ABSTRACTS

270 Impact on Anti-endotoxin Immunity of Allergen Specific Immunotherapy and Probiotics in Asthma Patients

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RATIONALE: Systemic and local anti-endotoxin immunity in patients with bronchial asthma may impact the efficacy of allergen specific immunotherapy (ASIT).

METHODS: 98 patients with asthma (GINA Step 1 and 2) were randomized into 3 clinical groups. Group 1 - 25 patients given standard asthma therapy; Group 2 - 49 patients given ASIT; Group 3 - 24 patients given ASIT and simultaneous administration of probiotics. Anti-endotoxin antibodies and lipopolysaccharide binding protein (LBP) were assessed in peripheral blood and induced sputum.

RESULTS: ASIT in responders gave increased blood levels anti-ET-IgA in Groups 2 and 3, normalized induced sputum levels of anti-ET-IgA, and decreased sputum LBP levels and blood IgE levels. Significant changes in anti-endotoxin immunity parameters were not found in patients who failed ASIT (nonresponders). Treatment with ASIT led to improved asthma control in 79.6% of patients. Simultaneous usage of probiotics with ASIT increased the clinical effectiveness of ASIT in 12.1% of patients and normalized sputum LBP levels and diminished blood IgE levels significantly greater compared with patients who received only ASIT.

CONCLUSIONS: ASIT efficacy in asthma may be associated with changes in anti-endotoxin immune parameters, enhanced by simultaneous administration of probiotics.

271 Use Of Omalizumab To Treat A Nine-year Old, With Steroid-dependent, Allergic Asthma, Adrenal Insufficiency And Vertebral Compression Fractures Due To Steroid-induced Severe Osteoporosis

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RATIONALE: In US and Canada, Omalizumab is indicated for adults and adolescents with moderate to severe persistent allergic asthma, but not for pediatric use (<12 years of age). A 9-year-old boy with steroid dependent, allergic asthma, multiple ICU admissions and severe back pain from compression fractures was referred to our centre. IgE was 1337 IU/ml. Skin prick testing showed multiple positive reactions. Asthma treatment included inhaled corticosteroids and frequent courses of oral prednisone.

METHODS: After obtaining necessary approvals and informed consents, Omalizumab treatment, 375mg every 2 weeks, was initiated in September 2010. Serum cortisol levels, bone density, spirometry, and pediatric asthma quality of life questionnaire (PAQLQ) were used to monitor clinical response.

RESULTS: Prior to Omalizumab therapy, the patient was taking daily prednisone 30mg. His daily ICS dose was 800mcg with a FEV1 of 1.45L. After 11 months, he was no longer taking prednisone and his ICS dose was 200mcg. FEV1 was 1.98 L. Serum cortisol levels were 12nmol/L (N: 185-624) prior to therapy. His total hip bone density scan was 0.626g/cm². Cortisol levels and bone density increased significantly over 11 months. Cortisol levels were within range at 215mol/L and total hip density was 0.686g/cm². PAQLQ was also obtained from patient monthly for 6 months from starting Omalizumab and at the 11 month mark. Also noted were decreased pain levels.

CONCLUSIONS: Prednisone treatment in young asthmatic children can be associated with serious side effects. Omalizumab therapy can permit steroid withdrawal and resolution of side effects.

272 Overturning The Conventional Notion Of Bronchial Asthma Treatment

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RATIONALE: While existing methods of treating bronchial asthma (BA) are unsatisfactory, a complete remission was achieved in nearly all patients treated with Chinese herbal medicines (kampo).

METHODS: Of 278 persistent BA sufferers (moderate: severe=89:189), 52 were given standard treatment and 226 were treated with kampo. The degree of severity and treatment effects were assessed (GINA Report, 2006. 2008) at the start of treatment and when symptoms stabilized. The subjects were also observed using ACT (Asthma Control Test). In standard treatment, all subjects were treated with inhaled corticosteroids + long acting β2-adrenergic agent (ICS+LABA). Medicinal kampo were compounds containing Glycyrrhiza, Atractylodes lancea, and others without ephedrine. The results were analyzed by chi square test and t-test.

RESULTS: There was no statistical difference in BA severity at the start of treatment. After treatment, symptoms completely disappeared in 93.5% (200/214) (dropouts=12) of the kampo group and 26.9% (14/52) of the standard group. The complete remission was 93.5% of the kampo group and 25% of the standard group; thus, treatment by kampo had significant effects in both cases (P<0.01). ACT at the start of treatment and when symptoms became stabilized was 14.7±2±4.8 in the kampo group and 14.0±2.3 in the standard group, again showing that treatment by kampo was significantly more effective (P<0.01). In the kampo group, symptoms disappeared after 16.2 days (n=200).

CONCLUSION: Treatment by kampo drugs is clearly superior to standard treatment; this is a very serious problem that could completely overturn the conventional notion of BA treatment.

273 Reductions In Oral Corticosteroid Use In Patients With Allergic (IgE- Mediated) Asthma Receiving Omalizumab

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RATIONALE: Oral corticosteroid (OCS) use is an indicator of poor asthma control. Long-term OCS use is associated with a major burden on patients and healthcare resources as a result of their significant adverse effects. Treatments that limit their use are important in asthma management.

METHODS: eXpeRience is a 2-year, multicenter, non-interventional, single-arm, andobservational registry collecting data from patients receiving omalizumab for uncontrolled persistent allergic asthma. Interim data are available from 876 patients (mean age, 44.9 years) receiving 8 months’ omalizumab therapy. Here we report the effect of omalizumab treatment on maintenance OCS use in this population.

RESULTS: With omalizumab, the proportion of patients using maintenance OCS was 22.9% at Week 16 and 17.4% at Month 8, compared with 28.9% at baseline. Mean (SD) OCS doses (prednisolone equivalent mg) at baseline, Week 16 and Month 8 were 15.1 (12.40), 10.5 (11.33), 8.4 (11.12), respectively, equating to a mean reduction of 25.2% at Week 16 and 40.4% at Month 8. At Week 16 and Month 8, OCS was reduced in 38 (18.1%) and 35 (18.7%) respectively, and discontinued in 42 (20.0%) and 69 (36.9%) respectively. Only five patients (2.4%) at Week 16 and nine patients (4.8%) at Month 8 had an increase in OCS dose.

CONCLUSIONS: This interim analysis from the eXpeRience registry indicates that patients receiving omalizumab had substantial reductions in maintenance OCS use. The decreased OCS use reflects improved asthma control and may reduce the risk of adverse effects associated with their use.
Impact of Omalizumab on Emergency-Department Visits, Hospitalizations and Corticosteroid Use in Patients with Uncontrolled Asthma Using High-Dose Inhaled Corticosteroids

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RATIONALE: This study evaluated the impact of omalizumab on emergency-department (ED) visits, hospitalizations and corticosteroid use among uncontrolled asthma patients using high-dose inhaled corticosteroids (ICS) prior to initiating omalizumab.

METHODS: Health insurance claims from the MarketScan database (2002Q1-2009Q1) were analyzed. Patients with ≥12 months of continuous insurance coverage prior to and after the first omalizumab dispensing, ≥8 weeks of high-dose ICS use, ≥8 weeks of long-acting beta2-agonist (LABA) use, and uncontrolled asthma at baseline were included. A retrospective analysis was conducted to quantify the impact of omalizumab on resource use by comparing ED visits, hospitalizations, and corticosteroid use one year before and after omalizumab initiation. A one-year period was chosen to cover any potential seasonality impacts.

RESULTS: A total of 644 patients (mean [median] age: 49.9 [53.0]; female: 59.2%) formed the study population. Omalizumab was associated with a 48.6% reduction in the proportion of patients with ≥1 asthma-related ED visits (pre vs. post-omalizumab period: 21.4% vs. 11.0%, P<0.001) and 40.8% reduction in asthma-related hospitalizations (25.0% vs. 14.8%, respectively, P<0.001). Compared to the pre-omalizumab period, the use of ICS decreased significantly after omalizumab initiation (7.8 vs. 6.5 dispensings, P<0.001; 41.9% of patients had a reduction in ICS use). A similar reduction in oral corticosteroid use was observed (5.0 vs. 3.6 dispensings, P<0.001; 53.3% of patients had a reduction in oral corticosteroid use).

CONCLUSIONS: The results of the current study showed that omalizumab treatment initiation was associated with statistically significant reductions in ED visits, hospitalizations and corticosteroid use.

Characterization and Predictors of Asthma Exacerbations in Patients on Steps 4, 5 And 6 Therapy in the TENOR Cohort

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RATIONALE: To characterize patients with severe or difficult-to-treat asthma on guideline-recommended Steps 4/5/6 asthma therapy and assess risk for future asthma exacerbations.

METHODS: Adolescent/adult patients ages ≥12 years (N = 1,293) from the TENOR observational study with medications data at baseline and month 12 were included. Patients were categorized into Steps 4/5/6 treatment groups based on the NHLBI (EPR-3) guidelines. Odds ratios (OR) and 95% confidence intervals (CI) for risk of asthma exacerbations at month 12 were generated using multivariable logistic regression.

RESULTS: The mean age of patients was 45.2 (±17.4) years. The majority were female (66.9%) and white (79.0%); 71.3% had private insurance. Most patients (72.8%) were treated by an allergist, and over 60.0% reported ≥3 allergic triggers. Mean pre-bronchodilator % predicted FEV1 was 70.5 (±21.9). About 75.0% of patients were classified as very poorly controlled (VPC), 23.1% as not well-controlled (NWC), and 0.8% as well-controlled (WC). After adjustment for covariates, (1) a baseline exacerbation (oral corticosteroid burst: OR = 2.85 [2.18-3.74]; emergency department visit: OR = 3.80 [2.51-5.76]; hospitalization: OR = 6.08 [3.55-10.42]); (2) reduced %predicted FEV1 (per 10 unit decrease) (OR = 1.07 [1.01-1.14]); (3) NHLBI asthma control group (VPC vs. NWC: OR = 1.83 [1.31-2.56]; WC vs. NWC: OR = 0.62 [0.07-5.47]); and (4) treatment setting (pulmonologist vs. allergist: OR = 1.36 [1.04-1.79]) were each associated with an increased risk of exacerbation.

CONCLUSIONS: These regression analyses suggest that two baseline factors, smoking history and FEV1 %predicted, significantly influence the percent of days with asthma control in active smokers treated for 6 months with montelukast or fluticasone.

Predictive Factors for Therapeutic Response to Asthma Treatment with Montelukast or Fluticasone in a Randomized Controlled Trial with Asthmatic Smokers

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RATIONALE: We conducted an analysis to identify factors predictive of therapeutic response to montelukast or fluticasone (each vs. placebo) in a large, randomized controlled trial in a population of smoking asthmatics.

METHODS: Actively smoking patients with poorly controlled asthma were randomized to placebo, montelukast 10mg QD, or fluticasone 250mcg BID. The primary efficacy endpoint was Percent of days with asthma control during the 6-month treatment period. Regression analysis (using backward selection) was used to determine factors predicting a therapeutic response. Factors considered were: history of allergic rhinitis, season at randomization (i.e., summer, fall, winter, spring), age, baseline airway reversibility, smoking history pre-study (pack-years [PK-Yrs]), smoking frequency (packs/day) at baseline, smoking frequency on treatment, baseline FEV1 %predicted, and baseline peripheral blood eosinophil level.

RESULTS: Using p≥0.15, the following factors were found in the regression model: smoking history (p<0.001), FEV1 %predicted (p<0.001), summer (season) (p=0.081), and smoking frequency at baseline (p=0.15); thus, smoking history and FEV1 %predicted were the most important. Patients with smoking history ≥1 PK-Yrs (median) tended to show more benefit with fluticasone; patients with >11 PK-Yrs tended to show more benefit with montelukast. For FEV1 %predicted, montelukast and fluticasone showed similar efficacy in patients with impaired lung function, while fluticasone tended to have a larger response in patients with FEV1 >75%-predicted.

CONCLUSIONS: These regression analyses suggest that two baseline factors, smoking history and FEV1 %predicted, significantly influence the percent of days with asthma control in active smokers treated for 6 months with montelukast or fluticasone.

Withdrawn

Guideline-defined asthma control appears to accurately capture clinical measures associated with future exacerbations risk.
**278** Oral Corticosteroid Use Increases the Risk of Glucocorticoid-related Adverse Events in Asthmatics

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**RATIONALE:** Oral corticosteroids (OCS) are a mainstay of asthma therapy for managing severe symptoms and exacerbations. Prolonged systemic exposure to glucocorticoids is known to be associated with increased risk of glucocorticoid-related adverse events (GAEs). Our objective was to determine the risk of GAEs associated with cumulative OCS exposure in asthmatics.

**METHODS:** Retrospective cohort analysis of adult asthmatics using a HIPAA-compliant claims database. Patients were continuously enrolled during 2002-2003 and were followed until the end of enrollment or study end (2007) whichever was earlier (median = 1,461 days observation). Patients with potential GAEs in the first year were excluded. Cox regression models estimated adjusted hazard ratios of the risk of GAEs (osteoporosis, fracture and cataracts) for different OCS exposure levels, measured by cumulative prednisone-equivalent dose as recorded on pharmacy claims, while adjusting for age, gender, region, usual care physician specialty, and chronic conditions.

**RESULTS:** We examined 37,891 asthmatics (mean age = 44.9 years, 65.7% female). For every 1,000 mg increase in prednisone-equivalent OCS exposure, the risk of a claim for osteoporosis was 3% higher (HR = 1.03, P < 0.001), the risk of a claim for fracture was 2% higher (HR = 1.02, P = 0.001), and the risk of a claim for cataracts was 2% higher (HR = 1.02, P < 0.001).

**CONCLUSIONS:** In asthmatics, greater cumulative OCS exposure is associated with statistically significant increased risks of osteoporosis, fracture and cataracts.

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**279** Onset and Duration of Action of Mometasone Inhalation Powder Measured by Impulse Oscillometry (IOS)

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**RATIONALE:** IOS is a noninvasive, unobtrusive alternative to spirometry in assessing airway impairment. IOS was measured in 21 asthmatics at intervals during a 4 week period with repeated mometasone dosing in order to determine the onset and duration of action as measured by IOS versus spirometry.

**METHODS:** Either 220 mcg or 440 mcg MIP was given for 28-36 days in steroid-naïve adult asthmatics. The primary variable was low frequency reactance (AX) measured by the IOS. The secondary variable was forced expiratory volume in one second (FEV1) measured by spirometry. IOS testing was done at baseline and 30, 60, 90, 120, and 300 min after mometasone. Spirometry was done at 120 and 300 min. Patients returned for IOS and spirometry testing on the next 2 mornings and then weekly for 4 weeks.

**RESULTS:** 21 patients were randomized to either 440 mcg or 220 mcg with the former group doing best. Significant decrease in AX was observed from baseline at 60 and 120 minutes Day 1 and on Days 2 and 3, and Weeks 1, 3 and 4. A significant increase in FEV1 was observed starting at Week 1 and continued over the 4 weeks of treatment.

**CONCLUSIONS:** Improved airway function is seen earlier with IOS versus spirometry. The effect was maintained as the treatment continued. These early changes are consistent with what is seen with bronchial vasodilitation.

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**280** Association Between The STIP1 Polymorphism And The Response To Inhaled Steroid In Children With Asthma

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**RATIONALE:** Bronchial hyperresponsiveness is usually measured by bronchial challenges using direct stimuli by methacholine or indirect stimuli by adenosine 5’-monophosphate (AMP). Airway inflammation is more closely reflected by AMP challenge test. The stress-induced-phosphoprotein 1 (STIP1) is one of the genes in steroid pathway. And polymorphisms in STIP1 are correlated with lung function after corticosteroid treatment in white subjects. We investigated whether polymorphism (rs4980524) of the STIP1 was associated with clinical phenotypes and responsiveness to inhaled steroid in Korean asthmatic children.

**METHODS:** We enrolled 84 asthmatic children. We prescribed budesonide (Pulmicort®) turbuhaler 800 µg/day for 8 weeks, and analyzed the responsiveness by AMP challenge test at 4 weeks after treatment and methacholine challenge test at 8 weeks after treatment. AMP and methacholine challenge test, spirometry and blood test for total IgE and eosinophil (total count, percent in white blood cells) were conducted. Polymorphism was genotyped by TaqMan assay. Subjects showing more or equal PC_{20}, than baseline before treatment were defined as ‘good’.

**RESULTS:** There were 70 good respondents and 14 poor responders. When we analyzed characteristics of subjects, there were not significant differences between good and poor responder. And there was no association between the STIP1 polymorphism and steroid response by AMP or Methacholine challenge.

**CONCLUSION:** The polymorphism (rs4980524) of STIP1 may be not a good predictor for responsiveness to inhaled steroid therapy in Korean children with asthma.

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**281** The Ability and Predictive Factors of Preschool Children to Use Swinghaler Device

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**RATIONALE:** The type of inhaler device affects adherence to asthma medication. DPI is a device which does not require hand-lung coordination but has limited data in preschool populations. Swinghaler required low peak inspiratory flow rate (PIFR). We examined the ability to use Swinghaler in preschool children compared to Turbuhaler and the predictive factors for the use of device effectively.

