP from NEC. Stratifying by genotype, stimulation with polylC resulted in decreased TSLP secretion in NEC obtained from heterozygous (CT; 1.8-fold) and homozygous minor allele (TT; 2.5-fold) individuals compared to NEC from homozygous major allele individuals (CC: p<0.05).

CONCLUSIONS: We show for the first time that SNP rs1837253 in TSLP may be involved in the regulation of TSLP secretion, which may help explain its protective associations with asthma, atopic asthma and airway hyper-responsiveness. Identifying functional consequences of SNPs in genes with previously reported clinical associations will eventually pave the way for novel therapies targeting the source of inflammation rather than life-long therapies aimed at dampening inflammation and easing symptoms.
499 Mycoplasma Pneumoniae Cards Toxin Regulates NLRP3 Inflammasome Activation
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RATIONALE: Mycoplasma pneumoniae is a common bacterial airway pathogen that possesses the Community-Acquired Respiratory Distress Syndrome (CARDS) toxin. CARDS toxin is capable of reproducing the robust inflammation and cytopathology associated with M. pneumoniae infection. We hypothesized that CARDS toxin interacts with components of the host innate immune response to trigger inflammation mediated by the cytokine interleukin-1β (IL-1β).

METHODS: WT or NLRP3 knockout bone-marrow derived macrophages were treated with WT CARDS toxin or different CARDS mutants for different time points. Supernatants and lysates were assayed for IL-1β maturation and caspase-1 activation. NLRP3-overexpressing 293 cells were treated with CARDS toxin and immunoprecipitation was performed on the cell lysates to study the interaction of CARDS toxin with NLRP3. NLRP3-overexpressing 293 cell lysate was also used for an ADP-ribosylation assay.

RESULTS: Treatment of macrophages with CARDS toxin triggers inflammasome complex formation, resulting in caspase-1 activation and IL-1β secretion. CARDS mutants deficient for cellular entry or ADP-ribosylation failed to activate the inflammasome complex. CARDS toxin was found to interact directly with NLRP3. Furthermore, CARDS toxin modifies NLRP3 by ADP-ribosylation.

CONCLUSIONS: We have uncovered a novel mechanism by which M. pneumoniae CARDS toxin ADP-ribosylates NLRP3, triggering inflammasome activation and enhanced inflammation. This is the first report of ADP-ribosylation as a post-translational modification that mediates inflammasome activation.

500 Fcγ-Fragment and IgG Monoclonal Antibody Polarization of Human Macrophages: A Novel Immunomodulatory Mechanism
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RATIONALE: Omalizumab (mAb), a humanized IgG1 monoclonal antibody, is an indicated treatment for allergic asthma and chronic urticaria. Efficacy in non-atopic asthma patients suggests alternative non-IgE mechanisms. Our laboratory has shown mAb helps to drive polarization of THP-1 human monocytes. We investigate if this polarization is a Fc or Fcγ mediated phenomenon.

METHODS: THP-1 monocytes were differentiated with PMA, then stimulated with IFNγ/LPS and IL-4 to polarize them into the M1 and M2 state, respectively. Cells were stimulated with mAb or human Fcγ or IFNγ/LPS or IL-4 (during and after polarization). Then, mRNA was isolated and subjected to QRT-PCR to examine profile markers for M1 (CCR7 and IL12), and M2 (CD163 and CCL17). Data was normalized to GAPDH.

RESULTS: Expression of CCR7 was increased over IFNγ/LPS controls in cells costimulated with IFNγ/LPS+mAb (26-fold, p<0.0001) and IFNγ/LPS+Fcy (31-fold, p<0.0001). CD163 expression was reduced in cells costimulated with IL-4+mAb (by 59%, p<0.0001) and IL-4+Fcy (by 56%, p<0.0001) versus IL-4 alone. Expression was also reduced in cells polarized first with IL-4 then stimulated with mAb (by 36%, p<0.0005) and Fcy (by 70%, p<0.0001).

CONCLUSIONS: The upregulation of M1 marker CCR7, and down-regulation of M2 marker CD163 is consistent with our previous study with mAb alone. Fcγ closely paralleled the use of mAb and suggests that the polarizing shift observed is Fcγ mediated. To our knowledge this study is the first to demonstrate Fcγ mediated polarization of human macrophages. This may represent a novel immunomodulatory mechanism for monoclonal antibody and IVIG therapy.

501 Effects of Maternal Geohelminth Infections on the Risk of Allergy during the First 3 Years of Life: Findings from a Birth Cohort in Rural Ecuador
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RATIONALE: Maternal geohelminths during pregnancy may protect against allergy development in childhood. We investigated the effect of maternal geohelminths during pregnancy on the development of eczema, wheeze, and atopy during the first 3 years of life in children.

METHODS: A cohort of 2,404 neonates followed to 3 years of age in rural Ecuador. Data on wheeze and eczema were collected by questionnaire and physical examination at 13, 24, and 36 months, and allergen skin prick test reactivity (SPT) to 10 allergens was done at 36 months. Maternal stool samples were examined for geohelminths by microscopy. Data on potential confounders was collected at birth by questionnaire.

RESULTS: Geohelminths were observed in 46.1% of mothers. Eczema and wheeze during the first 3 years was reported for 17.7% and 25.9%, respectively, of 2,069 (86.1%) children with complete follow-up. At 3 years, SPT to any allergen was present in 17.2% and 22.6% of mothers, respectively, of 2,069 (86.1%) children with complete follow-up. At 3 years, SPT to any allergen was present in 17.2% and 25.9% of mothers, respectively, of 2,069 (86.1%) children with complete follow-up. At 3 years, SPT to any allergen was present in 17.2% and 25.9% of mothers, respectively, of 2,069 (86.1%) children with complete follow-up. At 3 years, SPT to any allergen was present in 17.2% and 25.9% of mothers, respectively, of 2,069 (86.1%) children with complete follow-up. At 3 years, SPT to any allergen was present in 17.2% and 25.9% of mothers, respectively, of 2,069 (86.1%) children with complete follow-up. At 3 years, SPT to any allergen was present in 17.2% and 25.9% of mothers, respectively, of 2,069 (86.1%) children with complete follow-up. At 3 years, SPT to any allergen was present in 17.2% and 25.9% of mothers, respectively, of 2,069 (86.1%) children with complete follow-up. At 3 years, SPT to any allergen was present in 17.2% and 25.9% of mothers, respectively, of 2,069 (86.1%) children with complete follow-up.

CONCLUSIONS: Our data do not support a protective effect of maternal geohelminths on development of eczema and wheeze in early childhood, although in utero exposures to ascariasis may reduce allergic sensitization.
**502 Relationship Between Domestic Mouse Allergen Exposure Assessed in Settled Dust and Mouse Specific IgE, IgG and IgG4 Antibodies in Asthmatic Children**

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**RATIONALE:** The relationship between domestic exposure to animal allergens has been inconsistent across studies, with some reporting an IgE response and others a 'modified Th2' response (characterized by increased IgG4).

**METHODS:** Children with asthma were evaluated for enrollment in a mouse allergen intervention trial. Mouse specific IgE (n = 379) and Mus m 1 specific IgG and IgG4 (n = 268) were measured in serum. Mus m 1 was measured in bed (n = 384), bedroom floor (n = 337) and kitchen (n = 183) settled dust. Data were logarithmically transformed before Pearson’s correlations and linear regressions were tested.

**RESULTS:** All allergen measures correlated (P < 0.001; bedroom floor vs. kitchen: r = 0.39; bed vs. kitchen: r = 0.41; bed vs. bedroom floor: r = 0.74). IgE correlated with allergen measured from bed (r = 0.13, P < 0.009) and bedroom floor (r = 0.22, P < 0.001), but not kitchen (r = 0.072, P = 0.34). IgG and IgG4 correlated with allergen in dust (r = 0.18-0.34; bedroom samples highest). IgG and IgG4 were correlated with anti-mouse IgE (r = 0.77 and 0.54, P < 0.001). The ratio of IgG4/IgG was not correlated with allergen measures, but was inversely correlated with IgE (r = -0.44, P < 0.001). In a model with both variables, bedroom allergen (β = 0.23, P = 0.003) and the IgG4/IgE ratio (β = -6.2, P < 0.001) were independently associated with anti-mouse IgE.

**CONCLUSIONS:** Allergen measured in bed showed stronger correlations with IgE and IgG antibodies than did allergen measured from the kitchen. The inverse relationship between the ratio of mouse specific IgG4/IgG and IgE, independent of current mouse exposure, suggests that in addition to current allergen exposure other susceptibilities may be important in determining the IgE response to mouse.

**503 Maternal and Birth Characteristics Are Associated with Infant Gut Microbial Composition**

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**RATIONALE:** The mechanism linking breastfeeding to childhood allergic outcomes is not well understood. The infant gut microbiome may play an important role in this association, as breastfeeding influences microbial composition and function, features of which directly impact immune response.

**METHODS:** Data from 298 infants enrolled in the Wayne County Health Environment Allergy and Asthma Longitudinal Study (WHEALS) birth cohort were analyzed. Using infant stool samples collected over the first 6 months of life (N = 130), gut microbiome was profiled by 16S rRNA sequencing. Allergic-like response to pets at age 4 was defined as parental report of any coughing, wheezing, tightness, shortness of breath, runny nose, sneezing, or itchy eyes around pets. Compositional differences in the microbiome were evaluated using permutational multivariate analysis of variance. Tests of differential operational taxonomic unit (OTU) abundance were performed using zero-inflated negative binomial regression with false discovery rate adjustment (significance threshold q-value < 0.05).

**RESULTS:** Babies currently breastfed at 1 month were at decreased risk of developing allergic-like response to pets (p-value = 0.028). Both breastfeeding and allergic-like response to pets were significantly related to compositional variation in gut microbiome (p-value < 0.001, p-value < 0.023, respectively). Of the 109 OTUs significantly associated with both breastfeeding and allergic-like response to pets, 77 (71%) were negatively associated with breastfeeding but positively associated with allergic-like response to pets. This subset of risk-increasing bacteria suppressed by breastfeeding were predominantly members of the family Lachnospiraceae [51 (66%)].

**CONCLUSIONS:** Breastfeeding in infancy may protect against gut enrichment of specific Lachnospiraceae bacteria which are associated with allergic-like response to pets in early childhood.
505 Environmental Estrogens Alter Signaling in Immune Cells That Promotes the Development of Childhood Asthma

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RATIONALE: The goal of this project is to test the hypothesis that fetal exposure to environmental estrogens (EEs) enhances allergic sensitization initiating cell signaling in antigen-presenting and/or T cells, which leads to epigenetic alterations that promote the development of asthma.

METHODS: Cord blood mononuclear cells (CB-MNCs) were separated into CD4+ and CD8+ populations, incubated overnight in media with steroid-depleted serum, and then exposed to various concentrations (FM-pM) of estradiol, bisphenol A (BPA) and Bisphenol S (BPS). Supernatant from cell culture was collected for quantifying immunomodulatory cytokines. Intracelular signaling was assessed in a plate assay in cells that were fixed, permeabilized, and blocked, and incubated with anti-phospho ERK (pARK), anti-phosphoAKT (pAKT) or anti-phospho EZH2 histone methyl transferase (pEZH2). Phosphatase activity was assessed using secondary antibody and colorimetrical measurement. The signals were normalized to the number of cells in each well.

RESULTS: Current experiments suggest that estradiol, BPA and BPS significantly increase the phosphorylation of ERK, AKT and EZH2 in human MNCs, including CD8+ and CD4+ cells. The dose response to BPA and BPS indicate high sensitivity and typical non-monotonic responses.

CONCLUSIONS: The concomitant increases in the exposure of EEs and prevalence of asthma, raise the possibility that EEs may be a factor in the increasing prevalence of childhood asthma. Understanding the molecular and cellular basis for EEs’s asthma promoting effects in animal models and human epidemiological studies will inform public policy concerning EE exposures, and may ultimately allow the design of future prevention measures, and potentially, new molecular-based treatments for childhood asthma.

506 High Rate of Sustained Unresponsiveness with Early-Intervention Peanut Oral Immunotherapy

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RATIONALE: Data from oral immunotherapy (OIT) trials demonstrate limitations in safety, tolerability, and disease-modifying outcomes. We hypothesized that early-intervention OIT (EI-OIT) would enhance outcomes by capitalizing on permissive clinical and immune factors in the preschool years.

METHODS: We enrolled 40 children aged 9-36 months who had peanut-specific IgE (pIgE) > 5 kU/L and/or a recent allergic reaction. Qualifying subjects reacted during baseline oral peanut challenge and were block-randomized 1:1 to receive OIT at goal doses of 300 or 3000 mg/day in a double-blinded fashion. The primary endpoint, sustained unresponsiveness (SU), was assessed one month after stopping OIT, either upon achieving SU by blinded fashion. The primary endpoint, sustained unresponsiveness (SU), was assessed one month after stopping OIT, either upon achieving SU by intent-to-treat. 80% experienced likely- or possibly-related AEs (mean 2.8% per dose, 95% CI: 0.8, 4.8%). pIgE declined from baseline to end-of-study OFC while psIgE increased [median 13.6 kU/L (IQR 3.49) to 1.8 (0.7, 3) and 0.5 mg/L (0.2, 1) to 2.9 (2, 10)].

CONCLUSIONS: The high rates of SU and favorable safety we observed during EI-OIT have not been previously described. The data suggest that even at relatively low doses, early-intervention immunotherapy may enhance long-term food allergy outcomes. This concept is being further studied in an ongoing multicenter, randomized, placebo-controlled trial.

507 Peanut Sublingual Immunotherapy (SLIT) Results in Sustained Unresponsiveness in a Subset of Peanut Allergic Children

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RATIONALE: There are no approved active treatments for peanut allergy. Peanut SLIT potentially offers a safe and easily administered treatment for peanut allergy.

METHODS: We previously reported desensitization after 12 months of treatment in a SLIT trial of children ages 11-11 years with a clinical history of peanut allergy and a peanut IgE > 7 kU/L. Subjects continued daily maintenance dosing with 2 mg of peanut SLIT for the duration of the study. The first 11 subjects completed 3 years of treatment after which the study was extended to 5 years. After completing treatment, subjects underwent a double-blind, placebo-controlled food challenge (DBPCFC) to 5000 mg of peanut protein to assess desensitization. Subjects consuming the entire challenge without symptoms were rechallenged 1 month after discontinuing therapy to assess for sustained unresponsiveness (SU).

RESULTS: Thus far, 25 of 47 subjects completed the end-of-study desensitization DBPCFC, 11 subjects after 3 years and 14 subjects after 5 years of treatment. Nine subjects passed the desensitization DBPCFC and 8 of these subjects demonstrated SU, 5 subjects after 3 years and 3 subjects after 5 years (32% SU). Overall, peanut-specific IgE decreased significantly (mean 120.39 kU/L to 43.61 kU/L, p<0.01) and peanut-specific IgG4 (mean 0.41 mg/L to 37.52 mg/L, p<0.001) increased significantly but no differences were seen between the SU and non-SU groups.

CONCLUSIONS: Long-term treatment with peanut SLIT induced SU in a subset of subjects. Peanut-specific IgE, peanut-specific IgG4, and skin prick testing was not predictive of SU. The results are limited by the lack of a control group.
508 Combined Probiotic and Peanut Oral Immunotherapy for the Treatment of Peanut Allergy: A Randomised Trial
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RATIONALE: Co-administration of a bacterial adjuvant with oral immunotherapy (OIT) has been suggested as a potential treatment for food allergy but such combined therapy has not previously been evaluated by randomized controlled trial.

METHODS: A double-blind placebo-controlled randomized trial of probiotic Lactobacillus rhamnosus CGMCC 1.3724 and peanut OIT (PPOIT) in children (1-10 years) with peanut allergy. Primary outcome was induction of sustained unresponsiveness (2-5 weeks after discontinuation of treatment). Secondary outcomes were desensitization, and peanut skin prick test, sIgE, and sIgG4.

RESULTS: Fifty-two children were randomized, stratified by age (<5yr and 5-10yr) and peanut skin test wheal size (<5mm and 5-10mm); 56 reached trial end. Baseline demographics were similar across groups. Two-week sustained unresponsiveness was achieved in 82.1% receiving PPOIT and 3.6% receiving placebo (p < 0.001). Nine children need to be treated for seven to achieve two-week sustained unresponsiveness (NNT 1.27; 95% CI 3.6; <0.001). PPOIT was associated with reduced peanut SPT and sIgE and increased peanut sIgG4 (all p < 0.001). PPOIT participants reported a greater number of adverse events, mostly with maintenance phase home dosing.

CONCLUSIONS: This is the first randomized placebo-controlled trial evaluating the novel co-administration of probiotic and peanut OIT and assessing for sustained unresponsiveness in children with peanut allergy. PPOIT was effective in inducing two-week sustained unresponsiveness, and immune changes that likely reflect reprogramming of the peanut-specific immune response. Further work is required to confirm sustained unresponsiveness after a longer period of secondary peanut elimination and to clarify the relative contributions of probiotic vs OIT.

509 Monitoring Major Peanut Allergen Levels in Foods and in Therapeutic Preparations Used for Oral Immunotherapy
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RATIONALE: ‘Generic’ immunoassays for peanut cannot discriminate between allergen levels in peanut derived food products or therapeutics. Clinical trials of oral immunotherapy are strengthened by using standardized peanut preparations, with defined doses of major allergens. This paper describes measurement of Arah1, Arah2 and Arah6 in peanut foods and in peanut flour extracts used for oral immunotherapy (OIT).

METHODS: Two-site monoclonal antibody based ELISA for Arah1 (LOQ 31.5ng/ml), Arah2 (LOQ 2ng/ml) and Arah6 (LOQ 0.8ng/ml) were used to measure allergen levels in peanut (n = 19) and tree nut (n = 16) butters, and peanut flours (n = 11); oils (n = 8); flour extracts used for oral immunotherapy (n = 4); and the NIST Peanut Butter Standard Reference Material® 2387.

RESULTS: Roasted peanut butters contained 2000-42,000µg/g Arah1 and exceeded Arah2 and Arah6 levels by 2-4fold. Similarly, NIST SRM2387 contained 22,543µg/g Arah1; 5044µg/g Arah2 and 4072µg/g Arah6. In contrast, peanut flours contained 1500-29,000µg/g Arah2 and exceeded Arah2 and Arah6 levels by 2-20fold. Flour extracts used for OIT contained 358-505µg/ml Arah1, 1187-5270µg/ml Arah2 and 1104-8092µg/ml Arah6. In most cases, the peanut allergens were not detected in tree nut butters or peanut oils.

CONCLUSIONS: The results clearly show marked differences in specific peanut allergen profiles in peanut butters, flours and OIT extracts. Roasting may influence increased Arah1 levels in peanut butters. Variability in allergen levels in OIT extracts could affect the outcome of clinical trials of peanut OIT, especially with respect to Arah1. The results indicate that specific allergen measurements could be used to standardize peanut preparations that are being used for oral and transdermal immunotherapy.

510 Predictors of Elevated Rates of Adverse Events While on Peanut Oral Immunotherapy
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RATIONALE: Though peanut oral immunotherapy (OIT) is a promising investigational therapy, its safety for clinical use is limited by significant adverse event (AE) rates. We aimed to identify baseline clinical characteristics that may predict which subjects are at higher risk of AEs.

METHODS: We combined three peanut OIT studies and all home AEs experienced by 104 peanut-allergic children. We fit a generalized linear model, assuming a Poisson distribution, to determine the influence of the baseline clinical characteristics (sex; age; current and past history of asthma, atopic dermatitis, or allergic rhinitis; baseline peanut IgE, baseline peanut skin prick test) on the counts of AEs, adjusting for time on therapy. We then fit similar models for the build-up and maintenance period.

RESULTS: After controlling for the other variables, the AE rate among subjects with allergic rhinitis is 2.8-fold higher than those without allergic rhinitis (p < 0.001) and rates of AEs increase by 1.4-fold for every 5 mm increase in peanut skin prick test (p = 0.007). Allergic rhinitis history and peanut skin prick test remained predictors of elevated AE rates during build-up, but during maintenance, asthma was also a predictor, increasing AE rates by 3-fold (p = 0.01).

CONCLUSIONS: This analysis suggests that the history of allergic rhinitis, asthma, and larger peanut skin prick tests may be predictive of higher rates of AEs. While these results need to be confirmed in larger prospective randomized controlled clinical trials, it is conceivable that this information may prove useful in appropriately screening high risk patients.
511 Empowering Students with Asthma in Chicago Schools through Photovoice and Videovoice
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RATIONALE: Asthma is a problem of epidemic proportions in Chicago with childhood prevalence and mortality rates above the national average. The objective of this study was to partner with adolescents to improve asthma management and increase community asthma knowledge and support.

METHODS: Middle School students with asthma (N=12) were recruited to engage in a 13-week program grounded in Community-Based Participatory Research (CBPR) principles. Students were given minitablets to investigate socio-environmental factors influencing their asthma by taking photographs, recording video Public Service Announcements to educate their communities, and implementing a targeted community intervention. Wilcoxon-Mann-Whitney and paired t-tests were used to analyze changes in student and caregiver asthma knowledge, self-efficacy, empowerment, quality of life, symptom frequency, severity, and adherence to management practices pre-/post-program implementation.

RESULTS: Students identified asthma triggers in their communities, including pollution, smoking and automobile idling. Participants showed significant improvement in self-efficacy and empowerment scores on the Asthma Belief Survey (p=0.03) and the Sociopolitical Control Scale (p=0.033). Caregivers demonstrated significant improvement in quality of life (p=0.006) and asthma knowledge scores (p=0.006) and increased use of peak flow meters (p=0.001), spacers (p=0.046), and asthma action plans (p=0.003). Students developed and presented videos to peers and caregivers and posted them to a website to disseminate results to the community.

CONCLUSIONS: Utilizing CBPR principles involves students in many aspects of the research process and allows students to actively take control of their asthma. Photovoice allows students to investigate how their asthma is impacted by their unique communities and to communicate information to the larger community.

512 Taking Advantage of Smartphones and Cloud Computing to Decrease the Cost of Asthma
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RATIONALE: Asthma is a growing disease burden and a substantial cost to society. Smartphone technologies interfacing with cloud computing platforms have the potential to improve asthma control by illuminating many environmental exposures eliciting asthma exacerbations, and thus enabling the design of effective personalized preventative strategies.

METHODS: We conducted a case study comparing asthma control before and after the regular use of AsthmaAlly, a cloud computing platform tracking asthma symptoms and automatically compiling temporally and spatially appropriate environmental data (e.g. air quality, climate, pollen, etc.). Efficacy was quantified by compiling emergency department and urgent care facility visits from insurance billing records over a four year time frame, up to two years prior and two years following the regular use of AsthmaAlly.

RESULTS: In the years prior to AsthmaAlly use, the mean number of urgent care visits per year was 3.0. Following AsthmaAlly use, the mean number of urgent care visits dropped to 0.5. The drop in urgent care visits represents an average annual savings of $345 per patient given the average cost of $138 per urgent care visit.

CONCLUSIONS: Preliminary results suggest that use of smartphone technology and improved understanding of the environmental factors triggering asthma events enabled better decision making and the design of effective personalized preventative strategies, thus decreasing the need for urgent care. Smartphone and cloud computing technology could be useful tools to improve disease control for individuals and decrease the costs of asthma in terms of human suffering and monetary expenditures.

513 Gestational Asthma and Eczema: A New Reality?
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RATIONALE: Gestational asthma and eczema occur frequently in pregnancy. However, the role of the mother’s asthma and eczema history in terms of new onset of asthma and eczema during pregnancy is poorly understood. High prevalence of asthma and eczema in pregnancy may impact childhood asthma and affect rates of asthma exacerbations and hospitalizations.

METHODS: Study participants included 85 women with asthma and 18 without asthma at 13 weeks of pregnancy. Participants were followed until the end of pregnancy.

RESULTS: Of 85 women without asthma at 13 weeks, 13 women developed new-onset asthma during the pregnancy. Of 18 women with asthma at 13 weeks, 7 had a history of asthma exacerbations. Of these 18 women, 7 had a history of asthma exacerbations during pregnancy. Of the 7 women with previous asthma history, 6 had a prior history of wheezing during pregnancy. Of the 7 women with previous asthma history, 6 had a prior history of asthma exacerbations during pregnancy. Of the 7 women with previous asthma history, 6 had a prior history of asthma exacerbations during pregnancy.

CONCLUSIONS: New-onset of asthma and eczema occurs in pregnancy in about 15% of disease-free women.

514 Evidence for Harm Reversal in Asthmatic Smokers Who Switched to Regular Electronic Cigarettes Use
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RATIONALE: Asthmatic smokers have more severe disease, inadequate disease control, and poor response to therapies. Here we retrospectively evaluate asthma outcomes in smoking asthmatics who switched to electronic cigarettes (EC) use.

METHODS: Changes in lung function, methacholine hyperresponsiveness, exacerbations and subjective asthma outcomes were assessed after 6 (±1) (V1) and 12 (±1.5) (V2) months of regular EC use compared to baseline (BL) as well 6-12 (pre-BL) months earlier.

RESULTS: Eighteen (11M, 7F; 38.8 ± 12.3 years) subjects were reviewed, of whom ten were single (EC only) and eight dual (EC and cigarettes) users. No differences were noted in any outcomes between BL and pre-BL. Compared to BL there were significant improvements in all objective and subjective asthma parameters at V2, and some at V1. FEV1 improved from 3.30 ± 0.78 L (BL) to 3.40 ± 0.73 L (V2); FVC improved from 4.28 ± 0.90 L (BL) to 4.43 ± 0.78 L (V2); PEFR25-75% improved from 2.75 ± 0.72 L/sec (BL) to 3.11 ± 0.57 L/sec (V2); PC20 Mch improved from 1.24 mg/ml (BL) to 2.56 mg/ml (V2); ACQ improved from 2.03 (BL) to 1.47 (V2). At both V1 and V2 compared to BL there were marked reductions in daily conventional cigarette consumption. No adverse events were reported with EC use.