**METHODS:** One hundred and eighty healthy children, aged 3-6 years, were included. Swinghaler tester and Turbutester were used. The PIFR was measured by In-Check Dial after they were trained.

**RESULTS:** The ability to use Swinghaler and Turbulhaler in children aged 3-4, 4-5, and 5-6 years was 72.2%, 80.7%, 84.8% and 48.9%, 61.4% and 78.8% respectively. The ability to use Swinghaler in children aged 3-4 and 4-5 years was significantly higher than that of Turbuhaler (p < 0.001 and p = 0.003, respectively). Eighty-six of 103 children (83.5%) aged 4 years or older and 108 of 132 children (81.8%) with height more than 100 centimeters can use Swinghaler (p = 0.02 and p = 0.015, respectively). Mean PIFR in children aged 3-4, 4-5, and 5-6 years was 35.2±13.1, 41.3±14.6, and 48.7±16.4 L/min, respectively. Factors which independently associated with PIFR were male gender and height (p = 0.01 and p < 0.001, respectively).

**CONCLUSIONS:** Children from the age of 4 years or a height from 110 centimeters were able to generate adequate PIFR to use the Swinghaler. This device can be considered as an alternative inhaler device to MDI for the well-trained preschool children.
**282** Comparative Sensitivity of Various Indices in Evaluating Improvement in Mild Persistent Asthma

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**RATIONALE:** Asthma is traditionally diagnosed and managed based on clinical history and pulmonary function testing however in recent years there has been an effort to develop markers to aid in the diagnosis and management. The fraction of exhaled nitric oxide (eNO) and eosinophil percentage (eos%) in induced sputum are two tests which have been used in clinical practice. We compared these measurements to the concentration of sputum hyaluronan, which is an important pro-inflammatory component of the lung extracellular matrix. Several previous studies have revealed that hyaluronan is increased in lung tissue and sputum of patients with asthma.

**METHODS:** We enrolled 10 patients with mild, persistent asthma, not using controller medications and measured eNO, sputum eos% and hyaluronan concentration. We compared these markers to the historical history, asthma control test (ACT) and FEV1% before and after 2-4 weeks of inhaled fluticasone propionate, at 220mcg 2 inhalations bid.

**RESULTS:** There was a significant decrease in the level of eNO (57.3 ± 48.7 vs 17.8 ± 6.7;p = 0.03) and a significant increase in FEV1% (79.6 ± 10.9 vs 85.2 ± 10.5;p = 0.02) following the treatment period. Treatment did not produce a significant decrease in mean sputum hyaluronan (240.9 ± 348.3 vs 137.3 ± 131.5;p = 0.4), ACT (15.6 ± 5.4 vs 19.1 ± 4.2;p = 0.7) or sputum eos% (0.7 ± 1.1 vs 0.1 ± 0.2;p = 0.1).

**CONCLUSIONS:** In mild, persistent asthmatics there was a significant decrease in eNO and an increase in FEV1% following fluticasone treatment, however there were no significant differences in ACT score, sputum eos% or hyaluronan. eNO appears to be the most sensitive biomarker for assessing asthma control in patients with mild persistent asthma.

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**283** Combined Mometasone Furoate and Formoterol in Patients With Moderate to Very Severe Chronic Obstructive Pulmonary Disease (COPD): Phase 3 Efficacy and Safety Study

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**RATIONALE:** We report findings from a phase 3 trial evaluating the effects of mometasone furoate/formoterol (MF/F), administered via metered-dose inhaler, on pulmonary function in moderate-to-very severe COPD.

**METHODS:** This 26-wk, multicenter, randomized, double-blind, placebo-controlled trial evaluated MF/F 400/10µg twice daily (BID) and 200/10µg BID in current/ex-smokers (≥10 pack-y) ages ≥40y with moderate—very severe COPD (mean baseline [BL] % predicted FEV1 < 80% or >120y). Subjects received BID treatment with MF/F 400/10µg, MF/F 200/10µg, MF/F 100/5µg, or PBO. Lung function was analyzed for mean changes from BL in FEV1 over 0–12hrs (AU20–12, FEV1) and pre-dose AM FEV1 at endpoint (EP) after 13wks or 26wks of treatment.

**RESULTS:** 1193 subjects received treatment. At 13wks, changes from BL in AUC20–12 FEV1 with MF/F 400/10µg, MF/F 200/10µg, MF/F 100/5µg, PBO were 179µL, +31µL, +53µL, 92µL, respectively (*P<0.004 vs PBO; †P<0.001 vs MF 400/10µg ‡P<0.001 vs F 10µg). At 26wks, changes from BL in AUC20–12 FEV1 with MF/F 400/10µg, MF/F 200/10µg, MF/F 100/5µg. PBO were 152µL, +10µL, +53µL, 63µL, respectively (*P<0.001 vs PBO; †P<0.001 vs MF 400/10µg, F 10µg; ‡P<0.027 vs MF 400/10µg, F 10µg; §P=0.038 vs PBO). At 26wks, changes from BL in pre-dose AM FEV1 with MF/F 400/10µg, MF/F 200/10µg, MF/F 100/5µg, PBO were 92µL, +69µL, 27µL, 10mL, 3mL, (*P<0.030 vs PBO, F 10µg; †P=0.017 vs MF 400/10µg). No unexpected AEs were observed; MF/F was generally well tolerated.

**CONCLUSIONS:** MF/F treatments significantly improved lung function and were well tolerated in subjects with moderate to severe COPD.

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**284** Efficacy of Budesonide/Formoterol Pressurized Metered-Dose Inhaler in Patients With Mild to Moderate Asthma With and Without Fixed Airflow Obstruction

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**RATIONALE:** The influence of fixed airflow obstruction on asthma treatment response is unclear. The efficacy of budesonide/formoterol pressurized metered-dose inhaler (BUD/FM pMDI) was evaluated in asthma patients with or without fixed airflow obstruction.

**METHODS:** This 12-week, randomized, double-blind, placebo-controlled study (NCT00651651) was conducted in mild-moderate patients with asthma (as defined by American Thoracic Society) aged ≥21 years with ≤10-pack-year smoking history (Clin Ther 2007;29:823-43) and included a 2-week placebo run-in. Mean changes from baseline to treatment average in efficacy data were stratified by fixed airflow obstruction category (screening post albuterol FEV1/FVC ratio <0.70[with] or ≥0.70[without]) in the twice-daily BUD/FM pMDI 160/9µg (n = 17[with], n = 112 [without]), BUD pMDI 160µg (n = 20[with], n = 107[without]), and placebo (n = 24[with], n = 1[without]) arms.

**RESULTS:** At baseline, patients with versus without fixed airflow obstruction were older (45 vs 33y), more likely male (50% vs 38%), with longer asthma duration (27.1 vs 17.5y). In BUD/FM patients, treatment effects (treatment minus placebo) were numerically greater in patients with fixed airflow obstruction versus without (FEV1: 0.29 vs 0.26L; FVC: 0.39 vs 0.15L; rescue medication use: -2.91 vs -1.0 inhalations/day; symptom score: -0.49 vs -0.24). In BUD patients, treatment effects were numerically lower for FEV1 (0.12 vs 0.17L) and greater for FVC (0.24 vs 0.10L), rescue medication use (-2.06 vs -1.01 inhalations/day), and asthma symptom score (-0.36 vs -0.28) with versus without fixed airflow obstruction.

**CONCLUSIONS:** Mild-moderate asthma patients with versus without fixed airflow obstruction were more likely male and older, with longer disease duration. Robust improvements across multiple control metrics were observed for BUD/FM pMDI irrespective of the presence of fixed airflow obstruction that were greater than for BUD.
Clinical Pharmacokinetics and Tolerability of AZD3199, a New Inhaled Ultra Long-acting β₂-adrenoceptor Agonist (uLABA) Bronchodilator

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Rationale: To describe the clinical pharmacokinetics and tolerability of AZD3199, a new ultra-long-acting β₂-agonist, in healthy volunteers and patients with asthma or chronic obstructive pulmonary disease (COPD).

Methods: Data from five studies are presented - one single ascending dose study (48 healthy men); two multiple ascending dose studies (27 healthy Caucasian men, 27 healthy Japanese men); two randomized, double-blind studies, using a crossover (37 asthma patients, 5 women) or a parallel-group design (329 COPD patients, 82 women). AZD3199 was administered via a nebulizer (Spira: lung doses 2-1280 μg) or a dry-powder inhaler (Turbuhaler®: single delivered doses 120-1920 μg, or repeated once-daily doses 240-1680 μg [lung dose ~40% delivered dose]). Pharmacokinetics were assessed using total plasma concentration and urinary excretion. Tolerability and bronchodilatation were assessed using clinical laboratory tests.

Results: AZD3199 appeared rapidly in the systemic circulation; Cmax was reached within 30 minutes. Outcomes indicated dose-proportional, time-independent pharmacokinetics. Plasma exposure was similar in healthy volunteers and patients with asthma, but was relatively lower in patients with COPD. Estimated terminal t1/2 was 142 hours. Urinary excretion of AZD3199 was <2% of total apparent clearance. Dose-dependent, systemically mediated effects were seen on potassium levels, heart rate, and QTc. AZD3199 showed a bronchodilatory effect with a duration of ≥24 hours in patients with asthma or COPD.

Conclusions: Plasma exposure of the novel uLABA AZD3199 suggested linear pharmacokinetics and a long terminal half-life. AZD3199 showed no, or only mild, systemically mediated effects; and sustained bronchodilatation in patients with asthma and COPD.

Long-term Cardiovascular Safety, as Evaluated by Electrocardiographic Monitoring, of Budesonide/Formoterol Pressurized Metered-Dose Inhaler in African-American Patients With Moderate to Severe Asthma


Rationale: Data are limited on the cardiovascular safety of combination inhalant corticosteroids (ICSs) and long-acting β₂-adrenergic agonists in specific racial/ethnic populations of asthma patients. The cardiovascular safety of budesonide/formoterol pressurized metered-dose inhaler (BUD/FM pMDI) versus BUD pMDI was assessed in African-American asthma patients using serial electrocardiogram (ECG) monitoring.

Methods: This 52-wk, randomized, double-blind, parallel-group study (NCT00419952) was conducted in 742 self-reported African-American patients ≥12 y with moderate to severe asthma previously receiving a medium- to high-dose ICS. After a 2-wk run-in period on twice-daily BUD pMDI 320/μg, patients were randomized 1:1 to twice-daily BUD/FM pMDI 320/μg or BUD pMDI 320/μg. Adjusted mean changes from baseline to treatment maximum and shifts in twelve-lead ECG assessments were evaluated.

Results: Changes from baseline to treatment maximum for BUD/FM versus BUD were similar for heart rate (9.87 vs 9.04 beats/min) and QT interval corrected for heart rate (QTc [Bazett]: 18.46 vs 18.18 msec; QTc [Fridericia]: 13.47 vs 14.73 msec), but lower for uncorrected QT interval (15.59 vs 19.12 msec; unadjusted P = .002). Percentages of patients with a heart rate >100bpm or an increase ≥20bpm were 11.4% for BUD/FM and 8.5% for BUD, consistent with known effects of β₂-adrenergic agonists. Percentages with QT, QTc (Bazett), or QTc (Fridericia) intervals ≥500 msec or increase ≥60msec were low for BUD/FM (1.3%, 2.4%, and 0.3%) and BUD (1.9%, 1.1%, and 0.3%).

Conclusions: In this African-American patient population with moderate to severe asthma, the cardiovascular safety profile of BUD/FM pMDI (evaluated by ECG assessments) was generally similar to BUD, with no clinically meaningful differences between treatment groups.

Efficacy of Budesonide/Formoterol Pressurized Metered-Dose Inhaler in Patients With Moderate to Severe Asthma With and Without Fixed Airflow Obstruction

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Rationale: Effect of fixed airflow obstruction on baseline characteristics and treatment response in asthma patients is unclear.

Methods: Mean changes from baseline to treatment average in efficacy data from a 12-wk, randomized, double-blind, placebo-controlled study (NCT00652002) in moderate-severe patients with asthma (as defined by American Thoracic Society) aged ≥12 years with ≤10-pack-year smoking history (Drugs. 2006;66:2255-54) were stratified by fixed airflow obstruction category (screening post albuterol FEV1/FVC ratio <0.70 [with] or ≥0.70 [without]). Results for twice-daily budesonide/formoterol pressurized metered-dose inhaler (BUD/FM pMDI) 320/9μg (n=47 [with], n=77 [without]), BUD pMDI 320μg (n=32 [with], n=77 [without]), and placebo (n=39 [with], n=86 [without]) arms are presented.

Results: At baseline, patients with versus without fixed airflow obstruction were older (46.6y vs 38.5y), more likely male (46% vs 34%), and had longer asthma duration (28.0y vs 20.0y). Treatment effects on pulmonary function (treatment minus placebo) were numerically greater with BUD/FM but similar with BUD versus without fixed airflow obstruction (FEV1: 0.37 vs 0.31L/BUD/FM; 0.22 vs 0.25L [BUD]; FVC: 0.35 vs 0.25L [BUD/FM]; 0.18 vs 0.19L [BUD]), with greater effects with BUD/FM versus BUD. Treatment effects were numerically greater with BUD/FM and lower with BUD in patients with versus without airflow obstruction for rescue medication use (-1.85 vs -1.71 inhalations/day [BUD/FM]; -0.89 vs -1.22 inhalations/day [BUD]) and lower with BUD/FM and similar with BUD for symptom score (scale: 0 [none]-3 [severe]) (-0.29 vs -0.38 [BUD/FM]; -0.20 vs -0.20 [BUD]), with greater effects with BUD/FM versus BUD.

Conclusions: Moderate-severe asthma patients with versus without fixed airflow obstruction tended to be older, male, and have a longer duration of disease. A robust treatment response to BUD/FM pMDI was retained in the presence of fixed airflow obstruction that was greater than for BUD.
Post-hoc Analysis Of Short-acting Beta₂-agonist Efficacy In Baseline Corticosteroid Users And Non-corticosteroid Users Following Treatment With Levalbuterol Metered-dose Inhaler, Racemic Albuterol Or Placebo In Asthma Patients Ages 4-11 Years

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RESULTS: DBAv % change FEV1 was 29.4% and 19.6% for non-steroid users and placebo (PBO) groups, respectively. Non-steroid users treated with racemic albuterol (RAC) had a 16.7% DBAv % change FEV1 and steroid users had a 26.4% change. AUC FEV1 percent change followed a similar trend as DBAv % change FEV1. Responses rates (response defined as a percent change in FEV1 of >15%) averaged over the double-blind period (DBAv % change FEV1). Patients 4-11 years with stable asthma for >6 months and FEV1 ≥80% predicted were randomized in a 2:1:1 ratio to receive treatment with levalbuterol trtarate 90mcg QID (XOPENEX HFA® Inhalation Aerosol, steroid users n=42, non-steroid users n=34), racemic albuterol 180mcg QID (Proventil HFA® Inhalation Aerosol, steroid users n=19, non-steroid users n=20) or placebo (steroid users n=13, non-steroid users n=22).

RESULTS: DBAv % change FEV1 was 29.4% and 19.6% for non-steroid users and placebo (PBO) groups, respectively. Non-steroid users treated with racemic albuterol (RAC) had a 16.7% DBAv % change FEV1 and steroid users had a 26.4% change. AUC FEV1 percent change followed a similar trend as DBAv % change FEV1. Responses rates (response defined as a percent change in FEV1 of ≥15%) averaged over the double-blind period were LEV 85.7%, RAC 94.7% and PBO 23.0% for steroid-users and LEV 93.7%, RAC 47.4% and PBO 50.0% in non-steroid users.

CONCLUSIONS: In this post-hoc analysis, non-steroid users treated with LEV had larger improvements in lung function compared to steroid users while the opposite was observed in patients treated with RAC.

The Relationship between Sales Data for Asthmatic Drugs and Asthma Events

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RATIONALE: Although the mortality rate of fatal asthma is decreased, over 2,000 patients still die of asthma every year in Japan. We analyzed the relationship between asthmatic drugs and asthma events by prefecture in Japan.

METHODS: Pharmaceutical companies cooperated with this study by providing sales data for total sales of inhaler corticosteroids (ICS), long acting beta-agonists (LABA), and leukotriene receptor antagonists (LTRA) for the period 2006-2009, and approximate 90% of data is available thus far. Data on asthma prevalence, numbers of cases of fatal asthma and hospitalization, and population sizes were obtained from government ministry publications.

RESULTS: The relationship between asthma prevalence and total asthmatic drugs sales per patient in 2009 showed negative correlations (ICS: r² = 0.58, p<0.01; LABA: r² = 0.49, p<0.01; LTRA: r² = 0.60, p<0.01). A positive correlation was found between hospitalization and ICS (r² = 0.28, p<0.01) but not LABA (r² = 0.00) or LTRA (r² = 0.05). In regard to fatal asthma cases among those hospitalized, a negative correlation was seen with ICS (r² = 0.09, p<0.05) but not LABA (r² = 0.00) or LTRA (r² = 0.00).