CONCLUSIONS: In asthmatics who smoke, EC use is well tolerated and may have a positive impact on asthma outcomes. We hypothesize that EC use in smoking asthmatics may be a safer alternative to conventional cigarette consumption and has potential for smoking cessation/reduction and respiratory harm reversal.
515 Identifying CpG Sites Associated with Eczema Via Random Forest Screening of Epigenome-Wide DNA Methylation

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RATIONALE: The prevalence of eczema among children of industrialized countries is increasing. There is some evidence that DNA methylation plays a role in eczema, however, there is lack of epigenome-wide study on their association.

METHODS: Association between eczema and DNA methylation was studied in 18-year-old subjects (n = 366) from the Isle of Wight (IOW) birth Cohort. To efficiently screen cytokine-phosphate-guanine (CpG) sites in genome-wide, we applied the approach of random forest (RF) ensemble. A total of 307,357 CpGs were subjected to random forest data reduction, repeatedly dropping 50% of variables with lowest variable importance measures until the misclassification rate showed a significant increase. Functional annotation and pathway analyses along with logistic regressions were performed to further evaluate the selected RF-CpG sites.

RESULTS: The RF method yielded 75 CpGs, 72 of which are linked to eczema corroborated by logistic regression. Eczema-associated CpGs, e.g., in genes PARD3 and GUCY1A3, were significantly enriched within the pathways ‘Tight junction’ and ‘Gap junction’ (P values 0.0008 and 0.0002, respectively, after correcting for multiple testing with false discovery rate of 0.05). It has been shown that genes in these pathways are correlated to allergic and immune-related diseases.

CONCLUSIONS: The RF Ensemble method was successfully utilized for epigenome-wide scanning to identify epigenetic loci associated with eczema, detecting both previously known and novel CpG sites. The significant enrichment of differentially methylated CpGs within tight and gap junction pathway genes implicates epigenetic regulation in epidermal barrier dysfunction in eczema.

516 Ara h 1 Peptide Immunotherapy Ameliorates Peanut-Induced Anaphylaxis

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RATIONALE: Peptide immunotherapy, a treatment that makes use of short peptides representing major allergen T cell epitopes, has been shown to be efficacious in reducing symptoms of allergic rhinoconjunctivitis in ongoing clinical trials. In the current study we evaluated whether this approach could prevent anaphylaxis in a murine model of peanut allergy.

METHODS: Mice transgenic for the human leukocyte antigen DRB1*0401 were sensitized to peanut epicutaneously and then treated with Ara h1 peptides. Mice were subsequently challenged intraperitoneally with peanut and clinical anaphylaxis was evaluated. Mice were sacrificed and samples of skin, spleen, inguinal LN, mesenteric LN, bone marrow, and peritoneal lavage were collected. HLA DRB1*0401 tetramers specific for the treatment peptides were used to identify peanut-specific T cells.

RESULTS: Peanut-sensitized mice treated with Ara h1 peptides demonstrated significantly decreased anaphylaxis following peanut challenge. Control mice treated with saline experienced a mean maximum temperature drop of 5°C, while mice receiving 100 µg of peanut experienced a drop of 2.4°C (p = 0.0065), and mice receiving 30 µg of peanut experienced a drop of 1.8°C (p = 0.0226). Mean hemocrit values for control mice were 49.9%, while values for the 100 µg group were 43.2% (p = 0.074) and the 30 µg group were 41.0% (p = 0.0113). Numbers of Ara h1-specific T cells were reduced in treatment group tissue samples.

CONCLUSIONS: The ability of peptide immunotherapy to ameliorate signs and symptoms of anaphylaxis in an experimental, murine model supports the further evaluation of this form of therapy in clinical peanut allergy.

517 Persistent Treatment Effect with Grass Synthetic Peptide Immuno-Regulatory Epitopes in Grass Allergy Symptoms in an Environmental Exposure Unit Challenge after a Second Season of Natural Pollen Exposure

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RATIONALE: Previously, we demonstrated a series of Synthetic Peptide Immuno-Regulatory Epitopes (SPIRE) derived from grass allergens resulted in a statistically significant reduction in rhinoconjunctivitis symptoms in an Environmental Exposure Unit (EEU), following a short course of treatment over 14 weeks and subsequent exposure to the natural grass pollen season. Here we evaluate whether the treatment effect persisted after a second grass pollen season without further dosing.

METHODS: This was a double-blind, optional follow-up study to a multi-centre, randomised, double-blind, placebo-controlled, parallel group study. Subjects who had mean baseline TRSS of ≥8 completing the original study in 2012 were invited to participate. EEU visits were scheduled after a second grass pollen season, approximately 1 year after the original post-treatment EEU challenge. There were no further administrations of Grass-SPIRE.

RESULTS: Eight administrations of 6 nmol Grass-SPIRE over a 14 week period showed a mean change in TRSS scores at EEU challenge, after two intervening grass pollen seasons off treatment, of -6.0 versus -3.6 on placebo (p = 0.038). The reduction in mean TRSS for the 6 nmol dose out performed two higher 12 nmol doses.

CONCLUSIONS: Treatment with Grass-SPIRE over 14 weeks showed a sustained treatment effect in grass allergy symptoms in an EEU model of grass allergy measured after two intervening grass pollen seasons following the cessation of dosing. These results for Grass-SPIRE showing a persistent treatment effect are similar to those obtained with other SPIREs, including cat and house dust mite.
518 Larger and Stronger Expression of Tregs Gut Homing Receptors with Epicutaneous Than with Sublingual or Oral Immunotherapy

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RATIONALE: Regulatory T cells (Tregs) plays a pivotal role in Epicutaneous Immunotherapy (EPIT) (Dioszeghy, Clin Exp Allergy 2014). Expression of homing molecules by Tregs could potentially modulate their response. The aim of this study was to compare the repertoire of homing receptors of EPIT-induced Tregs to Oral or Sublingual immunotherapy (OIT or SLIT).

METHODS: BALB/c mice were sensitized to peanut orally and treated by EPIT, SLIT, or OIT. After 8 weeks of treatment, the proportion of Tregs in spleen, inguinal and mesenteric lymph node (iLN or mLN) and the expression of homing receptors by Tregs were analyzed by flow cytometry.

RESULTS: In spleen, expression of CCR4 increased on Tregs induced by all 3 therapies whereas the expression of CXCX3, CCR6 and CCR8 increased on EPIT-induced Tregs only. Skin homing receptor (CLA) increased on EPIT- but not SLIT- or OIT-induced Tregs. Gut homing receptor CCR9 increased on EPIT- and OIT- but not on SLIT-induced Tregs. Interestingly, 81% of the CCR9+ Tregs induced by EPIT expressed CLA whereas only 43% did after OIT. In iLN, Tregs level increased only after EPIT with induction of CLA+CCR9 (22%) and CLA+CCR9+ (12%) Tregs. In mLN, EPIT and OIT significantly induced CCR9+ Tregs, 25% and 18% of them expressing also CLA in EPIT and OIT respectively.

CONCLUSIONS: EPIT induced a larger repertoire of homing receptors on Tregs than SLIT or OIT. EPIT was able to induce higher level of gut homing Tregs than OIT, strongly suggesting its relevance in food allergy.

519 Immunogenicity Evaluation of Subcutaneous Administration of Peptide Hydrolysate from Loliun Perene (gpASIT+) in Combination with Bacterial HSP70 (DnaK) in Patients with Seasonal Allergic Rhinitis: A Double Blind Placebo Controlled Trial

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RATIONALE: We have developed a peptide hydrolysate from Lolium perene (gpASIT+) for treating grass pollen-induced allergic rhinitis. Bacterial HSP70 (DnaK) can reduce inflammation and can be used as an adjuvant. The aim of the study was to assess immunogenicity, clinical safety and tolerability of gpASIT+ in the presence/absence of DnaK.

METHODS: In a phase IIa RDPCT, participants were subcutaneously treated over 4 weeks with gpASIT+ alone (n=9) or combined with equal amount of DnaK (n=9), a third group received placebo (n=9). SLG4 and blocking antibody responses were evaluated by ImmunocAP and IgE-FAB assay, respectively.

RESULTS: The mean change over the placebo for rhinoconjunctivitis symptom and rescue medication scores in subjects treated with ≥105μg gpASIT+ (PPS - 60 subjects) were -30% and -43% respectively. Post-hoc analysis showed 4/9 patients who received low-dose gpASIT+2/DnaK (<105 μg) had an improvement of symptom (-53% compared to placebo) and rescue medication scores (-46%). SLG4 and SLG levels increased following treatment in both groups and induced IgG-associated blocking antibodies: -30.1% [-51.7; -8.4] of FAB at the end of pollen season in the gpASIT+ group and -21.3% [-89.4; 46.8] in the gpASIT+/DnaK group.

The number of well-days correlated with levels of sLgG4 in gpASIT+/DnaK treated group but not gpASIT+ group. There was no difference between gpASIT+ and gpASIT+/DnaK groups for numbers of related unsolicited AE.

CONCLUSIONS: For the first time, we report that subcutaneous administration of peptide hydrolysate from Lolium Perene is safe and effective. Its effect in inducing long-term clinical benefit remains to be investigated.

520 The Evaluation of Efficacy and Adverse Effect in Intralymphatic Allergen-Specific Immunotherapy Against House Dust Mite, Cat, and Dog Allergens in Allergic Rhinitis

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RATIONALE: Recently, several clinical trials reported that intralymphatic immunotherapy (ILIT) against some allergens including cat dander and birch or grass pollen induces tolerance faster than conventional subcutaneous immunotherapy with comparable duration of effect after only 3 injections, but without serious local or systemic reaction. However, the efficacy and safety of ILIT against various allergens in allergic rhinitis still remains to be investigated. We evaluated the efficacy and adverse effect in ILIT against house dust mite, cat, and dog allergens in allergic rhinitis.

METHODS: A total of 10 patients with allergic rhinitis sensitized to Dermatophagoides fariniae, Dermatophagoides pteronyssinus, cat, or dog allergen were treated with 3 intralymphatic inguinal injections of causal allergen extract (HollisterStier, New Orleans, USA). Rhinoconjunctivitis quality of life questionnaire (RQLQ) in daily life and rhinitis symptoms after exposure to causal allergen were evaluated before and four months after initial treatment.

RESULTS: RQLQ in daily life was significantly improved after ILIT from 71.2 (range 50-105) to 52.3 (range 37-89) (P = 0.042). Rhinitis symptom after exposure of causal allergen including rhinorrhea, sneezing, nasal obstruction, and nasal itching were also significantly reduced (P = 0.017, P = 0.005, P = 0.027, and P = 0.041, respectively). We observed two cases of severe systemic reaction and one case of severe local reaction among three subjects.

CONCLUSIONS: ILIT can rapidly improve rhinitis symptom in daily life and after allergen exposure, however ILIT can also provoke severe systemic or local hypersensitivity reaction.

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**521** IL-18 Is Induced in Food Allergic Eosinophilic Esophagitis (EoE) Patients and Its Overexpression Promotes Disease Pathogenesis in Mice

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**RATIONALE:** Elevated levels of IL-18 are reported in a number of allergic diseases. Earlier, we reported enhanced expression of IL-18Rα mRNA in the esophageal biopsies of EoE patients. Accordingly, we tested the hypothesis that IL-18 induction promotes EoE.

**METHODS:** Quantitative PCR and ELISA analyses were performed to examine tissue mRNA and protein levels in non-EoE and EoE patients. We examined the cell surface molecules by flow cytometry, mouse tissue eosinophils by anti-MBP immunostaining, mast cells by chloroacetate esterase enzymatic staining, and human biopsy eosinophilia by H&E staining.

**RESULTS:** We demonstrate that blood IL-18 and IL-18Rα mRNA in the esophagus are induced ~4-fold in human EoE compared to non-EoE patients. This increased IL-18 level is highly significant in food allergen SPT+ human EoE compared to SPT- patients. Human blood IL-18 levels correlate with esophageal eosinophilia (p<0.01). The IL-18Rα+ cells and mRNA levels are induced in the esophageal biopsies of EoE compared to non-EoE patients. Further, we report that esophageal eosinophils and mast cells also correlate with induced ICAM levels in human EoE. Additionally, we showed that IL-18 intratracheal inoculation induces time and dose dependent esophageal mast cell and eosinophil inflammation and rTRACC10-IL-18 blazensigic mice develop (p<0.001) EoE. Mechanistically, we show that IL-18 in vitro stimulates iNKT cells (not conventional CD4+ T cells) and endothelial cells and induce eosinophil active cytokines IL-5, IL-13 and ICAM/VCAM, respectively.

**CONCLUSIONS:** Taken together, we provide evidence for the first time that IL-18 has a significant role in promoting food allergen-induced EoE.

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**522** H-PGD Synthase (H-PGDS) Gene Expression Increases in Eosinophils of Aspirin Exacerbated Respiratory Disease (AERD) Patients after Oral Graded Aspirin Challenge

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**RATIONALE:** 10% of adult asthmatics suffer from AERD, characterized by adult onset of moderate to severe asthma and nasal polyps, peripheral and tissue eosinophilia, and a hypersensitivity reaction (bronchospasm, rhinorrhea, and/or conjunctivitis) in response to cyclooxygenase-1 (COX-1) inhibition. They also have increased PGD2 metabolites in blood and urine that has been reported to come from mast cells. We hypothesized that eosinophils of AERD patients have a higher expression of the H-PGDS gene after aspirin-induced hypersensitivity reaction.

**METHODS:** We collected eosinophils from AERD patients (n=8) and aspirin-tolerant asthmatics (n=10) at baseline, and after oral graded aspirin challenge. Eosinophils were isolated to ~97% purity by depletion of non-eosinophils using magnetic labeling and conjugation to MicroBeads. RNA was extracted from eosinophils and RNA expression was measured in comparison to internal housekeeper transcripts using RNA-specific RT-qPCR. RESULTS: After an aspirin-induced hypersensitivity reaction, eosinophil H-PGDS gene expression significantly increased from baseline in AERD patients (p<0.01) in contrast to aspirin-tolerant asthmatics, where there was no change. Urine levels of PGD2 metabolite (tetranor PGDM) after aspirin challenge significantly increased in AERD patients (p=0.02) and accompanied the increases in H-PGDS gene expression. Tetranor PGDM decreased in aspirin-tolerant asthmatics (p<0.01).

**CONCLUSIONS:** Peripheral blood eosinophils differentially express the H-PGDS gene in response to aspirin challenges in AERD patients as compared to aspirin-tolerant asthmatics. In addition to the previously demonstrated PGD2 production by mast cells, our results suggest that eosinophils may contribute to the elevated PGD2 levels in this disorder.

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**523** Development of a Novel Peptide Nanoparticle Inhibitor for Human CCR3/Eotaxin-Mediated Eosinophil Migration

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**RATIONALE:** Asthma is treated using corticosteroids to inhibit airway inflammation by down-regulating the activity of inflammatory cells. While this approach is effective in eosinophilic asthma, it lacks efficacy in neutrophilic asthma. CCR3 is predominantly expressed by eosinophils, and binding by eotaxins mediates eosinophil tissue recruitment. CCR3 is also expressed by other cells comprising the asthma inflammatory infiltrate including basophils, mast cell subpopulations, activated Th2 cells, macrophages and epithelial cells. For unclear reasons, a small molecule CCR3 antagonist recently failed to show clinical efficacy in asthma. We have sought to develop novel peptide nanoparticle-based inhibitors of CCR3 that reduce signaling, chemotaxis and recruitment of multiple immune cells in allergic inflammation.

**METHODS:** We designed peptides containing transmembrane domains with/without extracellular loop portions of CCR3 that interact with its chemokine ligands. Peptide binding to CCR3 was analyzed by NMR. Inhibitory activity on CCR3 function was evaluated using CCL11/CCR3-mediated chemotaxis in CCR3+ lines and eosinophils from allergic asthma subjects.

**RESULTS:** A peptide inhibitor containing transmembrane and extracellular loop portions of CCR3 self-assembles into nanoparticles and directly binds the receptor. It inhibits CCL11/CCR3-mediated chemotaxis at low micromolar concentrations, comparable to small-molecule CCR3 antagonists. Inclusion of the extracellular loop enhances inhibitory activity.

**CONCLUSIONS:** We identified a novel peptide that inhibits CCR3-mediated eosinophil chemotraction. The peptide likely disrupts CCR3 conformation, impacting signaling and chemokine interactions. CCR3 peptide nanoparticle inhibitors are protected against degradation and may provide novel therapeutic approaches for asthma and other eosinophil-associated diseases. Importantly, understanding peptide-based inhibition could provide novel insights into the mechanism and roles of CCR3 signaling.
METHODS: To identify the IL-33 receptor-expressing CD34+ hemato-poietic cells, we analyzed the expression level of ST2 and IL-1RAcP. However, the detailed characterization of these CD34+ cells is not fully explored.

RESULTS: IL-33 receptor expression was restricted on eosinophil progenitors (EoPs), basophil progenitors (BaPs), mast cell progenitors (MCPs) and megakaryocyte / erythrocyte progenitors (MEPs) in hematopoietic stem/progenitor cells by multi-color flow cytometer. To evaluate the in vitro effect of IL-33, gene expression analysis (microarray and quantitative real-time PCR), multiplex cytokine analysis, proliferation assay and apoptosis assay were conducted. To clarify the in vivo effect, IL-33 was administrated to C57BL/6 mice intraperitoneally.

CONCLUSIONS: These results showed that IL-33 directly modulates the potential effector function of lineage-committed myeloid progenitors and positively regulates the eosinophil development in IL-5-dependent manner at the lineage-committed progenitor stage.

525 Global Expression and Epigenetic Analyses of Eosinophil Development Reveal Potential Novel Regulators

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RATIONALE: The mechanisms that direct granulocyte/monocyte progenitor (GMP) cell fate toward the eosinophil lineage and promote differentiation of eosinophil lineage-committed progenitors (EoPs) into eosinophils are not well defined.

METHODS: To identify regulatory pathways, we performed global expression and epigenomic analyses. GMPs, EoPs and eosinophils were sorted from pooled BALB/c bone marrow. Gene expression profiling was performed via RNA-Sequencing. Genomic distribution of the histone modification H3K4me3 was performed via Chromatin Immunoprecipitation- Sequencing (ChIP-Seq).

RESULTS: Expression analysis revealed 5779, 5016 and 4808 genes expressed by GMPs, EoPs and eosinophils, respectively. In each cell type, 75% of the expressed genes had the positive chromatin H3K4me3 modification. Eosinophils had a greater proportion of H3K4me3 marks located in gene introns than did GMPs or EoPs; the H3K4me3 peak in intron 10 of Ifi54 was the greatest in eosinophils and may mark a novel regulatory element. An eosinophil-lineage transcriptome comprising 938 genes expressed only by EoPs and/or eosinophils (not by GMPs or neutrophils) was identified. We noted that only EoPs and eosinophils (not GMPs or neutrophils) expressed the transcription factors Helios and Aiolos and confirmed Helios and Aiolos expression in murine and human peripheral blood eosinophils. Potential Aiolos binding sites were significantly enriched in the promoters of the top 100 genes expressed by EoPs and eosinophils. Notably, IL-5–stimulated eosinophil yield from Helios-deficient bone marrow cells was significantly decreased, suggesting a role for Helios in eosinophil development.

CONCLUSIONS: Combining expression and epigenetic analyses can identify potential novel regulators, such as the transcription factors Helios and Aiolos, of eosinophil development.

526 A Role of IL1RL1 in Epigenetic Transgenerational Transmission of Asthma

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RATIONALE: Genetic studies to date have been unable to fully explain the observed heritability of asthma. This study examined whether differential DNA methylation within IL1RL1, a candidate gene for asthma that encodes the receptor for the Th2 cytokine IL33, can link grand-maternal and maternal asthma to wheezing in grandchildren.

METHODS: In the Isle of Wight (UK) birth cohort, questionnaire data were obtained from children (F2), their mothers (F1) and grandmothers (F0) regarding asthma and wheezing. DNA methylation was measured in peripheral blood from F1 mothers (n=245) and in cord blood (n=116) from their F2 newborn children using the Illumina Infinium HumanMethylation450 beadchip. We focused on four cytosine-phosphate-guanine (CpG) sites within IL1RL1 and tested whether F0 asthma was associated with F1 methylation of IL1RL1, and whether this methylation was related to asthma within the F1. We repeated these analyses from F1 mothers to F2 infants with wheezing as the outcome. Analyses used linear, log-linear, and mixed linear models.

RESULTS: The IL1RL1 CpG site cg17738684 connects asthma in mothers to asthma in their offspring through the following chain of significant associations: F0 (grand-maternal) asthma — (p<0.028) —> F1 (maternal) cg17738684 methylation — (p<0.0001) —> F1 asthma status at 18 years — (p<0.0001) —> F1 asthma during pregnancy — (p=0.008) —> F2 (child) cg17738684 methylation — (p=0.01) —> F2 incidence of infant wheeze.

CONCLUSIONS: DNA methylation of IL1RL1 cg17738684 is significantly associated with asthma heritability across three generations. This suggests epigenetic marks within asthma associated genes may contribute to its transgenerational inheritance.
527 Circulating Micro-RNAs Are Biomarkers and Potential Therapeutic Targets in Asthma
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METHODS: After IRB approval, miRNAs were isolated from blood by guanidinium/isopropanol purification and profiled by real-time PCR in 38 asthmatics and 29 non-asthmatics. Secretion of exosomes from blood mononuclear cells was assessed in a healthy population after culture of cells in the presence or absence of T-cell stimulation or glucocorticoids. The effect of secreted miRNAs on cytokine production by airway epithelial cells was measured.

RESULTS: Profiling of blood revealed expression differences in 30 miRNAs (21 increased, 9 decreased) in asthmatics vs. non-asthmatics. Cluster analysis of asthmatics demonstrated the presence of two main miRNA expression clusters, which differed by peripheral eosinophil levels. Anti-CD3/CD28 T-cell stimulation of PBMCs from healthy subjects induced expression and exosome secretion of 17 of these miRNAs, and glucocorticoids inhibited production of 3 (miR-155, -374a, -570-3p). Transfection of miR-570-3p and -155 induced numerous cytokines from airway epithelial cells, and identified CCL5 as a central target.

CONCLUSIONS: Circulating miRNAs have potential to diagnose and phenotype asthma, and may be targets of anti-inflammatory therapies. We propose a model where miRNAs participate in communication between T-cells and airway cells such that activation of T-cells induce the production and secretion of miRNAs, which are taken up by airway cells to promote lung inflammation and eosinophilia.

528 Rhinovirus Species and Asthma Exacerbations in Inner-City Children
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METHODS: In a case-control study of asthma exacerbations, we collected blood samples from 328 children (164 controls, 164 exacerbations) with and without asthma. MiRNA expression was measured using TaqMan Gold microRNA Reverse Transcription and TaqMan Gold miRNA Assays (Applied Biosystems). We performed a two-sample t-test to compare miRNA expression between groups.

RESULTS: We found that miRNA expression was significantly different between control and exacerbation groups in children with asthma. In children with asthma, the most significantly upregulated miRNA was miR-155 (p = 0.001), while the most significantly downregulated miRNA was miR-30b (p = 0.002). These results suggest that circulating miRNAs may be a biomarker of asthma exacerbations in inner-city children.

CONCLUSIONS: Circulating miRNAs offer a promising biomarker for asthma exacerbations in inner-city children. Further studies are needed to validate these findings and explore the potential clinical utility of miRNAs as biomarkers.
**530 DNA Methylation and Childhood Asthma in the Inner-City**

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**RATIONALE:** Epigenetic marks, like asthma, are heritable, influenced by the environment, direct the maturation of T lymphocytes, and influence the development of allergic airway disease in mice. We hypothesize that epigenetic markers in circulating immune cells are associated with allergic asthma in humans.

**METHODS:** We compared DNA methylation patterns and gene expression in African American inner city children with persistent atopic asthma versus healthy controls, using DNA and RNA from peripheral blood mononuclear cells (PBMCs). Findings were validated in an independent population of inner city atopic asthmatics. We also examined asthma-associated methylation changes identified in nasal epithelia from a subset of the same subjects.

**RESULTS:** Comparing asthma subjects (N=97) to controls (N=97), we identified 81 regions that were differentially methylated. Several immune genes were hypomethylated in asthma, including IL-13, RUNX3, and specific genes relevant to T lymphocytes (TIGIT). Hypo- and hypermethylated genes were associated with increased and decreased gene expression respectively (P<0.05x10^-11). We further explored the relationship between DNA methylation and gene expression using an integrative analysis and identified additional candidates relevant to asthma (IL-4 and ST2). Methylation marks on genes involved in T cell maturation (RUNX3), Th1 immunity (IL-4), and oxidative stress (Catalase) replicated in an independent asthma cohort of African American children living in the inner city. 16 of the 81 DMRs are also differentially methylated in nasal epithelia of asthmatics (N=36) compared to controls (N=36) with larger percent methylation changes than PBMCs.

**CONCLUSIONS:** Our results define novel methylation-gene transcription relationships that may prove important in asthma.

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**532 Regulation and Production of Interleukin 35 Subunits, p35 and EBI3, in Human Bronchial Epithelial Cells**

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**RATIONALE:** Bronchial epithelial cells are able to control both activation and inhibition of T cell function. IL-35 is a heterodimeric anti-inflammatory cytokine consisting of IL-12A (p35) and Epstein-Barr virus-induced gene 3 (EBI3) and is known to be produced from regulatory T cells. The aim of this study was to investigate whether bronchial epithelial cells control T cell function by the production of IL-35.

**METHODS:** Primary normal human bronchial epithelial (NHBE) cells were stimulated with cytokines and TLR ligands and mRNA and protein expression of p35 and EBI3 were determined by real-time RT-PCR and Western blot.

**RESULTS:** Messenger RNAs for p35 and EBI3 were significantly up-regulated by stimulation with IFN-γ (6.2-fold, 5.3-fold respectively) and poly(I:C) (TLR3 ligand, 10.2-fold, 112-fold respectively) in NHBE (24 hr, n=5). Messenger RNA for EBI3 was also weakly up-regulated by TNF-α, IL-1β, Pam3CSK4 (TLR2/1 ligand) and FSL-1 (TLR2/6 ligand). In addition, mRNAs for p35 (167-fold) and EBI3 (472-fold) were synergistically enhanced by a combination of IFN-γ and poly(I:C) in NHBE (n=5, p<0.05). Proteins for p35 and EBI3 were detected in cell free supernatants of IFN-γ and poly(I:C)-stimulated NHBE but not in unstimulated cells by Western blot.