CONCLUSIONS: Prefectures where patients were treated sufficiently for asthma, including with the use of ICS, LABA, and LTRA, had a lower prevalence of asthma events. Although prefectures with a high percentage of hospitalization showed higher ICS usage, the percentage of fatal asthma events was decreased by such use. We will present the full results for all data including a combination inhaler at AAAAI 2012.
292 Flavonoid-Rich Alcoholic Extract of Leaves of Achyranthes Aspera Reduces Inflammation in a Murine Model of Ova-Induced Asthma

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RATIONALE: The leaves of Achyranthes aspera Linn. (AA) are used to treat wounds, injuries, intermittent fever, typhoid and as an anti-asthmatic. In this study we hypothesized that an extract of AA leaves may be effective for the treatment of asthma.

METHODS: The fresh leaves were extracted with 80% (v/v) ethanol (AAE). Female BALB/c mice were sensitized by intraperitoneal injection of OVA in alum. The extract was administered intranasally (500 or 750mg/kg) and intraperitoneally (500 mg/kg) from day 18 to 24. Airway hyperresponsiveness (AHR) was measured by methacholine challenge, and after 24 hours bronchial lavage was performed.

RESULTS: AAE diminished AHR more when given intraperitoneally than intranasally. The eosinophil count was lower after intraperitoneal than intranasal delivery, compared with OVA control group. The amount of IL-5 and TNF-α was reduced in both the groups but in a dose dependent manner in the intranasal group. Histopathology of the lung sections showed more protection intraperitoneally than intranasally.

CONCLUSION: AAE attenuates the infiltration of neutrophils and the production of inflammatory cytokines TNF-α and IL-5 in the lung. The anti-asthmatic activity of AA may be due to the presence of flavonoids that inhibit the NF-kB pathway.

293 The effect of vitamin D Status on Pediatric Asthma in a university hospital, Thailand

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RATIONALE: Hypovitaminosis D has been shown to associate with increased asthma severity, emergency visit and impaired pulmonary function in asthmatic patients in USA and Europe. In tropical countries, data on the effect of vitamin D status on asthma is lacking. This study aimed to determine the prevalence of vitamin D deficiency/insufficiency in asthmatic children in Thailand and the effect of vitamin D status on asthma control.

METHODS: Asthmatic children in allergy clinic were enrolled. Serum 25-hydroxyvitamin D, pulmonary function test and the level of asthma control classified by Global Initiative for Asthma guidelines were assessed.

RESULT: Ninety asthmatic children were recruited (61 boys and 29 girls). The mean age±SD was 11±3 years. The levels of asthma control were controlled 50%, partly-controlled 34.4% and uncontrolled 15.6%. Vitamin D deficiency (<20 ng/ml) and insufficiency (<30 ng/ml) were found in 26.6% and 61.1% of asthmatic children, respectively. The mean levels of vitamin D were 32.8 ng/ml in uncontrolled, 29.27 ng/ml in partly-controlled and 27.99 ng/ml in controlled patients (P=0.05). There were no differences in pulmonary function, asthma exacerbation, inhaled-steroid doses or other medications between vitamin D deficiency/insufficiency and vitamin D sufficiency groups. Compared to controlled asthmatic patients, the risk factors for uncontrolled/partially-controlled asthma were females (OR, 3.3; 95% CI, 1.1-9.84; p=0.02) and cockroach sensitization (OR, 2.71; 95% CI, 0.15-9.93; p=0.04).

CONCLUSION: High prevalence of vitamin D deficiency and insufficiency were found in asthmatic children in Thailand. However, there was no association between vitamin D status and the level of asthma control in these patients.

294 History Of Asthma Maintenance Medication Use And Asthma Exacerbation Risk Factors Before Initiation Of Inhaled Corticosteroid-long-acting β-agonist (ICS/LABA) Combination Therapy For Asthma: Comparison Of Budesonide/formoterol (BFC) And Fluticasone/salmeterol (FSC) In A US Commercially Insured Population

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RATIONALE: Expert Panel Report 3 (EPR-3) guidelines recommend that initiation of ICS/LABA combination therapy be considered in patients whose asthma is not well controlled on ICS or whose disease severity warrants. Differences in asthma medication use and asthma exacerbation risk factors ≥1 year before BFC and FSC initiation were evaluated.

METHOD: Retrospective cohort study identified commercially-insured patients (age, 12–64 years) with asthma diagnosis and first-time BFC or FSC prescription fills from 6/1/2007–12/31/2010. Patients with ≥1 year of health plan enrollment before initial treatment were categorized into BFC or FSC cohorts. Per EPR-3 guidelines, therapy initiation was considered appropriate if patient had ICS and/or leukotriene receptor antagonist prescription fill or asthma exacerbation risk factors (asthma-related emergency department visit or hospital admission, or ≥2 oral systemic corticosteroid or ≥6 short-acting β-agonist canister prescription fills) in year prior. Multivariate logistic regression was used to identify factors possibly associated with BFC or FSC initiation.

RESULTS: Among 1,538,077 asthma patients, 11,718 BFC and 38,697 FSC patients were identified (64%, female; mean age, 40 [BFC] and 38 [FSC] years). In each group, 32% of patients met asthma exacerbation risk criteria in the year before BFC/FSC initiation. Asthma maintenance therapy use was 42% for BFC and 31% for FSC patients in the year prior. Compared with FSC patients (52%), BFC patients (57%) had a higher likelihood of appropriate ICS/LABA therapy initiation (adjusted odds ratio, 1.21 [P<0.001]).

CONCLUSION: Overall, >50% of patients studied met EPR-3 guideline criteria for initiation of ICS/LABA combination therapy, with the likelihood slightly higher for BFC patients.

295 Positive Correlation Between Serum IL-5 and TNF-alpha Levels and Churg-Strauss Syndrome Activity in Patients Successfully Treated with Montelukast

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RATIONALE: Assessment of cytokines and expression of CD markers of eosinophils may be useful in Churg-Strauss Syndrome (CSS).

METHODS: 21 patients with CSS were studied. Concentrations of cytokines (IL-2, TNF-alpha, IL-4, IL-5 and IL-10) were measured by ELISA. Surface CD antigens on peripheral blood eosinophils were analyzed by flow cytometry.

RESULTS: High serum concentrations of IL-5 and TNF-alpha occurred in 16 of 21 cases. A positive correlation existed between: 1) IL-5 levels and CSS activity with intense pulmonary involvement; and 2) higher TNF-alpha levels with active systemic cutaneous CSS involvement. Newly expressed surface antigens such as CD25, CD4, and CD69 were observed on peripheral blood eosinophils in 17 of 21 cases with active CSS. HLA-DR was expressed in 10 of 21 patients with chronic CSS with skin symptom persistence. After 3 months therapy with montelukast decreased IL-5 and TNF-alpha levels occurred with reduced pulmonary symptoms in patients with active CSS and some reduction in skin symptoms.

CONCLUSIONS: Eosinophils in peripheral blood are activated in CSS likely dependent on IL-5 and TNF-alpha associated stimulation and related to the clinical stage of CSS.
Role of a Plasminogen Activator Inhibitor-1 4G/5G polymorphism in the development and clinical outcome of Idiopathic Sudden Sensorineural Hearing Loss

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RATIONALE: Idiopathic sudden sensorineural hearing loss (ISSHL) can be devastating. Although the pathogenesis of ISSHL is still unclear, recent studies suggest that vascular impairment, microthrombotic process, and autoimmune disorders may be involved. Plasminogen activator inhibitor-1 (PAI-1) is a key molecule in the fibrinolytic system and elevated plasma levels of PAI-1 are associated with ISSHL. Here we investigate the role of a 4G/5G PAI-1 polymorphism in the development and clinical outcome of ISSHL.

METHODS: We assessed 103 patients with ISSHL and 113 age and sex-matched controls from the University of Ferrara, Italy under IRB approval. ISSHL was diagnosed by experienced otolaryngologists after excluding other causes of sudden hearing loss. Genomic DNA was isolated from peripheral blood using the QIAamp kit and the 4G/5G polymorphism in the -675 promoter region was genotyped with an allele-specific PCR. Genotype distribution was tested in patients and compared to controls by chi-square and odd-ratio analysis.

RESULTS: In this population, 5G/5G genotype had a two-time lower frequency in ISSHL patients compared to healthy controls (15.5% vs 30.1%) and was associated with decreased odds compared to 4G/5G genotype (OR 0.37, 95% CI 0.19-0.75, p = 0.005). In addition, the patients with 5G/5G genotype showed 2 times higher ratio of hearing recovery (> 20 dB) after treatment compared to other genotypes, suggesting a better prognosis as a clinical outcome.

CONCLUSIONS: This study suggests that the 5G/5G genotype of PAI-1 may have a protective effect against developing ISSHL and function as a prognostic factor in recovery from ISSHL.

Interferon-Gamma Gene Polymorphism In Patients With Allergy

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RATIONALE: TH1-type cytokines such as interferon gamma (IFN-gamma) inhibit IgE synthesis and the development of TH2 immune responses. This study assesses SNP polymorphisms in the IFN-gamma gene in patients with pollen and lichen/mold allergy.

METHODS: The study included a control group (n = 17) of healthy subjects, patients with pollen allergy (n = 33) and patients with lichen/mold allergy (n = 12). The SNPs of IFN-gamma +874 were determined in DNA using allele-specific PCR.

RESULTS: The most common genotype for +874 was the heterozygous AT in 64.7% of control subjects, in 42.4% of pollen allergy patients and in 75% of lichen/mold allergy patients. The frequency of genotype [H[Unsupported Character - A]|H[Unsupported Character - A]] in position +874 of the gene interferon-gamma gene in lichen/mold allergy patients was 0% significantly less compared with the control group (23.5%) and pollen allergy patients (21.2%) (p<0.05).

CONCLUSIONS: Sequence changes in the non-coding regions of cytokine genes may influence production of the corresponding peptide due to linkage with another marker directly affecting gene expression. A probable influence of IFN-gamma gene polymorphisms +874 was seen in allergy patients suggesting that identification of variants of the IFN-gamma gene may demonstrate a role for these genes in the development of allergy and susceptibility to allergic disease.

Incidence of Immunodeficiency in Patients with 49-XXXX Chromosomal Variation

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RATIONALE: Boys with 49-XXXX sex chromosomal variation have been described to have high incidence of recurrent otitis media and asthma, the cause of which is unknown. We hypothesized that some degree of primary immunodeficiency occurs in patients with XXXY aneuploidy.

METHODS: The families of patients with known XXXY chromosomal variation were interviewed. Histories obtained included screening via the “10 warning signs of immunodeficiency”(Jeffrey Modell Foundation), as well as atopic or autoimmune conditions and a family history. All available prior immunologic testing and vaccine histories were reviewed with family consent.

RESULTS: Eighteen affected boys were evaluated. Thirteen had history of at least 2 of the 10 warning signs, and three had history of 4 or more warning signs. Seven boys had history of recurrent pneumonia, and four of these boys had received 23-valent pneumococcal booster vaccine in the past. Five patients had prior immunologic testing, and one boy had been diagnosed with specific antibody deficiency due to lack of response to pneumococcal booster. All patients had history of atopic conditions, with 14 carrying the diagnosis of asthma. None had history of autoimmune disease, and none had family history of immunodeficiency.

CONCLUSIONS: A high incidence of warning signs of primary immunodeficiency occurs in patients with XXXY chromosomal variation. The frequent occurrence of pneumonia, asthma, and the finding of a patient with specific antibody deficiency in this cohort suggest that humoral abnormalities may be increased in this population. Further studies of these patients are currently ongoing.

INTERLEUKIN 1 BETA +3953 C/T Gene Polymorphism In Patients With Asthma And Chronic Obstructive Pulmonary Disease In Venezuela

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RATIONALE: Asthma and Chronic Obstructive Pulmonary Disease (COPD) are chronic inflammatory diseases of the airways, resulting from the interaction of genetic and environmental factors. Interleukin (IL) 1 beta is a member of a group of proinflammatory cytokines. Most of the genes that encode for the IL-1 family members are located on the long arm of chromosome 2. The polymorphic loci of these genes seem to have a role on a broad range of illnesses. The aim of this study was to determine the potential association between the interleukin (IL) 1 beta (+3953) polymorphism and the presence of asthma and COPD in Venezuelan population.

METHODS: The groups in this study consisted in 100 patients with asthma, 100 with COPD and 100 age matched controls. Genomic DNA was purified from blood leukocytes by using a Qiagen® kit and subsequently Polymerase Chain Reaction (PCR), Restriction Fragment Length Polymorphism (RFLP), and agarose gel electrophoresis techniques were used to determine the IL 1beta (+3953) genotype. The strength of association between this polymorphism, in each of the patients groups and controls, was estimated by the odds ratio (OR) after performing Fisher’s exact test (2 x 2 contingency tables).

RESULTS: Significant differences were observed in the frequency of genotype CT between COPD and asthma: OR = 3.03, 95% CI (1.37-6.71), P= 0.0058 and between COPD and healthy controls: OR = 6.86, 95%CI (2.51-18.74), P= 0.0001.

CONCLUSIONS: The data suggest that CT genotype in IL-1B +3953 may be a major contributing genetic risk factor for COPD in the Venezuelan population.
300 Pregnancies in Women with Hereditary Angioedema due to Mutations in the F1Z gene
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RATIONALE: The two pathogenic heterozygous missense mutations, p.Thr328Lys and p.Thr328Arg in exon 9 of the factor XII (F1Z) gene are a recently recognized cause of recurrent angioedema (HAE-FXII). Symptomatic patients are females almost exclusively, as clinical manifestations seem to be connected with high estrogen levels. The condition is rare and the clinical course of pregnancies in affected females has not yet been investigated systematically.
METHODS: Twelve adult female patients with HAE-FXII have been identified at the HAE Comprehensive Care Center in Frankfurt, Germany. Nine of these have ever been pregnant. The course of 17 pregnancies of these 9 patients were documented, four of these have been followed prospectively.
RESULTS: Pregnancies in females with HAE-FXII were complicated by angioedema in 41% (7 pregnancies in 5 patients). In 59% of pregnancies (10) the patients showed no or questionable angioedema. Angioedema involved the intestine, extremities, face and upper airway. The onset of symptoms was between the 5th gestational week and the 4th gestational month, with symptoms mostly ongoing until delivery. Clinical severity varied between mild to severe. The attack frequency was up to once weekly. Most deliveries (13/17) were spontaneous vaginal deliveries. Angioedema during delivery was recorded in one case (swelling of the birth canal), whereas the post partum period was symptom-free in all 17 pregnancies.
CONCLUSIONS: The clinical course of pregnancy in females with HAE-FXII may be complicated by angioedema in a big proportion of cases. The risk of angioedema seems to be lower during the intra- and post partum period in these patients.

301 Lack Of Associations Between Tnf-α Genetic Polymorphism -308G/a And Anti-tuberculosis Drug-induced Maculopapular Eruption
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RATIONALE: Adverse cutaneous reaction to anti-tuberculosis drugs (ATD), such as maculopapular eruption (MPE), is one of the most common cause of discontinuation of scheduled treatment of tuberculosis (TB). Tumor necrosis factor-α (TNF-α) plays an important role in drug- or drug metabolite-induced immune responses and we recently reported that TNF-α genetic polymorphism -308G/A is significantly associated with ATD-induced hepatitis. In this study, we aimed to investigate associations between TNF-α -308G/A and ATD-induced MPE.
METHODS: Patients with ATD-induced MPE and controls without any adverse reactions to ATD were recruited from the database of the Adverse Drug Reaction Pharmacogenomic Research Group database of Korea. We compared genotype frequency of TNF-α -308G/A in patients with ATD-induced MPE and ATD-tolerant controls.
RESULTS: A total of 69 patients with ATD-induced MPE and 229 control subjects were enrolled for this study and genotyped for TNF-α -308G/A. There was no significant difference in genotype frequency between case and control subjects suggesting lack of associations between TNF-α -308G/A and ATD-induced MPE.
CONCLUSIONS: These results reveal that the TNF-α genetic polymorphism -308G/A is not related with the development of ATD-induced MPE, contrary to ATD-induced hepatitis. These findings suggest that associations between TNF-α -308G/A and ATD-induced adverse reactions are phenotype specific.
304 Classification of 26 Cases of Biopsy-proven Eosinophilic Myocarditis
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RATIONAL: Eosinophilic myocarditis is a rare disorder associated with heart failure. We sought to determine underlying causes of this condition.
METHODS: We performed a retrospective chart review of subjects with biopsy-proven eosinophilic myocarditis at a tertiary care institution. Data was collected and analyzed under an IRB-approved protocol.
RESULTS: Twenty-six cases of eosinophilic myocarditis were identified in 16 males and 10 females with a mean age 57 ± 20 years. The majority of these subjects were Caucasian. Nine cases were found after heart transplantation; two were diagnosed following myectomy; the remaining cases were diagnosed by an endomyocardial biopsy. Sixteen of 26 (62%) subjects were diagnosed with hypersensitivity myocarditis, although in most cases drug causality was difficult to prove. Furosemide (68%), Digoxin (62%), Dobutamine (37%) and Aspirin (37%) were the most commonly prescribed drugs in these subjects. Two of 26 subjects were diagnosed with Churg-Strauss syndrome and were treated with Prednisone and Methotrexate. These subjects had a peripheral blood absolute eosinophil count >1800 cells/mm³. Two subjects had suspected parasitic infection with a peripheral blood absolute eosinophil count >9000 cells/mm³. Pathology was suggestive of Loeﬄer’s syndrome in 2 subjects and Amyloidosis in 1 subject. The underlying etiology remained unclear for 3 subjects. Overall mortality was 50% among all subjects.
CONCLUSIONS: In this series, eosinophilic myocarditis was most commonly identified incidentally after heart transplantation. Significant peripheral blood eosinophilia was associated with Churg-Strauss or parasitic infection and was not commonly seen with hypersensitivity myocarditis.