**CONCLUSIONS:** Expression and production of IL-35 subunits p35 and EBI3 are induced in bronchial epithelial cells by stimulation with IFN-γ and TLR3 ligand. However whether bronchial epithelial cells produce heterodimerized IL-35 or monomeric or homodimeric subunits is still not clear. Further study will be required to clarify the structure of p35 and EBI3 proteins produced from bronchial epithelial cells.

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**531 Dust Mite-Induced Dectin-2 Pathway Triggers IL-33 Generation in Leukotriene C4 Synthase- and CAR9D- Independent Manner**

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**RATIONALE:** House dust mite (HDM) activates the C-type lectin receptor Dectin-2, leading to the generation of leukotriene C4 synthase-(LTC₄S)-dependent cysteinyl leukotrienes (cys-LTs), LTC₄S-dependent Th2 pulmonary inflammation, and CARD9-dependent cytokines. IL-33, a recently described product of Dectin-2 signaling, can also promote Th2 pulmonary inflammation, but the pathway by which it is generated is poorly understood. We sought to determine if IL-33 generation is dependent on the Dectin-2/LTC₄S and/or Dectin-2/CARD9 pathways.

**METHODS:** Bone marrow-derived dendritic cells (BMDCs) were grown from C57BL/6, Dectin-2–/-, CARD9–/-, LTC₄S–/-, and myeloid differentiation primary response 88–/- (MyD88–/-) mice. Day 7 BMDCs were stimulated with extract from the HDM, Dermatophagoides farinae (Df). Wild-type BMDCs were stimulated with Df in the presence or absence of inhibitors of spleen tyrosine kinase (Syk) and phosphoinositide 3-kinase (PI3K). IL-33 in cell lysates was measured by ELISA and quantitative RT-PCR.

**RESULTS:** Df-induced IL-33 is dependent on the Dectin-2 receptor and a Syk and PI3K signaling pathway. IL-33 generation is independent of the Dectin-2/LTC₄S and Dectin-2/CARD9 pathways. In addition, the MyD88 adaptor protein is not required for IL-33 generation in BMDCs.

**CONCLUSIONS:** Dectin-2 signals through divergent pathways after Fc receptor γ chain and Syk activation. The mechanism of IL-33 generation is unique and independent of the well-described Dectin-2/LTC₄S and Dectin-2/CARD9 pathways. This provides a second mechanism by which Dectin-2 can drive Th2 immune responses to HDM.
533 How Well Does Whole Genome Sequencing Improve Ability to Detect Association with Asthma in Candidate Genes Compared to Existing GWAS Platforms in African American Populations?

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RATIONALE: Rhinovirus (RV) is a major cause of common cold. Over 100 RV serotypes have been identified to date, and infections with different strains occur repeatedly. Despite this, pre-existing T cells that might confer protection have not been characterized. We sought to develop a panel of MHC II tetramers displaying RV peptides with a view to phenotyping circulating RV-specific CD4+ T cells.

METHODS: T-cell epitopes of RV-16 and RV-39 capsid proteins were identified by tetramer-guided epitope mapping and their conservation assessed by Jalview. Circulating RV-specific CD4+ T cells were identified in uninfected subjects by enriching PBMCs for tetramer+ cells, counterstaining for other surface markers, and analyzing by flow cytometry.

RESULTS: T-cell epitopes of the RV capsid proteins, VP1 and VP2, mapped to regions that were highly conserved across all RV strains. Additionally, identical and overlapping RV capsid peptides were recognized in the context of multiple HLA alleles, indicating HLA promiscuity among RV epitopes. In healthy subjects, circulating RV-specific CD4+ T cells were predominantly inactivated (CD127+, CD25neg) memory cells restricted to common SNPs (MAF>0.01) with r2>0.8, forcing inclusion of any GWAS SNPs.

CONCLUSIONS: To date, 225 genes from 32 genome-wide association study (GWAS) manuscripts have shown association with asthma, yet only 2 GWAS manuscripts were from African ancestry populations. As part of the Consortium on Asthma among African-ancestry Populations in the Americas (CAAPA), 328 African Americans were whole genome sequenced (WGS). Because current GWAS arrays are inadequate for African populations, we propose analyzing candidate genes in CAAPA will help detect new variants associated with asthma.

METHODS: Candidate genes were selected searching for asthma in GWAS catalog and phenome databases with p-value<1E-5. Logistic regression adjusting for population stratification for common single nucleotide polymorphisms (SNPs) and Fisher’s exact test for rare SNPS was performed in asthma candidate genes on 168 asthma and 160 non-asthmatic African Americans on WGS samples sequenced with Illumina HiSeq 2000 and GWAS samples genotyped with Illumina Omni 2.5. Haplotyping tag coveraging was performed using Haplovigner tagging restricting to common SNPS (MAF>0.01) with r2>0.8, forcing inclusion of any GWAS SNPS.

RESULTS: Analysis was performed on 80 candidate genes analyzed for 231,548 and 17,586 SNPS from WGS and GWAS panels, respectively. The OMNI panel captured 15,098 of 37,739 tag SNPS. WGS analysis uncovered associations with asthma not tagged by OMNI panel in IL13 (rs2243200; P=3.7E-6; MAF=0.04), SCR3 (rs35776517; P=2.3E-4; MAF=0.24), PRKG1 (rs4576762; P=2.7E-4; MAF=0.05), and C11orf71 (rs181308097; P=4.8E-4; MAF=0.03).

CONCLUSIONS: In a sample of 328 African Americans in 80 candidate genes, we demonstrate that a standard GWAS panel captures only 40% of common variation. Association testing with asthma in additional populations of African descent is ongoing.

534 Circulating Rhinovirus-Specific CD4+ T Cells in Uninfected Subjects Recognize Conserved Epitopes

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RATIONALE: Rhinovirus (RV) is a major cause of common cold. Over 100 RV serotypes have been identified to date, and infections with different strains occur repeatedly. Despite this, pre-existing T cells that might confer protection have not been characterized. We sought to develop a panel of MHC II tetramers displaying RV peptides with a view to phenotyping circulating RV-specific CD4+ T cells.

METHODS: T-cell epitopes of RV-16 and RV-39 capsid proteins were identified by tetramer-guided epitope mapping and their conservation assessed by Jalview. Circulating RV-specific CD4+ T cells were identified in uninfected subjects by enriching PBMCs for tetramer+ cells, counterstaining for other surface markers, and analyzing by flow cytometry.

RESULTS: T-cell epitopes of the RV capsid proteins, VP1 and VP2, mapped to regions that were highly conserved across all RV strains. Additionally, identical and overlapping RV capsid peptides were recognized in the context of multiple HLA alleles, indicating HLA promiscuity among RV epitopes. In healthy subjects, circulating RV-specific CD4+ T cells were predominantly inactivated (CD127+, CD25neg) memory cells restricted to common SNPs (MAF>0.01) with r2>0.8, forcing inclusion of any GWAS SNPs.

CONCLUSIONS: To date, 225 genes from 32 genome-wide association study (GWAS) manuscripts have shown association with asthma, yet only 2 GWAS manuscripts were from African ancestry populations. As part of the Consortium on Asthma among African-ancestry Populations in the Americas (CAAPA), 328 African Americans were whole genome sequenced (WGS). Because current GWAS arrays are inadequate for African populations, we propose analyzing candidate genes in CAAPA will help detect new variants associated with asthma.

METHODS: Candidate genes were selected searching for asthma in GWAS catalog and phenome databases with p-value<1E-5. Logistic regression adjusting for population stratification for common single nucleotide polymorphisms (SNPs) and Fisher’s exact test for rare SNPS was performed in asthma candidate genes on 168 asthma and 160 non-asthmatic African Americans on WGS samples sequenced with Illumina HiSeq 2000 and GWAS samples genotyped with Illumina Omni 2.5. Haplotyping tag coveraging was performed using Haplovigner tagging restricting to common SNPS (MAF>0.01) with r2>0.8, forcing inclusion of any GWAS SNPS.

RESULTS: Analysis was performed on 80 candidate genes analyzed for 231,548 and 17,586 SNPS from WGS and GWAS panels, respectively. The OMNI panel captured 15,098 of 37,739 tag SNPS. WGS analysis uncovered associations with asthma not tagged by OMNI panel in IL13 (rs2243200; P=3.7E-6; MAF=0.04), SCR3 (rs35776517; P=2.3E-4; MAF=0.24), PRKG1 (rs4576762; P=2.7E-4; MAF=0.05), and C11orf71 (rs181308097; P=4.8E-4; MAF=0.03).

CONCLUSIONS: In a sample of 328 African Americans in 80 candidate genes, we demonstrate that a standard GWAS panel captures only 40% of common variation. Association testing with asthma in additional populations of African descent is ongoing.

535 Role of Natural Killer (NK) Cell Surface Receptor –NKp46– in Primary Influenza A Infection

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RATIONALE: Components of the pulmonary innate immune system NK cells and the lung collectin surfactant protein D (SP-D) may collaborate in inflammatory and infectious processes but the underlying mechanisms are unclear. We hypothesized that a membrane surface receptor, NKp46 is important in mediating NK cell and SP-D dependent anti-viral immunity.

METHODS: Wild type (WT) and NKp46–/– mice were infected with a lethal dose (1000 pfu) of Influenza A/34/P4/R8 or treated with PBS intranasally. Weight loss, survival and lung function (arterial oxygen saturation; SpO2) were recorded every day post infection. Post-mortem, total and native bronchoalveolar lavage (BAL) SP-D expression was studied by Western blot and native gel electrophoresis in WT and NKp46–/– mice. Pulmonary viral load was determined using plaque assay.

RESULTS: NKp46 Fc stimulated IL-12p70 production from splenic dendritic cells (DC) suggesting the involvement of NK cells in DC function. Seven days post infection total and native SP-D expression decreased ~50 and ~70 fold, respectively, and mice displayed similar weight loss in both the WT and NKp46–/– strains. NKp46–/– mice showed a significantly increased mortality and hazard ratio (p<0.001), lower oxygen saturation (SpO2; p<0.01) and 50% lower expression of total BAL SP-D compared to WT mice. NKp46–/– mice displayed a trend of heightened viral load in the lung compared to WT mice.

CONCLUSIONS: Lack of NKp46 is associated with increased susceptibility to lethal influenza A infection suggesting the importance of NKp46 dependent mechanisms in immune defense. NKp46 dependent anti-viral responses may involve impaired SP-D expression and IL-12p70 production.
536 Bla g 2 Hypoallergens Retaining the Native Fold and Capacity to Modulate T Cell Reactivity Provide Candidates for Cockroach Immunotherapy
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RATIONALE: Modified allergens that display reduced IgE reactivity along with T-cell activating properties, are strong candidates for immunotherapy, because of the potential to decrease side-effects due to IgE cross-linking, but still retain immunogenicity.

METHODS: Single and multiple Bla g 2 mutants were designed according to prior knowledge of the antigenic structure of the allergen and expressed in Pichia pastoris. Folding of the mutants was assessed by CD spectrometry or X-ray crystallography. IgE reactivity was measured by antibody binding and mast cell release assays. T-cell responses were assessed by analyzing Th1/Th2 cytokine production and CD4+ T-cell phenotype in PBMC cultures.

RESULTS: Single and multiple mutations of residues implicated in binding to monoclonal antibodies (K132A, K251A and/or F162Y) reduced IgE reactivity but did not influence the native molecular fold, as proven by comparing the triple mutant with wild type Bla g 2 by X-ray crystallography. As compared with wild type allergen, mutants KK and KKF showed from at least 100-fold to a total reduction in IgE antibody binding. Whereas similar T-cell activating capacity was retained based on CD25 expression, both mutants were weaker inducers of the Th2 cytokine, IL-13. Furthermore, both mutants induced high levels of IL-10 from a non-T-cell source, and levels induced by the triple mutant exceeded those induced by Bla g 2 (p = 0.004).

CONCLUSIONS: A rational design of site-directed mutagenesis was effective in producing candidate molecules for immunotherapy that maintain the same fold as wild type Bla g 2, but display reduced IgE reactivity with T-cell modulatory potential.

537 Protective Role of Hydrogen Sulfide in Paramyxovirus Infection
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RATIONALE: Hydrogen sulfide (H₂S) is a novel gaseous mediator that has gained increasing recognition as an important player in modulating acute and chronic inflammatory diseases, but its role in viral-induced lung inflammation is currently unknown. Respiratory syncytial virus (RSV) and human metapneumovirus (hMPV) are major causes of upper and lower respiratory tract infections in children, for which no vaccine or effective treatment is available. We hypothesized that administration of H₂S during RSV and hMPV infection would reduce viral-induced proinflammatory mediator release and viral replication.

METHODS: Airway epithelial cells were infected with RSV or hMPV, treated with either the slow-releasing H₂S donor GYY4137 or propargylglysin (PAG), an inhibitor of intracellular H₂S production, and harvested to measure cytokine and chemokine secretion, and viral titers. In vivo efficacy was tested in a mouse model of RSV infection treated with GYY4137 by assessing viral replication and disease.

RESULTS: RSV- and hMPV-induced secretion of several cytokines and chemokines, such as IL-8 and RANTES, and viral titers were significantly decreased by GYY4137 treatment, and increased by PAG. Infected mice treated with GYY4137 showed reduced viral titers and attenuated RSV-induced body weight loss, with faster recovery, compared to the untreated mice.

CONCLUSIONS: Modulation of cellular H₂S significantly impacts cellular responses and viral replication in an in vitro and in vivo model of RSV/hMPV infection. Our results underscore an important role of H₂S in regulating virus infection and host defenses that could lead to a novel treatment strategy for paramyxovirus infections, and possibly other respiratory viral infections.

538 Accurate Assessment of Personal Air Pollutant Exposures in Inner-City Asthmatic Children
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RATIONALE: Inner-city environments seem particularly toxic for children with asthma, in part due to air pollutant exposures. Personal monitors that sample from an individual’s “breathing zone” offer a more accurate method than stationary monitors to measure air pollutant exposures and minimize misclassification bias.

METHODS: We performed an observational study to measure inner-city children’s exposure to PM₁₀ (particulate matter <10μm), black carbon (BC), and brown carbon (BrC) using the MicroPEM™ (RTI International), and to NO2 using an Ogawa™ passive badge (Ogawa USA). Fifteen inner-city children (8-15 years) participated in this study, including 12 asthmatics. Eight participants reported exposure to cigarette smokers. Participants were instructed to wear personal monitors during waking hours. Stationary monitors (PEM™, MSP Corp.) were installed into each participant’s bedroom. Targeted sampling period was 72 hours. Exposure levels between personal and stationary monitors were compared via linear mixed models with random intercepts for sibling pairs.

RESULTS: Waking wearing compliance (WWC) was excellent in 87% of participants, with a median WWC of 83% for those with acceptable WWC (>60%). Personal monitor levels were significantly higher than, and correlated variably with, stationary monitor levels for PM₁₀ (>30% higher, p = 0.023; intraclass correlation coefficient (ICC) = 0.342, 95% confidence interval [0.254;0.399]), BC (>7-fold higher, p = 0.0001; ICC = 0.082 [-0.429;0.594]), and BrC (>4-fold higher, p = 0.0001; ICC = 0.635 [0.166;1.103]). NO₂ levels did not differ significantly.

CONCLUSIONS: Accurate exposure assessment using personal exposure monitors, such as the MicroPEM™, are feasible for use with inner-city children. Stationary monitors inaccurately estimate personal exposure to PM, as they do not capture the higher concentrations found near strong PM emission sources.
539 High Dose Acetaminophen Fails to Promote Airway Hyper-Reactivity Ex Vivo and Is Both Bronchoprotective and Bronchodilatory

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RATIONALE: Epidemiologic studies demonstrate an association between acetaminophen (APAP) use and the development of asthma symptoms in children. No studies have examined potential relationship between APAP exposure and mechanisms for asthma exacerbations. Given established hepatotoxicity of APAP, we hypothesized that acute APAP exposure, APAP metabolism and APAP-protein adduct formation enhances bronchoconstrictor responses thereby causing airway hyperreactiveness (AHR).

METHODS: APAP metabolism and adduct formation in lung tissue was evaluated by immunocytochemistry after in vivo APAP exposure in mice and ex vivo exposure in human precision cut lung slices (PCLS). Airway bronchoconstriction and bronchodilatation were measured in human PCLS airways by microscopy after acute exposure to APAP ex vivo.

RESULTS: In mouse and human airways, exposure to APAP generated APAP protein adducts in airway epithelial cells, verifying drug metabolism. Neither mouse nor human airways were hyper-responsive to bronchoconstrictor agents after exposure to APAP. Carbadox-induced bronchoconstriction was reduced by 92% and 70%, respectively, in airways from mice treated in vivo with 200mg/kg APAP for 1 or 24 hours and 5mM APAP treatment ex vivo reduced bronchoconstriction in human PCLS airways by 42%. Treatment with APAP after bronchoconstriction with carbadox, histamine or IgE cross-linking resulted in dose-dependent bronchodilatation (63-/+-10% at 5mM) that was sustained for hours. APAP also led to bronchodilatation of airways that were previously desensitized to bronchodilator by salmeterol.

CONCLUSIONS: APAP is metabolized in airway epithelial cells both in vivo and ex vivo leading to protein-adduct formation. Acute exposure to APAP does not promote AHR, is protective against bronchoconstriction, and is mildly bronchodilatory.

540 Agreement Between Caregiver Report and Hospital and School Records

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RATIONALE: Both self-reported, asthma-related medical services utilization and school absenteeism and claims data/hospital/school records have been used in intervention studies to determine outcomes and identify cases of poorly controlled asthma. We examined the concordance between self reports and claims/hospital/school data for children enrolled in a real-world school-based asthma program (“Building Bridges”).

METHODS: Parents of children with asthma were recruited from 3 elementary schools in Hartford. CT. Caregiver reports of asthma-related school absences, emergency department (ED) visits, and hospitalizations were collected by questionnaire and compared to school records and hospital data from the only pediatric hospital in Hartford. The unweighted Kappa statistic (κ) was computed to examine how often caregiver reported results agreed with actual medical and school records accounting for chance.

RESULTS: For the 2012-13 school year, parents of 67 students (58%M; age 8.4±2.2 yrs (mean ± SD), 76% Hispanic (primarily Puerto Rican), 18% African American) reported 3.2±4.3 school days missed; 0.7±0.9 asthma-related hospitalizations and 2.1±1.6 ED visits. The concordance between caregiver and hospital report for hospitalizations was fair (κ = 0.291, 95% CI,0.093,0.488); school absence concordance showed slight agreement (κ = 0.135, 95%CI, -0.005, 0.276). ED visits (κ = 0.051, 95%CI,0.045, 0.146) demonstrated the poorest agreement between hospital records and caregiver report.

CONCLUSIONS: Overall, concordance between self-reports and hospital/school records ranged from fair to poor. Investigators should consider these differences when using data from different sources to target students with poorly controlled asthma for enrollment into school-based asthma programs.

541 RNA Sequencing Identifies ANKRD1 As a Novel Anti-Viral Gene Downregulated in Atopic Dermatitis Complicated By Eczema Herpeticum

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RATIONALE: A subset of atopic dermatitis (AD) patients is prone to disseminated herpes simplex virus (HSV) infection, i.e. eczema herpeticum (AHE+). To search for novel AHE+ gene signatures, a RNA-sequencing (RNA-seq) approach was applied to evaluate global transcriptional changes of peripheral blood mononuclear cells (PBMCs) between AHE+ and AD without a history of EH (AHE-).

METHODS: RNA-seq was used to compare transcriptomic changes of PBMCs between AHE+ and AHE-. Differentially expressed genes were validated by qPCR in 20 AHE+ and 20 AHE-. Various molecular biology methods were performed to characterize candidate genes.

RESULTS: Distinct transcriptomic changes were found between AHE+ and AHE- PBMCs following HSV-1 stimulation with 792 genes differentially expressed at a false discovery rate < 0.05 (ANOVA). The Ankyrin repeat domain 1 (ANKRD1) gene, not previously implicated in host anti-viral defense, was significantly induced by HSV-1 stimulation in both ADEH- and ADEH+, however, its induction in ADEH+(143 fold greater in HSV-1-stimulated than sham treatment) was significantly lower as compared to ADEH+(~1000 fold greater in HSV-1-stimulated than sham) (p<0.05). ANKRD1 was induced by HSV-1 only in antigen presenting cells (APCs), but not in T cells and NK cells. Silencing ANKRD1 in APCs led to increased HSV-1 viral loads and decreased type 1 and type III interferon production. Using co-immunoprecipitation method, ANKRD1 was pulled down with IRF3, an important transcription factor required for anti-viral signal pathways.

CONCLUSIONS: ANKRD1 is involved in IRF3-related anti-viral innate immune response in APCs. Its reduced expression in ADEH+ subjects may serve as a biomarker to identify AD patients prone to ADEH+.
542 Immunization with ARA h1,2,3-Lamp-Vax Peanut Vaccine Blocked IgE Mediated-Anaphylaxis in a Peanut Allergic Murine Model

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RATIONALE: The prevalence, severity and life-long nature of peanut allergy (PNA) lend particular urgency to develop strategies to treat this disease. This study determined the effects of ARA h1,2,3-LAMP-Vax, a peanut vaccine, on peanut allergic mice.

METHODS: Five-week-old C3H/HeJ mice were sensitized with 10 mg peanut and 20µg cholera toxin (CT) for 5 weeks and boosted with 50 mg peanut and 20µg CT at weeks 6 and 8. Mice with established PNA were treated with ARA h1,2,3-LAMP-Vax or control vector intradermally (i.d.), weekly for 4 weeks. Three weeks post-therapy, mice were challenged intragastrically with peanut. Anaphylactic reactions, plasma histamine, peanut-specific IgE levels, and cultured splenocyte (SPC) and mesenteric lymph node (MLN) cell cytokine production were measured.

RESULTS: Prior to treatment, there was no significant difference in serum peanut-specific IgE levels between peanut sensitized groups. Three weeks post therapy, ARA h1,2,3-LAMP-Vax treated mice exhibited approximately 70% lower serum peanut-specific IgE levels than vector control treated mice (2,911.3 Vs 887.9ng/ml, p<0.05). ARA h1,2,3-LAMP-Vax treatment increased peanut-specific IgG2a (p<0.05), but not IgG1 levels. ARA h1,2,3-LAMP-Vax-treated mice also showed significantly lower symptom scores, higher core body temperatures and lower plasma histamine levels following challenge than control vector-treated mice (p<0.05 for all). SPCs and MLN cells from ARA h1,2,3-LAMP-Vax-treated mice produced less IL-4 and more IFN-γ and IL-10 than cells from control vector-treated mice.

CONCLUSIONS: ARA h1,2,3-LAMP-Vax administration produced significant protection against peanut induced anaphylactic reactions in peanut allergic mice. This study shows that ARA h1,2,3-LAMP-Vax has potential as a novel therapy for peanut allergy.

543 Clinical and Immunological Effects of Aspirin Desensitization in Patients with Aspirin Exacerbated Respiratory Diseases; A Randomized, Double Blind, Placebo Controlled Trial

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RATIONALE: The effect of aspirin desensitization (AD) on clinical and immunological profile of patients with aspirin exacerbated respiratory diseases (AERD) has been poorly understood.

METHODS: This randomized double blind placebo controlled trial comprised of 32 adult patients with AERD (NCT01867281). Participants were randomly assigned to equal ratio active and placebo groups. Active group underwent AD over a 2-day period with increasing doses of aspirin (ketorolac sprays, 60, 125, 325 and 625 mg) and followed by receiving aspirin 625 mg twice daily for 1 month and 625 mg for 5 months. Frequency of asthma attacks, Sino-Nasal Outcome Test (SNOT22) scores, pulmonary function tests, Lund Mackay scores, medication scores, and serum levels of interleukin (IL)-10, transforming growth factor beta (TGF-β) and interferon gamma (IFN-γ) were investigated at baseline and end of first and sixth months of follow up.

RESULTS: Symptoms scores and medication needs of patients with AERD underwent AD were significantly lower compared to placebo group after 6 months (7.5±3.5 vs. 10.6±3.8 and 9.3±2.2 vs. 11.0±3.1, respectively, all p<0.05). Forced expiratory volume in one second (FEV1) was significantly higher in active arm after 6 months1±.6.8 vs. 80.1±7.4, p=0.009). Frequency of asthma attacks was lower in active versus placebo group25% vs. 50%, p=0.137). However, no significant difference was observed in serum concentration of IL-10, IFN-γ and TGF-β between two groups neither at baseline nor at the end of study.

CONCLUSIONS: The clinical benefits of AD on patients with AERD are not coupled with any significant change in systemic anti-inflammatory regulation markers.

544 Dupilumab Improves Patient-Reported Outcomes (PROs) in a Phase 2 Study in Adults with Moderate-to-Severe Atopic Dermatitis

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RATIONALE: Atopic dermatitis (AD) is associated with substantial patient burden. Dupilumab, a fully-human monoclonal antibody against the interleukin-4 receptor-α, has demonstrated symptomatic efficacy in AD.

METHODS: 380 adults with moderate-to-severe AD were randomized 1:1:1:1:1 to 16-week treatment with subcutaneous placebo or dupilumab 100mg every 4 weeks (q4w), 300mg-q4w, 200mg every 2 weeks (q2w), 300mg-q2w, or 300mg weekly (qw) (NCT01859988). Assessments included PROs of Pruritus Numeric Rating Scale (NRS), SCORing Atopic Dermatitis (SCORAD), Patient Oriented Eczema Measure (POEM), Hospital Anxiety and Depression Scale (HADS), Dermatology Life Quality Index (DLQI), and EuroQol-5D (EQ-5D).