305 Basophils Autoinduced Degranulation (BAD) Test: A New Variant Of Basophils Activation Test (BAT) As Reliable In Vitro Tool For The Diagnosis Of Chronic Autoimmune Urticaria (CAU)
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RATIONAL: It is well known that 15-30% of chronic idiopathic urticaria (CIU) have autoimmune aetiology. The diagnosis of CAU is based on the demonstration of the presence of anti-FceRIα and/or anti-IgE autoantibodies in the serum. The commonly screening procedures is represented by Basophil Histamine Release test (BHR) and Autologous Serum Skin Test (ASST) associated, due to their low sensitivity and specificity (70%-80%). The aim of this study is to develop a new method more reliable and faster to diagnose CAU.
METHODS: 100 CIU patients were recruited. Serum and peripheral whole blood were collected. The serum was divided into two parts, one of which was decomplemented (DS), and the other was used whole (WS). These sera were used to stimulate autologous blood cells in order to perform the BAD test by flow cytometry. Our method uses a two-colours approach with anti-human CCR3 and CD63, subsequent to the stimulation of basophils with DS and WS.
RESULTS: 24 of 100 CIU patients showed a significant increase of CD63 on basophils stimulated with whole and/or decomplemented serum. Of these 12 patients showed degranulation only when stimulated with whole serum, 1 with decomplemented serum and 11 with both stimuli.
CONCLUSIONS: BAD test is useful for the quantitative determination of the basophils degranulation. Recent studies conducted with similar methods confirmed that the sensitivity and specificity of BAD test are approximately of 95% and 90%. Our method is more reliable, faster and cheaper than previous that used leucocytes separation or three-colours procedures. Also the sensitivity of BAD could be enhanced associating the activation marker CD203c.

306 Levels Of Inflammatory Cytokines And Chemokines In Bronchoalveolar Lavage Fluid In Patients With Idiopathic Interstitial Pneumonitis And Collagen Vascular Disease Associated Interstitial Pneumonitis
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BACKGROUND: Inflammatory cytokines and chemokines have been reported to play important roles in the pathogenesis of interstitial lung diseases. However, their individual roles in idiopathic interstitial pneumonitis (IIP) and in the other types of IP, including collagen vascular disease associated (CVD)-IP, remain unknown.
METHODS: BAL fluid levels of IL-1β, -2, -4, -5, -6, -7, -8, -10, -12, -13, -17, G-CSF, IFN-γ, MCP-1, MIP-1, TNF-α, MCP-1, MIP-1β, PDGF, and EGF were measured using a bead suspension array in 16 patients (8 men, 8 women; mean age, 60.1±9.3 years) with idiopathic nonspecific interstitial pneumonitis (NSIP), 7 patients (5 men, 2 women; mean age, 71.6±3.3 years) with idiopathic usual interstitial pneumonitis (UIP), 5 patients (3 men, 2 women; mean age, 67.2±4.4 years) with rheumatoid arthritis (RA), and 3 patients (1 man, 2 women; mean age, 52.7±14.2 years) with dermatomyositis (DM) in CVD-IP as well as in 13 patients (3 men, 10 women; mean age, 46.6±16.9 years) with sarcoidosis, as a disease control. RESULTS: Levels of IL-7 were highest for DM (19.0±6.8 pg/ml), compared with other IPs (9.4±6.3 pg/ml for UIP, 8.6±3.7 pg/ml for NSIP, 8.4±5.4 pg/ml for RA) and sarcoidosis (3.8±2.8 pg/ml). On the other hand, levels of TNF-α were highest for RA (17.3±29.9 pg/ml), compared with other IPs (2.7±1.5 pg/ml in UIP, 3.1±3.9 pg/ml in NSIP, 10.7±9.3 pg/ml in DM) and sarcoidosis (5.2±6.2 pg/ml). Interestingly, levels of IL-17 were detectable in RA.
CONCLUSIONS: Differences seen in the level of each cytokine and chemokine between patients with IIPs and CVD-IP might reflect the pathogenesis of the IP.

307 Terrelysin, a Potential Biomarker of Exposure to Aspergillus terreus
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RATIONAL: Exposure to airborne fungi has been associated with a variety of adverse health effects ranging from allergies and asthma, to opportunistic infections. However, not all species are equally problematic and improved diagnostics are essential to develop exposure and surveillance information. Here, we describe the development of monoclonal antibodies (mAbs) to a hemolysin (terrelysin) using Aspergillus terreus as a model species for development of a specific biomarker of exposure.
METHODS: Sixteen murine IgG mAbs were developed after immunization of mice with recombinant terrelysin and tested for reactivity in ELISA (screening) and Western blot assays (cross-reactivity). Epitopes for 3 of the mAbs were determined using synthetic peptides immobilized onto celllose supports. Microscopic methods were used to monitor fungal growth and immunohistochemistry methods were utilized to study immunolocalization of terrelysin.
RESULTS: mAbs showed reactivity towards multiple strains of Aspergillus terreus and cross-reactivity analysis identified 3 mAbs to be species-specific. Furthermore, epitope analysis showed that 2 mAbs of different isotypes recognize the same epitope while the other mAb recognizes a different epitope. Immunolocalization studies demonstrated that terrelysin is localized more abundantly at hyphal tips. Time course studies showed that unlike fungal proteolytic enzymes, terrelysin was expressed during early hyphal growth but reduced after mycelial expansion. Terrelysin was also detected early during growth in the culture supernatant, suggesting secretion.
CONCLUSIONS: Our goal is to better understand the adverse health effects caused by fungal exposures. The species-specific mAbs will be useful tools in determining the potential of terrelysin as a potential biomarker for Aspergillus terreus exposure.
308 Type III Hereditary Angioedema: First Description of a Mutation in Factor XII Gene and Clinical Features in a Brazilian Family

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RATIONALE: Hereditary Angioedema (HAE) type III has been described mostly in women, being influenced by exogenous estrogen exposure. Patients with HAE type III present normal levels and function of C1-inhibitor (C1-INH), and normal C4. Our aim was to describe characteristics of patients with HAE type III in a Brazilian family.

METHODS: A 26-year-old female patient, with a history of recurrent episodes of angioedema was evaluated. Whole blood and serum were collected for measurements of C1-INH and C4 by nephelometry. Genomic DNA was isolated from whole blood from the patient, her father and a paternal aunt who have experienced recurrent angioedema attacks, and one maternal uncle with no history of angioedema. PCR was performed with 50 ng genomic DNA, and sequencing of exon 9 from the F12 gene was performed.

RESULTS: Patient’s symptoms started at age 17, with episodes of edema of extremities, face, tongue, lips, larynx, and genitalia, abdominal pain, ascitis and dyspepsia. Episodes occurred once a month with severe intensity, being triggered by stress, sexual intercourse and oral contraceptive agents. Diagnosis was made at age 26, and symptoms were controlled after withdrawal of oral contraceptives. C4 and C1-INH levels were normal on at least two occasions. Genetic analysis revealed a missense mutation in exon 9 of the F12 gene, previously identified as p Thr309Lys, on the index case and her paternal relatives, but not on the asymptomatic maternal uncle.

CONCLUSIONS: This study demonstrates for the first time in Brazil the presence of a genetic mutation in factor XII as a cause of HAE type III.

309 Parasite-related IgE Antibodies, Including IgE to Galactose-alpha-1,3-galactose, in Sera from Virginia and Ecuador

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RATIONALE: IgE antibodies to galactose-alpha-1,3-galactose (alpha-gal) have become increasingly prevalent in the southeastern United States. These antibodies have recently been linked to bites from the tick Amblyomma americanum, but the possibility of a role for other parasites remains.

METHODS: Assays for IgE were performed on patients with delayed urticaria or anaphylaxis to meat and controls in Virginia (n=125), as well as a cohort from Esmeraldas Province, Ecuador (n=295) where Echinococcus is not endemic.

RESULTS: The significant association between IgE antibodies to alpha-gal and IgE to Echinococcus was strongest in Virginia (r=0.74 vs r=0.62 for Ecuador, both p<0.001). Further, only 3/37 Echinococcus IgE-positive subjects in Virginia were not positive for IgE to alpha-gal. In Ecuador, 118/223 Ascaris-positive sera were negative for IgE to alpha-gal; by contrast, in Virginia 52/79 alpha-gal positive sera were negative for Ascaris and only one sera was Ascaris-positive and alpha-gal negative. Virginia sera with IgE to Echinococcus (class 2 and 3) were absorbed with alpha-gal linked to Sepharose beads, which removed all detectable IgE to Echinococcus.

CONCLUSIONS: We think that the limited number of positive assays for Echinococcus and Ascaris in Virginia can be explained by cross-reactivity with alpha-gal, which may also be relevant for Echinococcus in Esmeraldas. By contrast, many sera in Ecuador had high titer IgE to Ascaris with negative responses to alpha-gal. None of these IgE antibodies were significantly associated with asthma in the United States, while IgE to Ascaris was significantly associated with asthma in Ecuador.

310 Analysis Of 4,610 Patients With Elevated Serum Immunoglobulin E

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RATIONALE: This study was designed to describe the demographic characteristics of patients with elevated total immunoglobulin E (IgE). METHODOLOGY: The period 2003 to 2011, we identified 4610 patients whose total IgE levels exceeded the upper limit of normal at least once. Medical records were retrospectively reviewed to check their age, sex, maximum total IgE values, maximum eosinophil counts, specific diagnoses, i.e. food allergy, bronchial asthma, allergic rhinitis, anaphylaxis, atopic dermatitis, urticaria and non-allergic disease.

RESULTS: In 4610 patients, total of 8610 diagnoses were identified. Most of the patients (96.6%) had at least one allergic diseases, and allergic rhinitis was the most common diagnosis (2438 out of 4610 patients, 52.9%) followed by bronchial asthma (45.0%) and atopic dermatitis (32.8%). The IgE level was significantly higher in men than women (p<0.001), and also higher in allergic diseases than non-allergic diseases (p<0.001). Among allergic diseases, only atopic dermatitis showed especially higher IgE than the others (p<0.001). In 97 patients who showed very high IgE, greater than 10,000 IU/mL, atopic dermatitis was the most common diagnosis (85.7%). Eosinophil counts significantly correlated with IgE in all allergic diseases (p<0.001), while they didn’t show any correlation in non-allergic disease.

CONCLUSIONS: Allergic diseases were overrepresented in populations with elevated serum total IgE. Allergic diseases, especially atopic dermatitis, tended to have higher value than non-allergic disease.

311 Relationship Of Toll-like Receptors 2 And 4 Gene Polymorphisms With Elevated Production Of Specific Immunoglobulin E

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RATIONALE: Polymorphisms of genes encoding TLR2 (NP_003255,2) and TLR4 (NP_61256,4,3) increase production of IgE.

METHODS: Three single nucleotide polymorphisms of TLR2 (rs5743708) and TLR4 (rs4986790, rs4986791) were genotyped in the control group which of 95 volunteers and 38 patients allergic diseases: 19 asthmatics, 3 atopic dermatitics and 6 allergic rhinitics. Selection required presence of high concentrations of allergen-specific IgE (> 3.5 ku / I) to at least one inhalant or food allergen. Allergen-specific IgE were determined by ELISA ("Rolycheck", Germany). Isolation of genomic DNA from blood used “rapid DNA-blood” (NPF "Lytech", Moscow). Genotyping of specific regions of the genome was performed by polymerase chain reaction.

RESULTS: Analysis of the frequency of polymorphic variants of the gene TLR4 (rs4986790, rs4986791) and TLR2 (rs5743708) showed the presence of significant correlation between the presence of a polymorphic allele of the gene and increased production of TLR2-specific IgE (p = 0.028). Allergic patients with elevated levels of specific IgE had significantly more common genotypes carrying the polymorphic allele G (AG and GG) of the gene TLR4 (rs4986790), than the control group (p = 0.013). The second single-nucleotide polymorphisms TLR4 (rs4986791) showed a possible link between the presence of at least one polymorphic allele T (genotype CT and TT) with elevated levels of specific IgE (p = 0.07).

CONCLUSIONS: A relationship of polymorphisms of TLR2 (rs5743708) and TLR4 (rs4986790) with a high level of production of specific IgE was seen in patients with allergic diseases, suggesting single-nucleotide substitution as an additional predictor of individual propensity for these diseases.
312 B, T, and NK Cell Antigen Expression in Patients with Common Variable Immunodeficiency

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RATIONALE: Common variable immunodeficiency (CVID) is known to affect immune cell phenotype and function. The purpose of this study was to assess the expression of CD45, CD16, CD27, CD38, CD44, and CD138 on CD56 bright, CD56 dim and CD3+CD19+ cells from patients with CVID.

METHODS: We evaluated 21 patients with CVID from 14 academic medical centers. Flow cytometry of CD56 bright, CD56 dim and CD3+CD19+ cells was performed. Data were compared to healthy controls using Student’s t-test.

RESULTS: CD56+CD19− cells were decreased compared to controls in CVID patients. No significant differences were found in CD16, CD27, CD38, CD44, or CD138 expression.

CONCLUSIONS: CD56+CD19− cell number may be decreased in CVID patients. Further study is needed to determine the mechanism and clinical significance of this finding.

313 Clinical and Molecular Characterization of Autosomal Recessive Hypo IgE Syndrome in Saudi Arabia

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RATIONALE: Autosomal-recessive hypo-IgE syndrome (AR-HIES) is a combined immunodeficiency characterized by susceptibility to viral infections, eczema and high serum IgE. Mutations in DOCK8 are responsible for many, though not all cases. We aim to characterize clinical, immunological and molecular features of AR-HIES in Saudi patients.

METHODS: Clinical data of 25 patients diagnosed with AR-HIES were collected. Eighteen patients screened for STAT3, Tyk2 and Dock8 mutations.

RESULTS: We found statistically significant lower absolute median numbers of CD19+, class-switched memory, and CD19+CD27 IgD+ mature naïve B cells in patients with CVID compared to normal controls with 95% confidence intervals. There was a statistically significant difference in the median percentage of CD3+ T cells, possibly relative to the decrease in B cells. There was no statistically significant difference in absolute median number of T and NK cells.

CONCLUSIONS: Studies have shown reduced class switched memory B cells. We also found differences in mature naïve B cells and percentage of T cells. This may indicate chronic IVIG therapy does not affect relative numbers of various B cell populations. Additionally, some of our patients have recurrent viral infections, which could not be attributed to a decrease in median absolute T or NK cell counts. We hope further research in this area will lead to screening tools for classification and prognosis.

314 Safety, Tolerability, and Efficacy of Hizentra Over An Extended Period for the Treatment of Primary Immunodeficiency Disease

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RATIONALE: Previously, a 52-week study demonstrated that 20% immune globulin (Hizentra®), CSL Behring administered subcutaneously to subjects with primary immunodeficiencies was associated with protection from serious bacterial infections (SBIs) and was well-tolerated. This extension study assessed longer-term (11–104 wk) outcomes, including efficacy, tolerability, and health-related quality-of-life (HRQL) in 21 subjects treated at home.

METHODS: Maximum infusion rates were 35 mL/h (1 pump) or 70 mL/h (2 pumps; maximum volume = 40 mL/site; ≤ 4 sites/infusion). Premedication was not given. Study endpoints included: annual SBI rate; rate of any infections; days hospitalized; missed work/school/or inability to perform normal activities days due to any infection; and days of antibiotic use. HRQL and treatment satisfaction were evaluated using validated questionnaires. Local reactions were assessed by subjects 24 hours postinfusion and documented in a diary.

RESULTS: The SBI rate was 0.06/subject-year (2 pneumonia cases), and total infection rate was 2.4/subject-year. Subjects were hospitalized a mean of 0.55 days/subject-year and missed work/school or were unable to perform normal activities a mean of 4.3 days/subject-year. Antibiotics were used a mean of 84 days/subject-year. Mean IgG levels were 11.7–12.8 g/L. HRQL scores were consistent with US norms; treatment satisfaction remained high and stable. The local adverse reaction rate was 0.5/infusion; 99.1% were mild. Adverse event and local reaction rates were similar among subjects infused at low (<35 mL/h), medium (35–50 mL/h), and high (50–70 mL/h) rates.

CONCLUSIONS: Hizentra was well-tolerated and effective over an extended period in subjects with primary immunodeficiency. Adverse event rates were similar among low, medium, and high infusion rate patients.

315 Clinical Characteristics of Adult Patients with Isolated Low IgG and Abnormal Response to Pneumovax

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RATIONALE: ESID defines possible common variable immunodeficiency to include patients with findings compatible with CVID, but with deficiency in only one antibody isotype. We considered that patients with possible common variable immune deficiency limited to the IgG isotype (PCVI-IgG) have an infection-only phenotype, rarely having autoimmune-related complications.