RESULTS: Mean age was 37 years; mean disease duration was 28 years. At 16 weeks, dupilumab significantly reduced itch on NRS (P<0.005 all doses). Quality of life (QOL) improved at all dupilumab doses except 100mg-q4w (P<0.05), along with improvements in sleep on items of SCORAD and POEM measures (P<0.0005 all doses except 100mg-q4w). Dupilumab also significantly reduced symptoms of depression and anxiety on HADS; number of patients with scores >11 indicating probable cases of anxiety or depression decreased by 66.7% to 75% in dupilumab groups vs. 22.2% in placebo group (P<0.05 all doses). Quality of life (QOL) improved at all dupilumab doses except 100mg-q4w (DLQI, P<0.0001; and EQ-5D, P<0.05). The most common adverse events (dupilumab doses combined vs. placebo) were nasopharyngitis (20.6% vs. 21.3%), headache (11.1% vs. 3.3%), and injection site reaction (9.5% vs. 3.3%).

CONCLUSIONS: In adults with moderate-to-severe AD, dupilumab significantly reduced patient-reported itch relative to placebo, with concomitant improvements on PROs that evaluated sleep, mood, and QOL.
**545** Fpies Epidemiology in Australia: Results from a 2-Year Prospective Population Study

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**RATIONALE:** This is the first prospective population based study examining acute food protein induced enterocolitis syndrome (FPIES).

**METHODS:** Every month, between January 2012 until May 2014, clinicians notified the Australian Pediatric Surveillance Unit when a new diagnosis of FPIES was made in infants < 24 months of age.

**RESULTS:** 242 confirmed cases were recorded. Incidence of FPIES was 1 per 10,000 children < 2 years of age. 75% children presented ≤ 6 months of age. 8% of children had siblings with a history of FPIES. Children with vomiting and diarrhea were significantly more likely to require IV fluid resuscitation compared to those with vomiting without diarrhea (p < 0.05). 70% of children with FPIES reacted to only one food trigger. Rice was the commonest trigger, followed by cow’s milk, egg, oats, and chicken/fish. These foods caused 73% of all reactions. 21 out of 72 children with cow milk FPIES had soy and 12 (57%) reacted. Children with rice FPIES were more likely to react to oats (40%), compared to corn (18%) or wheat (7%) (p < 0.05 for all comparisons).

**CONCLUSIONS:** FPIES is not rare. FPIES in siblings occurred in 8% of cases. IV fluid resuscitation was more likely to occur in the presence of diarrhea. Rice was the commonest trigger in Australia, and most children reacted to only one food. Most children with rice FPIES tolerated other grains apart from oats, and in 50% of cases, soy was tolerated in children with cow milk FPIES.

**546** Indoor Tobacco Legislation and Emergency Department Visits for Asthma in Children

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**RATIONALE:** Since the first statewide indoor tobacco legislation was implemented in California in 1995, numerous cities and states have adopted laws that ban smoking in public indoor spaces. The rationale for these policies has been to protect nonsmokers from the adverse health effects of second hand smoke. We hypothesized that the implementation of indoor smoking legislation would be associated with a decrease in severe asthma exacerbation in children.

**METHODS:** This retrospective analysis utilized a natural experiment to estimate the impact of clean indoor air legislation on the rate of emergency department (ED) admissions for asthma exacerbation in children (< 18). Data were obtained from the Pediatric Health Information System (PHIS). A Poisson regression was used for analyses and controlled for age, gender, race, payer, seasonality, and secular trends.

**RESULTS:** Asthma ED visits were captured from 20 hospitals in 14 different states plus the District of Columbia from July 2000 to January 2014 (n=335,929). Indoor smoking legislation, pooled across all cities, was associated with a decreased risk of severe asthma exacerbation in each of the first 3 years post law implementation. [1st year post law: aRR = 0.92 (95% CI (0.90, 0.94) p < 0.0001; 2nd year post law: aRR = 0.89 (95% CI (0.87, 0.91) p < 0.0001; 3rd year post law: aRR = 0.83 (95% CI (0.82, 0.85) p < 0.0001].

**CONCLUSIONS:** Indoor tobacco legislation is associated with a decrease in emergency department visits for asthma exacerbation. States and municipalities that have yet to implement such protective laws should be called to action in order to protect the respiratory health of their children.
548 Breastfeeding Is Associated with Infant Gut Microbial Composition
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RATIONALE: Breastfeeding has been shown to influence the development of allergic disorders, with results varying by maternal history of disease and feeding exclusivity and duration. Breastfeeding may affect allergic outcomes through its effects on the infant’s gut microbiome. Therefore, we examined the stool microbiomes of breastfed versus non-breastfed babies in a population-based birth cohort.

METHODS: Microbiomes of infant stools (N=298) collected at 1 month (range 1-<6) and at 6 months (range 6-11) after birth from the Detroit WHEALS birth cohort were characterized by MiSeq sequencing of the 16S rRNA gene. Indices of stool microbial community composition richness, evenness and diversity were calculated by breast feeding exposure. Compositional differences in the microbiome were evaluated using permutational multivariate analysis of variance. Tests of differential operational taxonomic unit (OTU) abundance were performed using zero-inflated negative binomial regression with false discovery rate adjustment (q-value<0.05 considered significant).

RESULTS: Current breastfeeding at 1 month was associated with lower stool richness, evenness and diversity, and distinct microbial compositions at both 1 and 6 months (all p<0.002). Similar associations were found for current breastfeeding at 6 months and the concurrent stool samples. In both the 1 and 6 month stools, non-breastfed babies were primarily significantly enriched with Lachnospiraceae, while breastfed babies were significantly enriched with a multitude of phylogenetically diverse bacteria.

CONCLUSIONS: Our results show that breastfeeding at age 1 and 6 months is associated with distinct microbiomes in infants, suggesting that feeding patterns could contribute to stool microbiome characteristics and subsequently to immune development and the evolution of allergic diseases.

549 Comparative Effectiveness of Stepping Down Asthma Medications in a Nationally Representative Sample
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RATIONALE: Limited data exist for outcomes after stepping-down asthma medications.

METHODS: Individuals were identified from the Medical Expenditure Panel Survey from between 2000-2010 by applying the Healthcare Effectiveness Data and Information Set criteria for persistent asthma; each individual was in the study for 2 years which were divided into 5 periods which were about 4-5 months each. Eligibility for stepping-down asthma medications was defined as having no hospitalizations for asthma and no emergency department (ED) visits for asthma in periods 1-3, and no systemic corticosteroid for asthma and <3 rescue inhalers dispensed in periods 2-3. Step-down was defined by a decrease of >1 steps when comparing period 4 to 3. The primary outcome of complete asthma control in period 5 was defined as no asthma hospitalizations, ED visits, systemic corticosteroid dispensing, and <2 rescue inhalers dispensed.

RESULTS: Controlling for age, sex, ethnicity, poverty, self-reported health, Charlson score, smoking status, geographic region, period length, metropolitan statistical area, specialty care, baseline step level, depression, COPD, GED, and rhinitis/sinusitis, the percentage of individuals who were eligible to step-down, did step-down, and had complete asthma control was 89.4% (95% CI 86.4-92.4%), which compares favorably with those similarly eligible for step-down who maintained their current step level, 83.5% (95% CI 79.9-87.0%).

CONCLUSIONS: In a nationally representative sample adjusted for available variables, stepping-down asthma medication in those who were eligible led to clinically similar outcomes compared to those who maintained their step level.

550 Breath Connection: A School-Based Telemedicine Program for Rural Children with Asthma
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RATIONALE: To improve translation of guidelines-based asthma recommendations to high-risk children living in rural regions.

METHODS: We are conducting a cluster randomized trial with rural children, ages 7-14 years, to compare a school-based telemedicine intervention to usual care. The intervention provides comprehensive asthma education via telemedicine to rural children with asthma, their caregivers and school nurses; prospectively monitors asthma symptoms and lung function; and provides primary care providers with evidence-based treatment prompts.

RESULTS: To date, 364/414 parent-child dyads have been enrolled from 17 school districts in the rural Mississippi Delta region of Arkansas. Median age of children enrolled is 9.6 years, with 54.6% being male, 81.8% African-American, 80% with state-issued insurance and 45.6% from a family with total household income <$15,000. At baseline, 72.2% children were classified as moderate-severe persistent asthmatics and 72.1% were uncontrolled according to national guidelines. At 3 months, days wheezing (P=0.03) and peak flow meter use (P<0.0001) significantly improved in the intervention group compared to usual care. At 6 months, peak flow meter use among intervention participants remained statistically significant (P=0.007) and intervention participants had 2.62 vs 1.83 days improvement in the number of symptom free days (SFD) in the previous 2 weeks compared to usual care participants.

CONCLUSIONS: The Breath Connection program aims to improve asthma outcomes for a high-risk, rural cohort through school-based telemedicine education and monitoring. We present results from a planned interim analysis of data. Results are encouraging and suggest positive benefits of the intervention.
**551 Investigation of Molecular Characteristics of Aspirin Exacerbated Respiratory Disease**
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**RATIONALE:** Aspirin Exacerbated Respiratory Disease (AERD) consists of chronic rhinosinusitis with nasal polyps (CRSwNP), asthma, and a hypersensitivity to inhibitors of the cyclooxygenase 1 enzyme. Compared to CrSwNP, patients with AERD tend to have more severe sinus disease, dependence on oral corticosteroids, and increased numbers of eosinophils present in nasal polyps (NP). The underlying mechanisms of AERD and CrSwNP pathogenesis are not entirely understood, and we have assessed whether differences in inflammatory mediators could distinguish between these diseases.

**METHODS:** Nasal polyp tissue was obtained during routine endoscopic sinus surgery from patients with AERD or CrSwNP. Asthma and steroid use were controlled to focus on differences unique to AERD. RNA was isolated from NP and a comprehensive microarray analysis was performed. Separately, NP mRNA and protein levels of various inflammatory mediators were measured by RT-PCR and Luminex bead array, respectively.

**RESULTS:** There were increased levels of eosinophil cationic protein, a marker of tissue eosinophilia, in NP from AERD (3,937ng/mg total protein) compared to CrSwNP (763ng/mg total protein, p<0.001). However, there was no significant difference in type 2 cytokines and CCR3 ligand protein expression between the two groups. Microarray analysis revealed at least 5 genes that appeared to be uniquely elevated in NP of AERD, including IL-5 receptor alpha (p<0.05) and 5-lipoxygenase activating protein (p<0.05) as well as 5 genes heretofore not associated with AERD. Confirmation of the microarray findings by RT-PCR and protein analysis is ongoing.

**CONCLUSIONS:** AERD is a complex disease with unique molecular mechanisms contributing to pathogenesis that may distinguish it from CrSwNP.

**552 Epithelial Cell-Derived Cytokines Contribute to the Pathophysiology of Eosinophilic Chronic Rhinosinusitis**
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**RATIONALE:** The epithelial cell-derived cytokines, TSLP, IL-25, and IL-33 induce T helper 2 cytokine-dependent immune responses and play key roles in allergic airway inflammation. Eosinophilic and non-eosinophilic chronic rhinosinusitis with nasal polyps display distinct patterns of inflammation in the western countries. This study was investigated to correlate between the presence of TSLP, IL-25, or IL-33 and the disease severity in ECRS.

**METHODS:** Nasal tissue specimens were collected from CRS patients, and assayed for TSLP, IL-25, IL-33, PAR-2, and P2Y2R by RT-PCR, ELISA, and immunofluorescence staining. Cytokine productions from cultured nasal epithelial cells (PNECs) were also examined by ELISA.

**RESULTS:** The mRNA expression of TSLP and IL-25 and the concentrations of IL-25 and IL-33 were significantly increased in PNECs from ECRS patients. Immunohistochemical staining demonstrated that TSLP, IL-25, and IL-33 were localized in the epithelial cells of nasal polyps, and the expression levels were increased in ECRS. The mRNA expression levels of TSLP and IL-25 were correlated with the clinical severity of ECRS, as per the CT score. The mRNA of TSLP and the protein of IL-33 were correlated with the number of eosinophils in nasal polyp of ECRS. The mRNA and tissue expression levels of PAR-2 and P2Y2R were significantly increased in cultured PNECs and nasal polyps from ECRS patients.

**CONCLUSIONS:** The results indicate that both increased induction and expression of TSLP, IL-25 and IL-33 from nasal epithelial cells contribute to the pathophysiology of ECRS.

**553 Increased ILC2s in the Eosinophilic Nasal Polyp Endotype Are Associated with Corticosteroid Responsiveness**
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**RATIONALE:** Group 2 innate lymphoid cells (ILC2s) have been identified in human nasal polyps. Whether numbers of ILC2s differ in nasal polyp endotypes (eosinophilic vs. non-eosinophilic), and whether ILC2 number is influenced by corticosteroids is not known.

**METHODS:** Nasal polyp or sinus mucosal samples were collected from 25 human subjects. ILC2s, eosinophils, and FceR1+ cells were quantified by FACS (in subjects treated or not treated with corticosteroids). Eosinophils were also confirmed by independent polyp section scoring. To determine ILC2 corticosteroid responsiveness in-vivo, wild-type mice were challenged with intranasal Alternaria alternata extract and received either oral dexamethasone or vehicle. Numbers of apoptotic lung ILC2s (Annexin V+) were quantified in vivo and in vitro.

**RESULTS:** Eosinophilic nasal polyps contained over double the number of ILC2s compared with non-eosinophilic polyps and sinus mucosa (p<0.05). Levels of eosinophils, but not FceR1+ cells, correlated with ILC2s (r²=0.36 vs. 0.0). Polyp ILC2s were reduced by 50% in patients treated with systemic corticosteroids (p<0.05). Topical corticosteroid use did not correlate with ILC2 numbers. Further, Ki-67+ proliferating and Th2 cytokine-producing ILC2s were greatly reduced in lungs of Alternaria-challenged mice receiving oral dexamethasone. Finally, ILC2 Annexin V staining revealed extensive apoptosis after corticosteroid treatment in vivo and in vitro.