METHODS: Records were analyzed in a retrospective cohort study, selecting subjects with the following criteria: IgG < 600 mg/dL and normal IgA and IgM, abnormal Pneumovax response, and no secondary causes of immunodeficiency. Pneumovax response was defined as positive if at least 70% of the 14 serotypes tested were at least 1 ug/mL and had a minimum 2-fold increase from pre-vaccination.

RESULTS: 26 subjects were identified with mean age of 52 (range 25–72). Mean IgG was 508 mg/dL (SD 15, range 338–590); mean IgA 138 mg/dL and mean IgM 97 mg/dL. Deficiency in IgG was mostly distributed to an infection-only CVID. Infections primarily involve the upper respiratory tract. IgG replacement may be indicated to reduce respiratory tract infections.
Abstracts

CD45 Deficiency Caused by Uniparental Disomy, a Novel Cause of Severe Combined Immunodeficiency

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RATIONALE: Analysis of the molecular etiologies of severe combined immunodeficiency (SCID) has led to important mechanistic insights into the control of immune cell development. Most cases of SCID result from either X-linked recessive or autosomal recessive inheritance of mutations in a known causative gene. However, in some cases, the molecular etiology remains unclear because an abnormal gene can be found in only one parent.

METHODS: To identify the molecular etiology of SCID in a case known to lack the protein-tyrosine phosphatase CD45, we employed a candidate gene approach, analysis of single nucleotide polymorphisms (SNP) and whole exome sequencing in a kindred.

RESULTS: We determined that while the mother was heterozygous for an inactivating mutation in CD45, the paternal alleles exhibited no detectable mutations. Nevertheless, the patient exhibited a single CD45 mutation identical to the maternal allele, suggesting either elimination of one paternal allele by microdeletion or inheritance by the patient of two mutant, maternal alleles. SNP array analysis of the patient revealed no change in copy number, but there was loss of heterozygosity for the entire length of chromosome 1 (Chr1), indicating that disease was caused by uniparental disomy (UPD) with isodisomy of the entire maternal Chr1 bearing the mutant CD45 allele.

CONCLUSIONS: We report here the first case of SCID caused by the novel mechanism of UPD. Moreover, further analysis of our patient cohort suggests that UPD may be prevalent in SCID and warrants consideration, particularly in cases where an abnormal gene is only found in one parent.

316 Clinical phenotypes and prognosis of patients with Common Variable Immunodeficiency (CVID) living in Rio de Janeiro, Brazil

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RATIONALE: CVID comprises a variety of clinical phenotypes that may influence the prognosis. Our objectives were to investigate the clinical phenotypes and prognosis of patients with CVID living in a developing country.

METHODS: We evaluated 11 patients with CVID according to the PAGID criteria in long-term clinical follow-up (>10 years). Clinical evaluation was performed monthly and laboratory tests every 6 months. Most patients were on regular use of intravenous immunoglobulin (IVIg).

RESULTS: The average follow-up was 21.9 years (12-34). Among the 11 patients, the mean current age was 39.8 years (16 to 62). The age at symptoms onset ranged from 4 to 31 years (mean=18) and diagnosis occurred between ages 11 and 47 (mean=28). Most patients (55%) had the phenotype of infectious complications only, 27% had infections and immune thrombocytopenic purpura and 18% had infections and solid neoplasias. None of the patients developed lymphoproliferative and/or inflammatory complications. The most common infections were recurrent sinusitis (100%), pneumonia (82%), giardiasis (36%) and tuberculosis (18%). Adherence to the use of IVIg was good/fair in 88% of patients. Two patients (18%) had mild adverse reactions to IVIg. All patients have good quality of life, performing their routine activities of study, work and leisure.

CONCLUSIONS: The most frequent phenotypes were infectious complications or infectious complications + autoimmunity. The use of IVIg was safe and cost-effective. With proper clinical management and good adhesion, patients with CVID in developing countries may have survival and quality of life similar to those described in developed countries.

317 An Evaluation of Pneumococcal Titers in Patients receiving Ig replacement for Immunodeficiency

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RATIONALE: Intravenous and subcutaneous immunoglobulin(Ig) is used as a replacement therapy for patients with primary characterized by absent or deficient antibody production. Despite Ig replacement therapy with appropriate doses some patients continue to get frequent sinopulmonary infections. This study examines the correlation between specific serotype pneumococcal antibody titers in those patients receiving Ig replacement therapy for immunodeficiency and occurrence of sinopulmonary infection.

METHODS: The study was a retrospective chart review. Charts of fifty-eight patients ages 1-80 years receiving Ig replacement for immunodeficiency were reviewed. The charts were reviewed for pneumococcal titers drawn while on Ig replacement, serum IgG level (at time of serum pneumococcal titers drawn), history of sinopulmonary infections, immune diagnosis, type of Ig replacement - product- and dose received. A positive serotype specific titer was defined by the particular lab resulting the data.

RESULTS: In the CVID patient group, pneumococcal titer were ≤50% in 11/23; sinopulmonary infection occurred in 8 of the 11 with low pneumococcal titers. Serum IgG levels were drawn on 5/8 instances and were all normal. In the selective antibody deficiency group, pneumococcal titers were ≤50% in 8/20; sinopulmonary infections occurred in 4 of the 8 with low pneumococcal titers.

CONCLUSIONS: We observed that patients on Ig therapy at appropriate replacement doses have a surprisingly high frequency of low(≤50%) specific serotype pneumococcal titers. There was no correlation between low pneumococcal titers and increased frequency of sinopulmonary infections in both the CVID patients and the selective antibody deficiency patients receiving Ig replacement for immunodeficiency.

318 Admissions for Primary Immunodeficiency at San Lucas Hospital, Ponce, Puerto Rico: A Pilot Project

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RATIONALE: Determine the prevalence of PID among admissions to a hospital in Ponce, Puerto Rico and evaluate admissions for common complications of immune deficiencies using a validated Immune Deficiency Related score (IDR).

METHODS: Subjects already diagnosed with primary PID within the age 0-59, who were admitted from January 1, 2005 to March 31, 2011 were evaluated. In the second group, subjects age 0-59 who were admitted for diagnosis included in the IDR score items documented on the medical discharge were evaluated. A score higher than 6 was suggestive of underlying PID. The age, sex, race, insurance payer, presentation, complications, morbidity, and mortality were compared among both groups.

RESULTS: 45,585 admissions or 7% of all admission to the hospital were evaluated. In the second group, subjects age 0-59 who were admitted for diagnosis included in the IDR score items documented on the medical discharge were evaluated. A score higher than 6 was suggestive of underlying PID. The age, sex, race, insurance payer, presentation, complications, morbidity, and mortality were compared among both groups.

CONCLUSIONS: Suspected but undiagnosed PID account for a significant percent of all admissions. Patient already diagnosed and treated for PID had lower disease related complications, than subjects with suspected PID. Immunologic test must be conducted to confirm diagnosis. The data support the establishment of an immunodeficiency registry in Puerto Rico.
320 Recombinant Human Hyaluronidase (rHuPH20) Facilitates Subcutaneous Infusion of Immunoglobulin, Increases Local Fluid Dispersion, and Reduces Induration in a Porcine Model


**Rationale:** Immunoglobulin (Ig) replacement therapy for primary immunodeficiency disease has traditionally been administered intravenously (IV), although the subcutaneous (SC) route of administration is demonstrating certain advantages over IV (e.g., no venous access required and reduced systemic adverse reactions). Current standard subcutaneous delivery of Ig (SCIG) is limited to frequent low-volume (≤10-20 mL) injections at multiple sites. In clinical studies, recombinant human hyaluronidase (rHuPH20) facilitated the SC infusion of up to a full monthly dose (≈300-600 mL) of Ig at a single site. A preclinical model was developed to quantitatively assess endpoints that measured fluid dispersion and induration during a large volume SC delivery of Ig in the absence and presence of rHuPH20.

**Methods:** Yucatan micro-pigs were infused with 300 mL of human Ig ± rHuPH20. Endpoints included objective evaluation of local induration, interstitial tissue pressure, skin viscoelasticity, Ig dispersion, cutaneous blood perfusion, and histopathology.

**Results:** Post-Ig infusion, swelling volume and area were reduced in the presence of rHuPH20 by 82% and 54%, respectively. The enzyme also minimized the incidence and severity of induration. Additionally, rHuPH20 reduced interstitial pressures by 39% while increasing local skin pliability. Local dispersion of Ig was improved with rHuPH20, as measured by immunofluorescent staining and histopathological assessments. Lastly, rHuPH20 reversed the negative effects of standard SC Ig infusion on cutaneous blood perfusion.

**Conclusions:** rHuPH20 improved SC dispersion of Ig in a porcine animal model. Co-administration of hyaluronidase during single, large volume delivery of Ig significantly reduced the incidence and severity of induration when compared to standard SC Ig delivery.

322 Cytokine Profile Shifts In Patients With Recurrent Herpes Simplex Of The Oral Mucosa And Lips

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**Rationale:** Relapses of Herpes Simplex may be related to cytokine expression.

**Methods:** 105 HSV patients during relapse and remission were assessed for changes in blood concentrations of IL-4, IL-10, TNF-α, IFN-γ.

**Results:** During a relapse there is a significant 2.5 fold increased concentration of Th2 cytokines (IL-4 - 50.3 ± 0.8 pg / ml; IL-10 - 67.2 ± 2.2 pg / ml) relative to the control group (20.1 ± 1.7 pg / ml and 55.1 ± 3.2 pg / ml; p < 0.01) and a 4 fold reduced level of Th1 cytokines (IFN-γ to 0.5 ± 0.2 pg / ml; TNF-α to 0.3 ± 0.2 pg / ml) in serum of HSV patients compared with the control group (p <0.05). In remission a significant 1.2 fold increase in the concentration of IFN-γ, a 1.7 fold increase in TNF-α and a significant increase in levels of IL-4 up to 25.1 ± 0.3 pg / ml and of IL-10 up to 56.01 ± 0.3 pg / ml compared with healthy controls but a decrease of IL-4 by 2.01 fold and IL-10 by 1.2 fold compared with HSV recurrent disease patients (p <0.05). During a relapse of HSV there are increased concentrations of Th2-cytokines (IL-4, IL-10) and lower concentrations of Th1-cytokines (TNF-α, IFN-γ), while for remission of HSV increased concentrations of IFN-γ and TNF-α occur.

**Conclusions:** Reduced synthesis of Th1 cytokines during remission of recurrent herpes simplex may indicate an approaching relapse.

323 A Novel Severe Combined Immunodeficiency (SCID) Mutation in Three Mexican Siblings with additional de novo Duchenne Muscular Dystrophy (DMD) mutation

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**Rationale:** Review a novel common gamma chain (IL2RG) mutation in a consanguineous kindred.

**Methods:** Chart review, gene sequencing (Correlagen). Children were assessed for immune competence.

**Results:** Two first cousins had 3 children with SCID with c.184T→C (p.cys62Arg) missense mutations in the IL2RG gene (not previously described) with the same father who is unrelated. Two maternal uncles of Pt1 and Pt3 died in infancy. Pt1 - Presented at 8 months with failure to thrive, diarrhea, pneumonia and adenoviruria. He received HSCT from an unaffected brother with resultant T, B and NK cell engraftment. Pt2 - Presented at birth with neutropenia without identifiable cause on bone marrow biopsy. He received maternal haploidentical HSCT with resultant T and NK cell engraftment but still receives immunoglobulin. Pt3 - Presented as healthy newborn. He received a matched unrelated HSCT with T and NK cell engraftment and still receives immunoglobulin. Elevated transaminases without other evidence of graft versus host disease (GVHD) and subtle lower extremity hypotonia led to muscle biopsy. DMD was diagnosed by absence of dystrophin staining and identification of a dystrophin gene mutation non-contiguous with his IL2RG mutation. No other family member shares the DMD mutation. He was started on steroid treatment at 13 months.

**Conclusions:** We report a kindred with a novel IL2RG mutation and successful HSCT correction. Evaluation for suspected GvHD in one infant revealed a second non-linked spontaneous DMD mutation not previously reported in the literature. The short term impact of pre-HSCT conditioning and prophylaxis and chronic DMD steroid treatment on outcome of both disorders is described.
Serum Immunoglobulin Levels in Healthy Thai Infants and Children Aged 0-2 Years Determined by Nephelometry

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RATIONALE: Normal serum immunoglobulin levels (Ig) are important for the diagnosis of immunologic disorders. Data of Ig evaluated by nephelometry are limited in healthy Asian children especially those under 2 years old.

METHODS: Healthy Thai children aged 0-2 years were tested for serum Ig G, A, M, and IgG subclasses by nephelometry. The geometric means for Ig were summarized and categorized by age groups; 1-3, >3-6, >6-8, >8-12, >12-18, and >18-24 months. Statistical analyses were used to compare Ig between gender and age groups, and to compare IgG in this study with the other published studies.

RESULTS: 101 children were enrolled, 44% were female with a median (IQR) age of 0.93 (0.48-1.37) years. The geometric mean IgG was 840 mg/dL, IgA was 36 mg/dL, and IgM was 102 mg/dL. The mean IgG1 was 648 mg/dL, IgG2 was 127 mg/dL, IgG3 was 46 mg/dL, and IgG4 was 17 mg/dL. The average ratios of IgG subclasses/IgG for IgG1:2:3:4 were 77:15:6:1.

CONCLUSIONS: This study illustrated the importance of having normal Ig values from age- and ethnic-matched controls by high precision nephelometry assay in order to correctly diagnose immunologic disorders.

Role of IgM in Pulmonary Complications of Common Variable Immunodeficiency (CVID)

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RATIONALE: Patients with CVID are treated with Ig replacement, however pulmonary complications may progress. As serum IgM varies in CVID and is not repleted by Ig therapy, we examined whether patients lacking serum IgM had more pulmonary complications.

METHODS: The electronic medical records of 33 CVID patients with little detectable IgM were compared to 31 patients with IgM levels greater than 25 mg/dl. Chronic lung disease was defined as pulmonary pathology on CT scan, interstitial disease on chest xray, abnormal pulmonary function tests, or a diagnosis of bronchiectasis or chronic lung disease made upon clinical grounds.

RESULTS: CVID patients with undetectable serum IgM had a significantly increased incidence of chronic lung disease compared to those with IgM greater than 25 mg/dL (78.8% vs 32.3%, 95% CI 1.423 - 4.191, p = 0.0012).

CONCLUSIONS: The absence of IgM may correlate with the progression of pulmonary disease in CVID. Further efforts to determine the protective role of IgM in pulmonary disease in CVID patients are necessary.

The Medical Awareness Concerning Primary Immunodeficiency Diseases (PID) in the City of Sao Paulo, Brazil

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RATIONALE: PID are a heterogeneous group of genetic illnesses that cause defective host defenses. Delay in diagnosis is thought to be caused by a lack of medical awareness concerning PID. We aimed to evaluate the degree of awareness on the part of physicians concerning PID in the city of Sao Paulo.

METHODS: A 14-item questionnaire was applied to physicians working at seven general hospitals. It included a card with the 10 warning signs for PID (adapted from the Jeffrey Modell Foundation). One of the questions described 25 clinical situations that could be associated with PID and a score was created based on percentages of appropriate answers.

RESULTS: A total of 746 physicians participated in the study, with 215 pediatricians (28.8%), 244 surgeons (32.7%) and 287 clinicians (38.5%). About 70% of physicians had learned about PID in medical school or residency training, 75% see patients who frequently take antibiotics, but just 34.1% have evaluated some of them and 77.8% were not familiar with the warning signs for PID. The mean score in the 25 clinical situations was 45.72% (±17.87). Only 26.6% of the pediatricians and 6.6% of both surgeons and clinicians appropriately answered at least 2/3 of these topics.

CONCLUSIONS: There is a deficiency in medical awareness concerning PID in the city of Sao Paulo, even among pediatricians, although doctors have been having more contact with the subject in recent years. An increase in awareness in regard to these disorders within the medical community is an important step towards improving recognition and treatment of PID.

Vitamin D Levels and Respiratory Infections in Patients with Common Variable Immunodeficiency

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RATIONALE: Common variable immunodeficiency (CVID) is the most prevalent symptomatic primary immunodeficiency in adults. While prior studies show association between vitamin D deficiency and increased risk of infections in the general population, the role of vitamin D deficiency in patients with CVID is not well understood.

METHODS: A standardized questionnaire was used to assess the severity of respiratory tract infections over the past two years. We performed a retrospective chart review to determine 25-OH Vitamin D levels, and compared the incidence and severity of infections in two groups.

RESULTS: Among 19 patients the average 25-OH Vitamin D level was 27ng/ml±13.33 (stdev). Using a threshold of 20ng/ml, the average infections per year for the below and above threshold groups were (3.75±2.73 vs 3.40±2.40, p = 0.93) respectively. The number of days per illness was (20.4±8.5 versus 14.2±10.9, p = 0.90), and the average infection severity on a 1 to 10 scale was (4.6±1.9 versus 4.7±2.1, p = 0.96).

CONCLUSIONS: Compared to national data published in the NHANES study, our cohort had similar average 25-OH Vitamin D levels (25ng/ml vs. 27ng/ml). Patients with levels below 20ng/ml trended towards longer duration of respiratory tract infections compared to the higher group. Notably, the two patients with the lowest levels of vitamin D were the only patients hospitalized in the past year for pneumonia. Inclusion of additional patients in this analysis will allow for the development of more robust data.
**328** Prolonged *Klebsiella* Sepsis and Meningitis in a Five Week Old Infant with an IKBKG Gene Mutation (NEMO)

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**RATIONALE:** Males with IKBKG mutations have impaired host defense, and some are at increased risk of severe bacterial infections early in infancy.

**METHODS:** IKBKG gene sequence by GeneDx.

**RESULTS:** A previously healthy male infant was hospitalized at 5 weeks of age for fever and irritability. WBC count was elevated at 46,000/mm3. Blood, spinal fluid and urine cultures were positive for *Klebsiella pneumoniae*. Immunologic workup revealed normal complement and decreased immunoglobulins. Flow cytometry showed marked lymphocytosis with a relatively normal distribution of lymphocyte subsets. Despite appropriate antibiotic therapy his blood cultures remained positive for *Klebsiella* greater than 3 weeks. Family history revealed that his mother and sister had been diagnosed clinically with Incontinentia Pigmenti. IKBKG gene sequence revealed a duplication of a single “C” nucleotide in exon 10 (c1167dupC) a frameshift that changes glutamate to arginine and causes a premature stop codon at position 5(p.Glu390ArgfsX5). The c1167dupC mutation in the IKBKG gene has been reported previously and is predicted to cause loss of normal function either through protein truncation or nonsense mediated mRNA decay. His hospital course was complicated by subclinical seizure activity in the setting of abnormal brain imaging, respiratory distress, anemia and thrombocytopenia. His bacteremia eventually resolved, and he was discharged home where workup continues for bone marrow transplantation.

**CONCLUSIONS:** Mutations in the X-linked IKBKG gene are associated with serious but variable infections in males. Severe mutations can present with early bacterial sepsis. Prolonged bacteremia, likely due to impaired phagocyte function in this disorder, may serve as a clue to the diagnosis.

**329** Common Variable Immunodeficiency Presenting with *Stenotrophomonas maltophilia* Pneumonia

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**RATIONALE:** *S. maltophilia* has emerged as an opportunistic respiratory pathogen in immunocompromised hosts, particularly in those diagnosed with cancer or AIDS. We describe a patient with common variable immunodeficiency (CVID) who initially presented with *S. maltophilia* pneumonia.

**METHODS:** Case report.

**RESULTS:** A 38 year-old, previously healthy Caucasian male presented with a three month history of persistent and productive cough. Despite a ten day course of oral antibiotics, his symptoms worsened to include dyspnea, chest pain, 101.5°F fever, and night sweats. Subsequent hospitalization included a chest CT that demonstrated multilobar pneumonia with areas of localized bronchiectasis and an initial respiratory culture that isolated *S. maltophilia*. Secondary immunologic workup was significant for panhypogammaglobulinemia (IgG 45 mg/dL, IgA < 4 mg/dL, and IgM 3 mg/dL). Qualitative antibody analysis demonstrated poor vaccine responses to both protein and polysaccharide antigens consistent with CVID.

**CONCLUSIONS:** CVID patients often present with recurrent respiratory infections, with *Streptococcus pneumoniae* or *Haemophilus influenzae* as the most commonly isolated pathogens. While other unusual or opportunistic infections have been reported, this is the first reported case of *S. maltophilia* pneumonia in a patient with CVID.
RATIONALE: The panallergen profilin is considered responsible for numerous patients’ cross-reactions to pollen and food allergen sources. Although sequence alignment has been widely used in allergy studies, the bases of the cross-reactivity are not well understood.

METHODS: Ara h 5, the peanut profilin, was expressed in E. coli, purified, crystallized, and studied by x-ray crystallography. Its structure was compared with that of the birch pollen allergen Bet v 2 and latex allergen Hev b 8. The sequence homologies between Ara h 5 and profilins from species for which cross-reaction cases have been reported and between Ara h 5 and profilins from other species were analyzed in conjunction with structure comparison.

RESULTS: The 3-dimensional structure of Ara h 5 was determined at 1.1 Angstrom resolution. The structures of Ara h 5 and Hev b 8 were superimposed with a root-mean-square deviation of 0.955 angstrom for the backbone-atoms. There was, however, significant difference at the N-terminal of the two allergens. The structures of Ara h 5 and Bet v 2 were superimposed with a root-mean-square deviation of 0.487 angstrom; missing residues in the reported structure of Bet v 2 were not included in the calculation.

CONCLUSIONS: Sequence alignment alone could not explain cross-reactivity of food allergy and pollen-food allergy syndrome. Knowledge of 3-dimensional structures of allergens and information about IgE recognition of conformational epitopes may shed light on the allergenicity of food allergens and the etiology of cross-reactivity, but such knowledge and information is very limited. Further studies of conformational IgE epitopes are required.

RATIONALE: The FDA lot release protocol review and testing program insures that the potencies of standardized allergenic extracts distributed in the U.S. are within established limits. For cat and short ragweed pollen allergenic extracts, potency is defined according to the concentration of Fel d 1 and Amb a 1, respectively, and is measured with the radial immunodiffusion assay (RID). The RID is labor intensive and subjective, and dependent on devices that are no longer manufactured. We have therefore developed a sandwich ELISA to more accurately, precisely, and reproducibly measure the potency of these standardized allergenic extracts.

METHODS: Three candidate single chain variable fragment antibodies (scFv) against each allergen were purified by affinity chromatography and used as the coating (capture) antibody. Purified native Fel d 1 (Indoor Biotechnologies) and Amb a 1 were used as antigens. Revealing antibodies were polyclonal sheep anti-cat or anti-short ragweed pollen sera in combination with HRP-conjugated rabbit anti-sheep antibody (KPL). Data were analyzed using a 4-parameter logistic model. The assay was validated with CBER’s current standardized cat and ragweed pollen reference standards, and with extracts purchased from the manufacturers.

RESULTS: Each combination of capture scFv antibody and revealing polysera was sensitive, highly specific, and linear within a wide range of concentrations.

CONCLUSIONS: Sandwich ELISAs for cat and short ragweed allergen extracts will enhance the FDA lot release protocol review and testing program’s ability to measure and confirm the potency of standardized cat and short ragweed pollen allergenic extracts.

RATIONALE: Nickel is the most frequent cause of contact dermatitis in North America, and can cause prosthetic joint failure. Based on our previous work with beryllium, we hypothesized that we could develop a sensitive and specific blood test for nickel sensitization and validate it by patch testing, the current gold standard.

METHODS: We recruited 22 normal controls, and 70 patients referred for evaluation of procreative joint failure. All provided informed consent. All patients were patch tested to 2.5% and 5% nickel sulfate hexahydrate (Allergeaze, Canada), and provided blood for nickel LPTs. Cells were incubated with 8 concentrations of nickel from 0.1 - 50 mcg/mL, and sampled for 3H-thymidine incorporation at 4 and 6 days. Subjects whose cells did not respond to positive controls PHA and Candida were excluded from the analysis. Threshold for a positive result was set above the highest mean control response, normalized to unstimulated counts, plus 3 SD. Patients with responses above the threshold at 2 concentrations were considered positive, and those with 0 or only 1 above threshold level were considered negative.

RESULTS: We compared nickel patch tests (pos/neg) to blood results (above/below threshold) for sensitivity and specificity. 67 (86%) of those with positive nickel patch tests had a positive nickel blood test. 21/24 with negative nickel patch tests had a negative nickel blood test (87.5%).

CONCLUSIONS: We have developed a blood test for nickel sensitization with 86% sensitivity and 87.5% specificity as compared to nickel patch testing. This test should be validated in a larger cohort of patients and controls.

RATIONALE: Immunoglobulin E(sIgE) at identifying those children with aeroallergen sensitization.

METHODS: Thirty-three highly atopic children with the mean age of 36 months (SD +/- 9 months) were analyzed. We collected SPT (ComforTen™) as well as allergen specific IgE (DPC Immulite 2000™) for 8 common aeroallergens. Mismatches between positive SPT and IgE were analyzed.

RESULTS: Our data showed an average mismatch between SPT and sIgE of 35% (median 34 and range 21-67%) when minimum value for serum IgE was 0.1 kIU/L. Forty-five percent (median 42% range 0-92%) of the mismatches were SPT positive and IgE negative and 35% were SPT negative and IgE positive (median 30% range 13-69%). The average mismatch was 27.6 % (median 27 range 21-50) when serum IgE minimum value was 0.35 kIU/L, of which 40.3% (median 50% range 14-57) were SPT positive and IgE negative and 59.7% (median 50% range 43-78%) were IgE positive and SPT negative. Age and eczema activity were not associated with mismatch.

CONCLUSIONS: Our study shows that a number of aeroallergen sensitizations are missed when testing with SPT or sIgE alone. Using both methods would optimize diagnostic yield. The implications of this could result in further research looking at early intervention and the determination of asthma prognosis in young atopic children.
336 Allergen Specificity Of 3 ScFv Antibodies Developed For A Multiplex Assay Of Blattella Germanica Extract Potency

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RATIONALE: We are developing a multiplex antibody-based assay for allergen potency. In previous work, the overall allergenicity of German cockroach (GCr) allergen extracts did not correlate with content of any known specific allergens. We have developed 14 avian scFv antibodies to GCr. In this study we begin to identify their target proteins.

METHODS: Targets of GCr-specific antibodies 2A1, 6A2 and 6A3, isolated by direct immunoprecipitation, were analyzed by SDS-PAGE and mass spectrometry (MS). The cDNA sequences of the target proteins of 2A1 and 6A2 were expressed in E. coli and purified using affinity chromatography. Allergenicity of these expressed proteins was determined by screening a GCr-allergic human serum pool (S1-Cr) for presence of specific IgE antibodies. Thermal stability of these proteins was determined by antibody binding following 1 min incubations at 40-80°C.

RESULTS: The target proteins of antibodies 2A1 and 6A2 have an apparent mass of ~33 kDa and ~78 kDa respectively. BLAST analyses of peptide sequences obtained from MS indicate the following closest matches: 2A1: Per 3 homologue (isofrom 2, GU086323); 6A2: Bla g 7 (AF260897); 6A3: vitellogenin (AJ000515). Targets of the other 11 antibodies remain unidentified. ELISA using S1-Cr indicates the presence of specific IgE for Per 3 homologue and Bla g 7. Per 3 homologue appears to be the only heat stable protein in GCr extract.

CONCLUSIONS: We have identified 3 specific target allergens in GCr: vitellogenin, Bla g 7, and a heat stable Per 3 homologue.

337 IgE Against Bed Bug (Cimex lectularius) Allergens Are Common Among Adults Bitten By Bed Bugs

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RATIONALE: Cimex lectularius, the bed bug, has had a resurgence in New York City (NYC). The potential health implications of this increase in exposure are unknown. Our goal was to develop assays for measuring IgE antibodies against crude C. lectularius extract and a salivary protein Cimex nitrophorin (cNP) in order to determine the prevalence of sensitization to bed bug allergens among adults with a report of bed bug bites.

METHODS: We recruited 30 adult subjects in NYC who reported being bitten by bed bugs within the past year and having an itchy, raised bump in response. Serum drawn from qualified subjects was used in the development of an immunoassay using the ImmunoCAP® system.

RESULTS: Seventeen (57%) subjects had detectable IgE (≥0.1 IU/ml) against the crude C. lectularius extract. Of those seventeen, nine subjects had detectable IgE against recombinant cNP. All subjects with IgE against cNP had measurable IgE against C. lectularius extract. Mean total IgE was higher among subjects with, as compared to subjects without IgE against C. lectularius extract (117 vs. 28.8 IU/ml, P = 0.002) and cNP (125 vs. 47.6 IU/ml, P = 0.05).

CONCLUSION: We developed assays for measuring IgE antibodies against C. lectularius extract and cNP. IgE antibodies to the crude extract and cNP were common among adults who reported being bitten by bed bugs. The dramatic rise in human exposure to bed bugs in NYC and the demonstration of an IgE response to allergens from C. lectularius potentially has important public health implications of bed bug exposure that should be further investigated.

338 Assessment of Allergenicity of GMO Maize Pollen

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RATIONALE: Farmers in a South African maize-growing area, Hartswater, Northern Cape Province, reported increased allergic symptoms as a result of contact with pollen of a specific GMO maize variety.

METHODS: Forty five subjects previously assessed and diagnosed with allergic rhinitis, asthma and chronic sinusitis by two Hartswater physicians were recruited to the study. Detailed questionnaires were completed. Skin prick tests (SPT) for maize pollen from 3 GMO varieties and the 3 isolines, a positive and negative control were performed on all subjects. Serum IgE for maize pollen, a variety of grasses and moulds, olive tree, house dust mites, and CCD was evaluated. Patients serum was used to search for novel or up-regulated allergens by immunoblot.

RESULTS: Thirty six patients (80%) were SPT positive for at least one variety of maize pollen. Nineteen subjects were SPT positive for at least six maize varieties. Fifteen subjects were shown to have maize pollen specific IgE >0.35 kU/L, of which 6 >0.5 kU/L. SPT was significantly more positive for a specific GMO variety in 6 patients. SPT resulted in anaphylaxis in 4 subjects requiring reversal with intramuscular adrenaline. Immunoblot analysis is proceeding.

CONCLUSIONS: Maize pollen is a common cause of hypersensitivity in the Hartswater region. Serum specific IgE determination for maize pollen does not appear to be useful in determining patients allergic to maize pollen suggesting that a maize pollen protein is not adequately represented in serum specific IgE assay. Some individuals appear to be more sensitised (reported history and SPT) to a specific GMO variety.

339 Clinical Usefulness Of Diagnosis By Molecular Components in Mite Allergy

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RATIONALE: In patients allergic to mites IgE antibody test with a natural pollen extract does not reveal the nature of the sensitizing molecules. Polisensitization to House Dust Mites (HDM) and Storage Mites (SM) is frequently observed. The aim of this study is to define whether determination of molecular patterns of sensitization is useful for optimizing the diagnosis of these patients.

METHODS: 101 patients attending our outpatient clinic suffering rhinoconjunctivitis and/or asthma sensitized to mites were included. Skin prick test (SPT, ALK-Abello) and specific IgE (ImmunoCAP) to the HDM Dermatophagoides pteronyssinus and to the SM Lepidoglyphus destructor were done. In parallel, IgE to a panel of allergen components including Der p1, Der p2, Der f1, Der f2, Lep d2 and Der p10 (mite tropomyosin) were performed in the Advia Centaur platform.

RESULTS: All subjects showed positive SPT and 99% positive IgE to D. pteronyssinus. In these patients 81.2% sensitization to Der p1 and 93.1% to Der p2 was found. SPT and specific IgE to L. destructor was positive in 84.2% and 85.1% respectively, although only 42.6% had sensitization to Lep d2. Positivity to Derp 10 was found in only 2% of patients.

CONCLUSIONS: In mite allergy the molecular diagnosis does not always coincide with the diagnosis performed with natural extracts, especially in SM. We consider important to know the molecular components responsible for the sensitization, in order to provide a more accurate diagnosis and etiological treatment to our patients.
340 Development Of A System For Pollen Forecasting In Vinnitsa, Ukraine

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RATIONALE: A system of pollen forecasting in Vinnitsa, Ukraine was developed based on pollen monitoring in 1999-2011, and in 2009-2010.

METHODS: Pollen counts from 1999-2000 were obtained by gravimetric sampling. Pollen collection in 2009 and in 2010 employed volumetric methods using a Hirst Type Burkard trap at Vinnitsa National Pirogov Memorial Medical University in association with the European Aeroallergen Network (EAN). Seasonal allergic patient symptoms at university clinics were correlated using a pollen diary assessing time and severity of ocular, nasal and lung symptoms. Meteorological conditions and solar geomagnetic storms were also noted.

RESULTS: Correlation between tree pollen peaks and solar geomagnetic storms was seen. Patients’ symptoms often occur the day before a pollen peak was noted by the Burkard trap especially for Betula allergy in 2010. Tree pollen peaks depend on weather conditions. Peaks of weed pollens were relatively stable but periods of grass pollination were earlier in May, 2010 and 2011 than in 1999 and 2000. A pollen forecast is provided free by text messages and emails to allergy patients at the university clinic and is printed in regional newspapers, broadcast on municipal radio and posted on websites.

CONCLUSIONS: The development of systematic analysis of trends in pollen counts and pollen forecast may assist patient with seasonal allergic symptoms in management of their disease.

341 Airborne Exposures To Allergen And Particles With And Without Carpeting

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RATIONALE: Health bodies and physicians advising on home allergen avoidance measures for asthmatics recommend removal of fitted carpets. Is this necessarily correct?

METHODS: Allergen Test Dust (ATD) of known composition was aerosolised into an AC-1 (28.5 m³) chamber under controlled conditions. During natural decay, room disturbance and vacuuming, serial airborne particle counts, surface and airborne allergen measurements (augmented ELISA), were undertaken for six different carpets and one hard floor.

RESULTS: Following ATD introduction and during room disturbance the greatest airborne particle counts (x10⁴/m³) were identified with the hard floor (1.0 x10⁴> m³, as well as the coarse PM, 5.0 x10⁴ m³). No significant correlation was found, however, between fine PM and coarse PM.

CONCLUSIONS: High concentrations of mouse and cockroach allergen were frequently detected in the homes of rural pediatric asthmatics; however, few home characteristics were predictive of allergen concentrations. Future studies should establish clinically relevant associations that might place these rural asthmatic children at risk for poor clinical outcomes.