**CONCLUSIONS:** ILC2s are elevated in the eosinophilic polyp endotype and systemic corticosteroid treatment reduced ILC2s. Further, allergen-challenged mice showed increased ILC2 apoptosis after corticosteroid treatment. Our studies suggest that ILC2s may be responsive to systemic, but not topical, corticosteroids in eosinophilic respiratory disease.
A New Strategy for Allergen-Specific Regulation of Allergic Rhinitis: The Use of Monoclonal Antibody Fab Fragments to Pathogenic Allergen

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RATIONALE: Antibody Fab fragments (Fabs) have the ability to bind to specific allergens but lack the Fc portion for binding to receptors on immune and inflammatory cells that play a critical role in allergic diseases. In the present study, we investigated whether Fabs of an allergen-specific IgG1 monoclonal antibody (mAb) inhibited allergic rhinitis in mice.

METHODS: BALB/c mice sensitized by intraperitoneal injections of ovalbumin (OVA) or Japanese cedar pollen (JCP) plus alum on days 0 and 14 were intranasally challenged with OVA or JCP on days 28-30, and 35. Fabs prepared by the digestion of an anti-OVA (O1-10) or JCP IgG1 (P1-8) mAb with papain were also intranasally administered 15 min before each OVA or JCP challenge.

RESULTS: Treatment with O1-10 or P1-8 Fabs significantly suppressed the sneezing frequency, associated with a decrease of OVA- or JCP-specific IgE in the serum and infiltration by mast cells in the nasal mucosa seen following the fourth antigenic challenge; additionally, the serum level of mouse mast cell protease-1, a marker of mast cell activation, was decreased. Furthermore, infiltration of eosinophils and goblet cell hyperplasia in the nasal mucosa at the fourth challenge were inhibited by treatment with O1-10 or P1-3 Fabs.

CONCLUSIONS: These results suggest that intranasal exposure to Fabs of a pathogenic allergen-specific IgG1 mAb may be effective in regulating allergic rhinitis through Fab capture by Fabs in the nasal mucosa before the interaction of the intact antibody and allergen.

Subcutaneous Allergen Immunotherapy in Patient with "Local Allergic Rhinitis" Sensitized to Dermatophagoides pteronyssinus

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RATIONALE: This study investigated the efficacy and safety of subcutaneous allergen immunotherapy (AIT) with Dermatophagoides pteronyssinus(DP) in patients with local allergic rhinitis (LAR).

METHODS: A randomized, double-blind, placebo-controlled, parallel-group, phase II study was conducted. Thirty-six subjects with LAR to DP were randomized to receive AIT (Pangramin PLAUS, ALK-Abelló, S.A., Dermatophagoides pteronyssinus) (AIT-DP) or placebo for 24 months. The primary endpoint was total symptoms (TSS) and total medication scores (TMS). Secondary endpoints included: total combined symptom+medication scores (TCS), daily symptoms score (DSS), daily medication score (DMS), medication free days (MFD), skin testing, nasal allergen provocation test (NAPT-DP), and adverse events. Serum and nasal lavage samples were obtained for immunological studies.

RESULTS: Twenty-eight patients completed the study. AIT-DP produced a significant improvement in the primary endpoints compared to placebo (a 47% of reduction in TSS (0.60 vs 1.14; p<0.001) and a 51.2% in TMS (0.65 vs 1.34; p=0.002). Moreover, at 6-12-18-24 months significant improvements in TCS (p=0.046; p<0.037; p=0.011; p=0.007) and DSS (p=0.003; p=0.012; p<0.001; p<0.001); and at 24 months in DMS (p=0.014), and MFD (p=0.031) compared to placebo were observed. AIT-DP induced an objective improvement in nasal tolerance to NAPT-DP at 6-12-18-24 months (p=0.003; p<0.001; p<0.001; p<0.001) compared to placebo, with negative responses in the 50% of patients. AIT-DP was well-tolerated, one patient had a local moderate reaction solved without pharmacologic treatment. No systemic reactions occurred.

CONCLUSIONS: We prove that AIT with Dermatophagoides pteronyssinus is an effective and well-tolerated treatment in LAR patients. With this work we provide the indication for AIT in LAR.

Respiratory Syncytial Virus Infects IL-13+ Group 2 Innate Lymphoid Cells Via TSLP

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RATIONALE: Respiratory syncytial virus (RSV) infection is the leading cause of infant hospitalization and is correlated with the subsequent development of childhood asthma. Clinical disease ranges from mild upper respiratory tract infection to bronchiolitis, viral pneumonia, and potentially death. Several lines of evidence support a role for inappropriate or excessive immune responses in the development of RSV-induced airway dysfunction. In the mouse model of RSV infection, IL-13 stimulates mucus production and enhanced airway reactivity. Recently described group 2 innate lymphoid cells (ILC2) are resident in the lungs and can potentially produce IL-13. We hypothesized that ILC2 contribute to early production of IL-13 during RSV infection.

METHODS: We infected 8-week old BALB/c, IL-33-deficient, or TSLP receptor-deficient mice with RSV clinical isolate strain 01/2-20 and harvested lungs for flow cytometry or ELISA in accordance with approved animal protocols.

RESULTS: We identified a threefold increase in the number of IL-13+ ILC2 in the lungs in RSV-infected mice compared to mice infected with vehicle or UV-inactivated virus at day 4 post infection. Concurrent with this finding, we identified an increase in the total concentration of IL-13 in the lungs. Additionally, we identified significant increases in the concentration of ILC2 stimulatory cytokines IL-33 and TSLP in the lungs by 12 hours post infection. Moreover, TSLP receptor-deficient mice, but not IL-33-deficient mice, showed reduced numbers of IL-13+ ILC2 after RSV infection.

CONCLUSIONS: These data suggest ILC2 are a significant source of IL-13 early during RSV infection via a TSLP-dependent mechanism.
557  Mast Cells Expressing the Germline HPS1 16-Bp Duplication (c.1470_1486dup16, Hermansky-Pudlak Syndrome-1) Defect Produce Extracellular Matrix Components

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RATIONALE: In the process of developing the Hermansky-Pudlak (HP) mastocyte (M) cell line from a patient with HPS-1, we noticed matrix production on culture flask surfaces which was reduced in clones transfected with wild type HPS1. Since human mast cells are associated with pulmonary fibrosis in patients with HPS-1, HPM cells were examined for production of matrix components.

METHODS: Stable transfection and HPS1 overexpression in HPM clones was performed using GeneCopoeia third generation HIV-based lentiviral vector system and human HPS-1 ORF cDNA lentiviral particles. Matrix ultrastructure was examined sequentially using scanning electron (SE) and transmission electron (TE) microscopy (M) of fixed control (cHPM) and transfected (tHPM) cells. Analysis of extracellular matrix-associated genes including collagen I, IV, V, laminin, fibronectin and galectin-3 from cHPM and tHPM was performed using cDNA and Affymetrix GeneChip RNA Array. Western blot (WB) was also performed for quantification.

RESULTS: SEM and TEM showed increasing formation of globular and fibrillar matrix forms over 8 weeks. Microarrays showed a shift in expression of genes coding for collagen, laminin, fibronectin and galectin-3 from cHPM when compared with tHPM. WB confirmed elevated fibronectin and galectin-3 from HPM cell lysates.

CONCLUSIONS: HPM cells in culture are thus capable of producing fibronectin and galectin-3 extracellular matrix components which are down regulated in HPM cells transfected with a normal HPS-1 gene. These observations support the possibility in vivo that human HPS-1 mast cells may be contributing directly to pulmonary fibrosis in certain patients, and that treatment targeting the mast cell compartment should be considered.

558  Leukotriene C4 Potentiates IL-33-Induced ILC2 Activation and Lung Inflammation through CysLT1R

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RATIONALE: Cysteinyl leukotrienes (CysLTs) and IL-33 are increased in asthma and have been shown to activate group 2 innate lymphoid cells (ILC2s). Whether IL-33 and leukotriene C4 (LTC4), the parent CysLT1, synergistically increase lung inflammation and ILC2 activation is unknown.

METHODS: Wild-type, CysLT1 receptor knockout (CysLT1R-/-), CysLT2 receptor knockout (CysLT2R-/-), and IL-7R knockout (IL-7R-/-) mice were challenged intranasally with 100ng of recombinant IL-33, 100ng of LTC4, or both compounds once per day for 3 days. Mice were euthanized one day after the last challenge. Bronchoalveolar lavage (BAL) and lungs were collected and levels of eosinophilia as well as ILC2 IL-5 production and proliferation were assessed by flow cytometry.

RESULTS: LTC4 synergistically increased IL-33 induction of lung and airway eosinophilia (2-fold above IL-33 alone) in wild-type mice. IL-33 and LTC4 did not induce lung or BAL eosinophilia in IL-7R-/- mice that lack ILC2s. LTC4 increased Ki-67+ proliferating ILC2s by nearly 3-fold and potentiated ILC2 and airway Th2 cytokine production above IL-33 alone. CysLT1R-/- mice showed significant impairment in the induction of lung eosinophilia and ILC2 proliferation after exposure to LTC4 and IL-33. In contrast, CysLT2-/- mice had similar levels of eosinophils and ILC2 proliferation compared with wild-type mice.

CONCLUSIONS: LTC4 synergistically increases IL-33-induced lung eosinophilia and ILC2 activation and is dependent on CysLT1R.

559  Immunoproteomic Analysis of German Cockroach (Blattella germanica) Reveals Antigens Differentially Recognized As a Function of Disease Severity

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RATIONALE: Sensitivity to German cockroach (GCR) is a major health problem in inner-city children and is strongly linked with development of asthma, however the T cell response to GCR has been largely uncharacterized.

METHODS: In this study, we used a transcriptomic/proteomic approach to identify novel GCR allergens. In addition, we characterized the CD4 T cell epitope response to the known and novel GCR allergens in 72 allergic adult donors with differing asthma severities (17 none, 33 intermittent, 4 mild, 5 moderate, or 13 severe) compared to 20 non-allergic controls.

RESULTS: We identified 13 novel GCR allergen through our transcriptomic approach. Interestingly, we found that greater than 50% of the response in allergic individuals is focused on only three allergens of the 38 total screened, Bla g 4, Bla g 5, and vitellogenin. Allergic donors with mild asthma had three-fold higher responses, primarily T cell, to alpha-amylase and Bla g 1 than other allergic donors. In contrast, the response to Bla g 4 was restricted to only those allergic donors with no clinical indications of asthma. Donors with severe asthma had the highest responses to the allergen Bla g 5.

CONCLUSIONS: Our data suggest that development of asthma in GCR allergic individuals is correlated with a loss of CD4 T cell response to Bla g 4 and an increase in the response to alpha-amylase, Bla g 1, and Bla g 5. These data raise the interesting possibility that controlling the responses to specific GCR allergens may assist in the treatment of GCR allergy-associated asthma.
Identification and Characterization of Leucine-Rich Repeat Containing Protein 31 (LRRC31) in Eosinophilic Esophagitis

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RATIONALE: Eosinophilic esophagitis (EoE), an allergic inflammatory disease of the esophagus, has increased esophageal expression of IL-13. Notably, IL-13-treated primary esophageal epithelial cells (PEECs) exhibit changes in gene expression that markedly overlap with genes differentially expressed in EoE esophageal biopsies. We identified leucine-rich repeat containing protein 31 (LRRC31), a novel gene with increased expression in EoE esophageal biopsies (12-fold, p<0.05) and IL-13-treated PEECs (26-fold, p<0.05). These data led us to hypothesize that IL-13-mediated induction of LRRC31 is important in EoE pathogenesis.

METHODS: We conducted bioinformatics analyses to characterize global LRRC31 tissue expression. We characterized esophageal LRRC31 expression in an independent cohort of patients with EoE, correlating LRRC31 and disease-associated gene expression levels. We characterized LRRC31 IL-13 dose-dependence and kinetic induction in primary esophageal epithelial cells. We overexpressed LRRC31 in EPC2 esophageal keratinocytes in differentiated air-liquid interface (ALI) cultures and evaluated epithelial barrier function by measuring transepithelial electrical resistance (TEER) and FITC-dextran paracellular flux.

RESULTS: At baseline, LRRC31 was specifically expressed in airway and colonic mucosal epithelium but not the esophagus. In EoE, esophageal LRRC31 mRNA expression increased (136-fold, p<0.05), normalized in patients responding to therapy, and significantly correlated with IL13 (R=0.55, p<10^-4) and CCL26 (R=0.68, p<10^-4) mRNA expression. In IL-13-treated PEECs, LRRC31 was induced in a dose-dependent manner, with peak expression between 24 and 48 hours. Overexpression of LRRC31 in ALI culture increased TEER (2.63-fold, p<0.05) and decreased paracellular flux (3.54-fold, p<0.05).

CONCLUSIONS: These data suggest LRRC31 is expressed in mucosal epithelium, is induced by IL-13 in EoE, and regulates esophageal epithelial barrier function.