342 Mouse and Cockroach Exposure in Rural Arkansas Delta Region Homes

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RATIONALE: Home characteristics and aeroallergen exposure among rural US children with asthma are poorly described.

METHODS: We examined the home environment of rural asthmatic children in the Arkansas Delta region. Home environment questionnaire, home inspection, and settled dust analysis were completed for each participant. Bedroom and kitchen dust samples were analyzed for concentrations of cockroach and mouse allergens.

RESULTS: Participants included 95 pediatric asthmatics (median age 9 years, 83% African-American) from Arkansas’ Delta region. The majority (78%) resided in detached (single-family) homes. Evidence of cockroaches (27%), evidence of mice/rats (23%), wall-to-wall carpeting (62%), smokers (27%), and lack of mattress/pillow encasements (100%) were recorded in the homes of participants. Allergen concentrations >1 U/g for Blag1 were detected in 71% of kitchens and 23% of bedrooms; Blag2 >1 U/g in 69% of kitchens, 37% of bedrooms; Mmu1 >1.6 μg/g in 43% of kitchens, 33% of bedrooms. Evidence of cockroaches in any room (OR7.27, 95% CI 1.39-71.08) and mildew on bedroom walls/windows (OR 4.71, 95% CI 1.06-20.22) were associated with Blag1 >1 U/g. Wall-to-wall bedroom carpeting was associated with Blag2 ≤1 U/g (OR = 5.26, 95% CI 1.61-20). Similar associations were found for Blag2. Home characteristics were not associated with Mmu1 >1.6 μg/g.

CONCLUSIONS: High concentrations of mouse and cockroach allergen were frequently detected in the homes of rural pediatric asthmatics; however, few home characteristics were predictive of allergen concentrations. Future studies should establish clinically relevant associations that might place these rural asthmatic children at risk for poor clinical outcomes.

343 The Relationship Between Indoor Particulate Matter and Home Ventilation

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RATIONALE: Indoor particulate matter (PM) concentrations have been shown to correlate with increased asthma symptoms, while poor home ventilation has been associated with elevated presence of asthma triggers. As such, we hypothesized that elevated PM concentrations would be associated with decreased ventilation in the homes of asthmatic children.

METHODS: This study was performed as a subanalysis of the Kansas City Safe and Healthy Homes Partnership, a program aimed at improving asthma through environmental home assessment and targeted intervention. Data from 491 rooms of the 81 unique homes with recorded ventilation and PM was included for analysis. PM concentrations (0.3 μm-10 μm) were averaged from readings taken 2 feet and 5 feet from the ground. Ventilation was measured in air changes per hour (ACH). Statistical analysis was performed using SPSS.

RESULTS: Both Pearson’s and Spearman’s correlation coefficients were generated. No significant correlation was found between total PM and ACH. Further, no significant correlation was found between individual PM sizes and ACH. A strong correlation was found between the fine PM, 0.3 μm-2.0 μm, as well as the coarse PM, 5.0 μm and 10 μm. No significant correlation was found, however, between fine PM and coarse PM.

CONCLUSIONS: Our data suggests that indoor PM is not significantly affected by home ventilation; rather, numerous variables are likely to influence home PM. As PM negatively affects asthma symptoms, comprehensive remediation strategies for PM in homes need to be developed.
**Abstracts AB91**

**344 The Effects Of Wind Conditions On Ragweed Counts**

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**RATIONALE:** Ragweed pollen is abundant in the Midwestern United States in late summer and early fall. Given the overall flat topography of this area, the impact of wind conditions on airborne ragweed concentrations was evaluated.

**METHODS:** For 13 successive years between August 1 and November 1, ragweed pollen was collected from the roof of a 5 story building in Kansas City using a Hirst spore trap. Pollen grains were enumerated microscopically every 4 hours. Weather parameters including wind speed and direction were recorded hourly on an Automated Weather Station. Data were entered into an Access database and values were analyzed.

**RESULTS:** Total counts of individual ragweed grains for each time period ranged from 0 to 1000. Total yearly counts ranged from 2,856 in 1998 to 17,899 in 2010. Mean ragweed counts from the first 5 years and the last 5 years were 4,238 (SD 1,747) and 10,501 (SD 5,205) (p < 0.02), respectively. Mean ragweed counts for each time period ranged from 12.5 to 13.4 for wind speeds between 0-5, 6-10, and 11-15 mph compared to mean counts that ranged from 20.3-21.8 for wind speeds between 16-20 and >21. The most common wind direction measured during ragweed season was 22 degrees from North in the clockwise direction.

**CONCLUSIONS:** Our data demonstrates a trend of increasing ragweed counts over recent years. A wind profile indicates that ragweed counts in the Kansas City area were increased when the wind direction is from the southwest. Finally, ragweed dispersal optimized at wind speeds >15 mph.

**346 Trends In Kansas City Tree Pollen: A 15 Year Perspective**

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**RATIONALE:** 55% of the U.S. population tests positive to at least one allergen. Tree pollen is a major allergen during spring in the Midwest. We have attempted to explore various tree pollen taxa seen in Kansas City, Missouri.

**METHODS:** Airborne pollen were collected using a Burkard spore trap positioned atop a 5 story hospital building in the urban core of Kansas City, Missouri. The collector operated from February to November from 1997 through 2011. Slides were stained with Calberlas stain and pollen grains were counted microscopically at 400X. Individual counts for pollen grains were generated every 4 hours for each 24 hour period. Pollen counters were certified by the National Allergy Bureau.

**RESULTS:** We had 2076 observations for tree pollen during this 15 year period. The highest count for any individual time period was 4927 for oak on 4/12/2010, followed by Mulberry 3206 on 4/13/2010 and Juniper 3074 on 3/21/2011. The top 5 trees for the total pollen production over 15 year period were Oak 32%, Mulberry 29%, Juniper 12%, Elm 6.5% and Ash 4.4%. The minor pollens were Birch 3.9%, Cottonwood 2.6%, Walnut 2.6%, Pine 2.5%, Maple 2%, Hickory 1.32%, Willow 1.3%, Sycamore 0.9%, Beech 0.2% and Alder 0.09%. Examination of the trend showed an increase in major tree pollen counts over 15 year period.

**CONCLUSIONS:** Kansas City tree pollen is dominated by Oak, Mulberry, Juniper, Elm and Ash. All tree pollen appears to be increasing over the last 15 years.

**347 Pilot Study on Inhalant Allergen filtration performance of newly developed intranasal filter**

J. Lee, J. Sohn, C. Hong, J. Park; Yonsei University College of Medicine, Seoul, REPUBLIC OF KOREA.

**RATIONALE:** Nasal inhalation of allergens is the first step of the development of respiratory allergic diseases including allergic rhinitis. However, avoidance of nasal allergen inhalation has not yet been established. We investigated the allergen filtration performance of newly developed intranasal filter.

**METHODS:** The allergen filtration performance of intranasal filter (NOK®) was measured with D. farinae and oak pollen allergen using the customized airflow chamber, Bradford protein assay and ELISA inhibition test.

**RESULTS:** The intranasal filter decreased the penetration of house dust mite allergens (84.3% by protein assay, 73% reduction by inhibition ELISA test) and oak pollen (95.0% reduction of oak pollen allergen by protein assay) particles as demonstrated through the customized airflow chamber.

**CONCLUSIONS:** Newly developed intranasal filter decreased effectively the penetration of house dust mite and oak pollen allergen in our investigation using the customized airflow chamber. Application of intranasal filter may contribute in avoiding of inhalant allergens. Further clinical studies with allergic rhinitis is needed for validation of clinical effect.
When Is It Safe To Go Outside? Nighttime Juniper Pollen Concentrations In Texas, Oklahoma, And New Mexico

L. D. Bunderson, P. Van de Water, J. Luvall; The University of Tulsa, Tulsa, OK, California State University, Fresno, Fresno, CA, NASA, Huntsville, AL.

**RATIONALE:** Juniperus pollen is a major allergen in Texas, Oklahoma, and New Mexico. While juniper pollen release is thought to be predominantly a daytime occurrence, overnight pollen concentrations can be very high. Determining sources of high overnight concentrations will aid the development of a pollen forecast system for *Juniperus*.

**METHODS:** Burkard volumetric pollen traps were established for two consecutive spring seasons at 6 sites in northern New Mexico and 6 sites for two consecutive winter seasons in Texas and Oklahoma. Standard methods were used in the preparation and analysis of slides.

**RESULTS:** The diurnal peak for Camp Classen, Oklahoma was at 10 pm for the winter of 2009/2010 and at midnight for the 2010/2011 season. Seasonal peak hour concentrations were at 10 pm and midnight respectively and overnight peaks were regular occurrences throughout both seasons. Texas sites peaked from 10 am to 12 noon and seasonal peak hours were during the daytime. Some of the detected pollen in Oklahoma was correlated with regular southerly winds that may have transported pollen from Texas. Two locations in New Mexico had nighttime diurnal peaks. The diurnal peak for Jemez Springs, NM was at midnight in 2010 and 8 pm for 2011. The seasonal peak hour for Jemez Springs was at 10 pm for 2010 and 8 pm for 2011.

**CONCLUSIONS:** Evidence supports the contribution of long-distance transport to nighttime peaks in Oklahoma. Canyon wind effects may contribute to overnight peaks in New Mexico. Average daily pollen concentrations do not reflect when exposure risk will be greatest.

A Recent Significant Increase in Ambrosia Pollen Abundance in Central Ukraine

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**RATIONALE:** Ragweed (*Ambrosia*) is an important allergen which is recorded in 19 from 26 regions of Ukraine.

**METHODS:** Volumetric methods used a Burkard trap placed at 25 meters above the ground on the roof of Vinnitsa National Medical University.

**RESULTS:** Highest concentrations of Ambrosia (80 grains/m³ and 103 grains/m³) were recorded on August 28, 2009 and August 13, 2010, with moderate concentrations September 4, 2009 (24 grains/m³) and September 8, 2009 (29 grains/m³). 2010 was characterized by 11 peaks of Ambrosia concentration > 20 grains/m³; but only 2 such peaks in 2009; and 9 peaks in 2011 with >174 grains/m³ recorded from August 24 to August 29, 2011 and a peak of 760 grains/m³ on August 25, 2011. This peak was 7.4 fold greater than the maximum peak seen in 2010. Timing of Ambrosia peaks remains stable for the last 13 years with greatest concentrations seen during the third ten day period of August and the first ten day period of September. Ambrosia was the 8th most abundant pollen in Vinnitsa during 2009; 7th most abundant pollen during 2010; and 1st most abundant pollen in 2011 accounting for 14% of all pollen collected in Vinnitsa in 2011.

**CONCLUSIONS:** Ambrosia pollen has been static in abundance from 1999 to 2009 in Vinnitsa, Ukraine, but has increased from 2009 to 2010 and now dramatically increased in 2011. This trend is consistent with other parts of southern and eastern central Europe which have seen an increase in ragweed allergic diseases.
Identification Of Increased Oral Eosinophils In Patients With Eosinophilic Esophagitis (EE) Using Oral Rinse Analysis: Proof Of Concept

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RATIONALE: Patients with suspected EE require endoscopic biopsy for confirmation. Evaluations of oral rinses from EE patients were assessed for increased Eosinophils (Eos) as a possible screening tool.

METHODS: EE Patients were asked to rinse with 10 ml of saline for 30 seconds. Expectorates were fixed with formaldehyde. Following filtration to remove epithelial cells, samples were centrifuged at 3000 rpm. Cell pellets were re-suspended in PBS. 50 microliters of cells were stained with Hematoxylin and Eosin. Eos and neutrophils (PMN) were visualized and quantified under light microscopy by characteristic nuclei, eosin containing granules and size. Quantification of Eos was assessed in microscopic fields. Control samples were obtained.

RESULTS: Six patients with biopsy proven EE showed an average of 19% Eos (range 10-12%) of all cells evaluated. This was significantly more than control, (mean 3.1% range 2-4%). There was a higher absolute number of PMN’s in control patients as compared to patients with EE (111 vs. 49).

CONCLUSIONS: There is evidence of increased amount of Eos isolated from oral rinses of EE patients. A combination of increased Eos and decreased PMN’s resulted in an increase relative proportion of Eos in patients with EE. The analysis of oral rinses may serve as a screening tool for patients suspected of having EE.

Fecal Eosinophil-derived Neurotoxin Is Significantly Elevated In Non-ige Dependent Gastrointestinal Allergies, Especially In Subtypes Showing Bloody Stool

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RATIONALE: Gastrointestinal allergies (GI-allergies), including food protein-induced enterocolitis syndrome (FPIES), proctocolitis, and enteropathy are thought to be non-ige-associated and cell-mediated food allergies. However, the precise pathogenesis of these allergies remains uncertain. In order to determine whether eosinophils are involved in these GI-allergies or not, we measured eosinophil-derived neurotoxin (EDN) levels in stool samples from patients with various GI-allergies.

METHODS: Stool samples were obtained from patients with GI-allergies (n=42) or healthy infants (n=230). Stool sample extracts were prepared using sample preparation kits (Roche) and the EDN levels in the stool sample extracts were measured by ELISA.

RESULTS: The EDN levels in the stool sample extracts from patients with GI-allergies were significantly higher than those from healthy infant. In addition, the EDN levels in the stool sample extracts varied among GI-allergies; the EDN levels in the stool sample extract from patients who exhibited bloody stools were higher than those without bloody stools.

CONCLUSIONS: Our results suggest that eosinophils are likely to be involved in the pathogenesis of GI-allergies, in particular, those with bloody stools. Our results also imply the involvement of Th2-type immune responses in the pathogenesis of GI-allergies through activation of eosinophils but not IgE synthesis.

Dynamics of Eosinophils in Non-IgE-mediated Gastrointestinal Food Allergies in Neonates and Infants, Differences Between 4 Clusters


RATIONALE: Non-IgE-mediated gastrointestinal food allergies are consisted from several syndromes; FPIES, proctocolitis, enteropathy and allergic gastroenteritis syndrome (AEG). AEG and enteropathy can be diagnosed only by histological examination. To establish effective initial diagnosis of whole patients at first presentation, we tried to classify those by simple clinical variables and then, 4 clusters were derived (JACI 2011:127:685-688).

In this paper, we tried to make comparison of eosinophils, important biological marker in GI allergy, between those 4 clusters.

Proof Of Concept


H. Morita1, I. Nomura1, T. Shoda1, H. Saito1, K. Matsumoto2; 1National Research Institute for Child Health and Development, Tokyo, JAPAN, 2Department of Pediatrics, Keio University School of Medicine, Tokyo, JAPAN.

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RESULTS: The EDN levels in the stool sample extracts from patients with GI-allergies were significantly higher than those from healthy infant. In addition, the EDN levels in the stool sample extracts varied among GI-allergies; the EDN levels in the stool sample extract from patients who exhibited bloody stools were higher than those without bloody stools.

CONCLUSIONS: Our results suggest that eosinophils are likely to be involved in the pathogenesis of GI-allergies, in particular, those with bloody stools. Our results also imply the involvement of Th2-type immune responses in the pathogenesis of GI-allergies through activation of eosinophils but not IgE synthesis.

Our 3-year Experience with Patch Testing for Eosinophilic Esophagitis

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RATIONALE: Treatment of eosinophilic esophagitis (EoE) consists of pharmacotherapy and/or dietary therapy. Food atopy patch testing (APT) for EoE is not standardized and its clinical utility is controversial. We hypothesize that our APT method is valuable adjunctive therapy to aid in treatment of pediatric EoE.

METHODS: Sixty-two patients (male=49, mean age=8.8 years) with eosinophils on esophageal biopsy (mean proximally=38/HPF, distally=41/HPF) who were evaluated by food APT between 01/2008-02/2011 were retrospectively identified by ICD-9 codes. APT was performed using powdered food, sterile water, and glycerin in a 12-food EoE panel. Biopsies, food-allergy tests [serum IgE (sIgE), percutaneous skin test (PST), APT], and swallowed steroid (SS) usage were recorded.

RESULTS: Combined APT and PST identified 1 food in 74%. For APT, 53% (n=33/62) were positive [peanut (n=13/55, 24%), corn (n=14/61, 23%), and chicken (n=11/58, 19%)], for PST, 57% (n=35/61; most commonly, peanut, wheat, and corn), and for sIgE, 67% (n=12/18). Eleven patients had negative PST, but positive APT. Those with surveillance biopsies (n=39) had 2 on average; 39% of surveillance biopsies (n=31/79) were on guided-elimination (GE) from food allergy testing. Those on SS (n=12/39, 30.8%), SS and GE (n=12/39, 30.8%), and GE (10.2%, n=4/39) had 62% (proximally) and 69% (distally) decrease in mean eosinophils/HPF on first surveillance biopsy after APT compared to diagnostic biopsy. 56% (n=10/18) of those on APT-based GE had <15 eosinophils/HPF on first surveillance biopsy, though 70% of those (n=7/10) were concomitantly on PST-based GE and SS.

CONCLUSIONS: Our APT method with subsequent food elimination aids in the complex treatment of pediatric EoE.
356 Food Extract Testing for Delayed-Type Hypersensitivity in Eosinophilic Esophagitis
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RATIONALE: Puree-food patch testing (PP) can identify cell-mediated hypersensitivity (CMH) to foods in Eosinophilic Esophagitis (EoE), but is not standardized, has variable predictive values for some foods, and patients may find it bothersome. Intradermal testing (IDT) read at 48-72 hrs is another technique to assess cell-mediated hypersensitivity. We explored a pilot feasibility study to determine if IDT with food extracts could also determine CMH in patients with EoE.

METHODS: 31 biopsy-proven EoE patients without a history of food anaphylaxis and 10 controls have been enrolled. Patients underwent PST and PP testing to a standard 12-food EoE panel. Dilute IDTs (1:1000 strength) were placed to foods negative on PST. IDTs were read at 20 minutes and 48-72 hours. Subjects completed a written survey detailing their experience.

RESULTS: No patients experienced systemic reactions during testing. Among controls, IDT was uniformly negative. 35% of patients had positive patch testing, compared 6.4% of delayed-IDT. All positive delayed-IDT were also positive on PP. There was a 94% concordance between negative tests. 60% had at least one positive immediate IDT. 56% of patients preferred IDT, 12.5% preferred PP. 57% of children reported needle fear was not a major issue.

CONCLUSIONS: This ongoing study suggests that in the context of EoE, food IDT appears safe and well tolerated. There was high concordance of negative tests between the two methods. Immediate reactivity to IDT may hold additional significance as a diagnostic test. Further evaluation is needed to better assess the sensitivity of IDT for testing in the context of EoE.

357 Quantifying Allergic Sensitivities among Adults with Eosinophilic Esophagitis
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RATIONALE: Eosinophilic esophagitis (EoE) patients have a high prevalence of allergic sensitivities. Our objective was to use serum measurements of specific IgE to food and inhalant allergens to quantify allergic sensitivities in adults with EoE.

METHODS: In a cohort of adults with EoE, we performed skin prick testing and measured total IgE and serum levels of specific IgE to a panel of common foods and inhalants (CAP FEIA).

RESULTS: The geometric mean (GM) total IgE for the whole group was 68.8 IU/ml. Serum measurements were positive in 78%; total IgE was significantly higher among these patients (GM 115 IU/ml) compared with those who were non-sensitized (13.2 IU/ml) (p<0.03). Serum IgE measurements were often different from skin test results. Specific IgE to wheat was found in 56% of patients [geometric mean titer (GMT) 1.28 IU/ml]; none of the patients had a positive skin test to wheat. Peanut and soy sensitivities followed in frequency and were often identified in the same patients. The prevalence of individual inhalant sensitivities ranged from 22-33% except for the most common [weeds (including ragweed) found in 55%]. The sum of specific IgE measurements correlated with total IgE (r=0.75, p<0.02).

CONCLUSIONS: Serum measurements can provide useful clinical information. Because of the large number of adult patients with specific IgE to wheat and negative skin tests, serum IgE measurements may be helpful in planning an avoidance diet. Non-sensitized patients had strikingly low levels of total IgE.

358 Evaluation of Skin Testing to Aeroallergens and Foods in the Management of Adults with Eosinophilic Esophagitis and Response to Comprehensive Therapy

RATIONALE: Assessment of aeroallergen and food sensitivity in patients with eosinophilic esophagitis and its value in treatment outcomes.

METHODS: Retrospective chart review of adult patients with eosinophilic esophagitis from 2007-2011 at an Allergy/Immunology referral center. Clinical history and endoscopic biopsy with ≥15 eosinophils/hpf were criteria for diagnosis.

RESULTS: Forty-nine patients were Caucasian (96%) and male (63%) with a mean age of 38±12 years. Thirty-eight (78%) had a history of atopic disease. Patients were skin tested to a standard panel of 41 aeroallergens; 87% were sensitive to ≥1 inhalant. Skin testing to 21 (IQR=14-24) foods showed 28 (58%) reactive to ≥1 food, with an average of 2 (1-3) positive tests per food-allergic patient. Patients were placed on: 1) swallowed inhaled steroids, 2) PPIs, 3) avoidance of relevant foods, and 4) aeroallergen avoidance plus intranasal/orally inhaled steroids when indicated for 3-4 months. Thirty-four patients followed up: 26 (77%) became asymptomatic, 7 had partial improvement, and 1 had no improvement. Twenty-seven of these patients were atopic, and 21 of these became asymptomatic. Thirteen atopic patients have been followed >4 months after swallowed inhaled steroid cessation. One patient had no improvement, symptoms recurred in 3 patients at 4, 5, and 12 months; three of these four were not fully compliant with therapy. Nine (67.7%) remain asymptomatic with 5 for ≥2 years, 2 between 1-2 years and 2 between 4-12 months.

CONCLUSIONS: A combination of swallowed inhaled steroids, PPIs, intranasal/oral steroids and avoidance of food and inhalant allergens appears to offer benefit in the management of eosinophilic esophagitis.

359 Clinical and Laboratory Characteristics of Adults with Eosinophilic Esophagitis
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RATIONALE: Understanding characteristics of adult patients with Eosinophilic Esophagitis (EoE) is critical for disease management and treatment advances. The aim of this study was to determine the baseline clinical and laboratory characteristics of adult EoE patients.

METHODS: A retrospective review of 49 patients with EoE evaluated in an adult allergy clinic was performed. Patients with suggestive histories and >15 eosinophils/hpf on biopsy were included. Data including baseline demographics, presenting symptoms, endoscopic findings, and available allergy test results were obtained.

RESULTS: 40/49 patients had skin prick testing performed (SPT) to both food and aeroallergens. 25/40 (63%) were male. 35/40 (88%) were sensitive to aeroallergens, 25/40 (63%) were sensitive to foods allergens, and 4/40 (10%) had negative SPT. Peanut and tree nuts were the most commonly positive foods, 62% and 60% respectively. Total IgE was elevated (mean 204 IU/ml) in patients with both food and aeroallergen sensitivity, compared to those with only food, only aeroallergen, or no SPT sensitivity (p=0.006). 35/40 (88%) had dysphagia. 17/40 (43%) reported oral symptoms (e.g. pruritus). 8/40 (20%) required esophageal dilations. There did not appear to be an association with age, gender, or total IgE level with respect to need for esophageal dilation.

CONCLUSIONS: To our knowledge, this is the largest descriptive study of adult EoE patients. There is a lack of standardized approach in the evaluation of adult EoE patients. Describing the characteristics of this population will not only aid in better defining this unique group of patients, but also assist in optimizing evaluation and management.
360 Serum Free Light Chain Levels In Children Are Higher In Females Than In Males With Eosinophilic Esophagitis

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RATIONALE: Eosinophilic esophagitis (EE) is an emerging disease worldwide, characterized by an eosinophilic infiltration of the esophageal wall. EE is closely associated with male gender and allergic disorders, such as food allergy, eczema and asthma. The objective was to investigate a cohort of 28 children with EE for the distribution of established and newly explored allergy markers, including immunoglobulin free light chains (Ig-fLC).

METHODS: Serum cow’s milk-specific IgE, thymic stromal lympho-poietin (TSLP), thymus- and activation-regulated chemokine (TARC/ CCL17) and Ig-fLC were analysed in a cohort of 21 boys (age 6.7 years ± 4.2 SD) and 7 girls (age 8.2 years ± 5.6 SD) suffering from EE as diagnosed by esophageal biopsy (eosinophils > 50/high power field).

RESULTS: Cow’s milk specific IgE levels were elevated when compared to clinical reference values in 9/21 (42.9%) males and 4/7 (57.1%) females. TARC was elevated in 1/21 males (4.8%) and TSLP in 4/21 males (20%); which was not the case in any female within this cohort (ns). Kappa Ig-fLC was elevated in 2/21 (9.5%) males versus 4/7 (57.1%) females (p=0.008) and lambda Ig-fLC was elevated in 1/21 (4.8%) males versus 5/7 (71.4%) females, (p=0.001).

CONCLUSIONS: Gender is a key factor in the biology of EE. Serum Ig-fLC appeared clearly increased in females as compared to males, thereby adding another gender difference in the biology of EE to the already described non synonymous polymorphism in the TSLP receptor (TSLPR) in males. The precise role for Ig-fLC in EE remains to be investigated.

361 The Combination Of Skin Prick Testing And Atopy Patch Testing Can Successfully Guide A Food Elimination/ reintroduction Diet In EE

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RATIONALE: Eosinophilic esophagitis (EE) is a disease diagnoses based on ≥1 eosinophils per high power field on esophageal biopsy after other diagnoses are ruled out. Foods have been shown to be the cause of EE via elimination diets and elemental formulas.

METHODS: We examined a group of patients in whom a causative food was identified by biopsy after treatment with proton pump inhibitor. Skin prick testing and atopy patch testing (SPT and APT) were performed by standard methods. Negative and positive predictive values (NPVs and PPVs) for SPTs and APTs were calculated. Causative foods were identified by: removal of an individual food leading to a normal esophageal biopsy (0 eosinophils/hpf) and/or addition of an individual food led to increased eosinophils (≥1 eosinophils/hpf) after a previously normal biopsy.

RESULTS: A total of 98 patients were studied. Milk, egg and wheat were the most common positive foods by esophageal biopsy, milk allergy was the most common positive foods by esophageal biopsy, milk allergy was identified by biopsy after treatment with proton pump inhibitor. Skin prick testing and atopy patch testing (SPT and APT) were performed by standard methods. Negative and positive predictive values (NPVs and PPVs) for SPTs and APTs were calculated. Causative foods were identified by: removal of an individual food leading to a normal esophageal biopsy (0 eosinophils/hpf) and/or addition of an individual food led to increased eosinophils (≥1 eosinophils/hpf) after a previously normal biopsy.

RESULTS: A total of 98 patients were studied. Milk, egg and wheat were the most common positive foods by esophageal biopsy, milk allergy was identified by biopsy after treatment with proton pump inhibitor. Skin prick testing and atopy patch testing (SPT and APT) were performed by standard methods. Negative and positive predictive values (NPVs and PPVs) for SPTs and APTs were calculated. Causative foods were identified by: removal of an individual food leading to a normal esophageal biopsy (0 eosinophils/hpf) and/or addition of an individual food led to increased eosinophils (≥1 eosinophils/hpf) after a previously normal biopsy.

CONCLUSIONS: The markers investigated included eosinophil derived neurotoxin (EDN), eotaxins-1,-2 and -3, IL-4, IL-5, IL-13, and CCR3, eosinophil peroxidase (EPX), absolute eosinophil count (AEC), fibroblast growth factor and mast cell markers. Eight studies have identified correlation between tissue eotaxin-3 and disease activity. There were two studies on tissue EDN and two on serum EDN. Eight studies on blood and tissue levels of IL-5 were identified. Three studies investigated tissue IL-13 expression. Mast cells and their products were investigated in six studies. There were four studies on eotaxins-1 and -2, two on stoll EDN and several with AEC as one of the parameters examined. Most of these studies have identified a positive correlation between disease activity and biomarker levels. A novel histologic scoring system based on a monoclonal antibody against EPX has been proposed.

CONCLUSIONS: Major studies have investigated tissue biomarkers. Eotaxin-3 has emerged as the most promising tissue marker for EE. More studies investigating peripheral and non-invasive biomarkers to monitor response to treatment will be beneficial. Larger prospective studies are needed to further characterize these biomarkers and define their place in the diagnostic algorithm of EE.
**AB96 Abstracts**

**363** Histology Scoring System (HSS) is Superior to Peak Eosinophil Count (PEC) to Identify Treated vs Untreated Eosinophilic Esophagitis (EOE) Patients

M. H. Collins1, L. J. Martin1, E. S. Alexander1,2, S. Pentiuk1, A. Ellision1, P. E. Purwok2, J. P. Franciosi1, J. P. Abonia1, M. E. Rothenberg1, Cincinnati Children’s Hospital Medical Center, Cincinnati, OH; University of Cincinnati College of Medicine, Cincinnati, OH.

**RATIONALE:** PEC ≥15 eosinophils per high power field in esophageal biopsies is an EOE diagnostic criterion. We hypothesize greater discrimination among EoE biopsies occurs using a HSS including additional pathology (eosinophil abscesses/surface layering, epithelial hyperplasia and necrosis/ apoptosis, dilated intercellular spaces, lamina propria fibrosis).

**METHODS:** Biopsy scores were scored retrospectively using an 8 point HSS to grade (measure severity) and stage (measure prevalence) histologic characteristics. Univariable data were analyzed using two-tailed Student t-tests at p<0.05. Wilcoxon 2-sample nonparametric tests were used to compare HSS parameters by treatment status at p<0.05. Logistic regression models (pg<0.05) with AICs were used to compare goodness of fit for models designed to predict treatment status.

**RESULTS:** A total of 46 proximal and 42 distal esophageal biopsies from 41 patients were scored. Demographics were 81% male, 100% white, age 10.16±4.35 years (range 1-18 years) without differences between treated (diet and/or topical steroids) (35 endoscopies) vs untreated (11 endoscopies). Using nonparametric tests, PEC in distal (PECD) and proximal (PEC) biopsies, maximum PEC in either site (PECMax), and maximum stage (MSS) and grade (MGS) scores were assessed with treatment status (p<0.01). Logistic regression models were significant for PECD and PECMax, and MSS and MGS, but not for PECD. Goodness of fit was superior for both MGS (42.79) and MGS (46.78), compared to PECD and PECMax (50.3, 50.4) with MSS having the best fit.

**CONCLUSIONS:** HSS is superior to eosinophil counts to identify EoE biopsies following therapy. HSS more completely evaluates mucosal healing and likely forms a better basis for making therapeutic decisions.

**364** The Potential Benefit of Immunotherapy in the Treatment of Eosinophilic Esophagitis in Adult Patients

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**RATIONALE:** This study examined 2 groups of adult patients with eosinophilic esophagitis to assess immunotherapy’s possible added treatment benefit.

**METHODS:** This was a retrospective chart review of 12 adult patients with eosinophilic esophagitis, diagnosed by esophageal biopsy. Group A included 6 patients (4 male, 2 female, ages 30-45), all receiving immunotherapy, medications, and diet changes. Group B included 6 patients (4 male, 2 female, ages 30-45), selected from 45 eosinophilic esophagitis patients to match age/gender distribution of Group A, on medications and diet changes alone.

All patients were asked to rate symptom severity on a 1-10 scale (1=none, 10=most severe).

**RESULTS:** Of Group A, all patients experienced pre-treatment dysphagia (daily to every few months). Pre-treatment symptom severity ranged 2-10/10 (average 6/10). All patients started swallowed fluticasone. Five started proton pump inhibitors (PPPIs). Five made diet changes. All patients were on immunotherapy to treat seasonal allergies, for a period from 6 months to greater than 2 years.

Post-treatment, 2 patients reported their dysphagia had completely resolved. Four reported infrequent dysphagia (every 2 months). All patients reported symptom severity of 1/10.

Of Group B, all patients experienced pre-treatment dysphagia (daily to every few weeks). Pre-treatment symptom severity ranged 3-8/10 (average 5.8/10). Four patients started swallowed fluticasone. Five started PPPIs. Five made diet changes.

Post-treatment, one patient reported his dysphagia had completely resolved. Four reported infrequent dysphagia. One reported weekly dysphagia. Symptom severity ranged 1-7/10 (average 2.5/10).

**CONCLUSIONS:** Immunotherapy may impart an added benefit to medical and diet changes in treatment of eosinophilic esophagitis in adult patients.

**365** Predicting Esophageal Biopsy Eosinophil Counts from Presenting Symptoms in Patients with Suspected Eosinophilic Esophagitis

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**RATIONALE:** Eosinophilic Esophagitis (EOE) is a clinicopathological condition hallmarkled by esophageal eosinophilia >15 per high powered field (hpf) on biopsy. EoE symptomatology has considerable overlap with gastroesophageal reflux disease (GERD). No symptom patterns accurately distinguish the conditions without a biopsy.

**METHODS:** Chart review of 504 consecutive pediatric and adult patients who underwent esophageal biopsy between 2008 and 2011. 19 symptoms were analyzed by logistic regression to determine their association with diagnostic cell counts indicative of EoE (>15/hpf).

**RESULTS:** 78.4% (388/495) had biopsies with diagnostic counts. Pediatric bivariate analysis indicated dysphagia to solids, duration of symptoms >6 months, and Caucasian race positively predicted; but weight loss/failure to thrive, vomiting, and abdominal pain negatively predicted diagnostic counts. On multivariate analysis, dysphagia to solids (OR 2.13, CI95%:3.35-3.37, p<0.001) and weight loss (OR 0.406, CI95%:0.21-0.784, p<0.007) predicted diagnostic counts. Adult bivariate analysis indicated regurgitation, atopy, and dysphagia to solids positively predicted; but early satiety, nocturnal awaking, weight loss, and reflux/heartburn negatively predicted diagnostic counts. On multivariate analysis, regurgitation (OR 2.29, CI95%:1.4-4.74, p<0.025), dysphagia to solids (OR 1.82, CI95%:1.3-2.93, p<0.013), early satiety (OR 0.359, CI95%:0.14-0.898, p<0.029), weight loss (OR 0.463, CI95%:0.249-0.861, p<0.015) and chest pain (OR 0.55, CI95%:0.31-0.975, p<0.041) predicted diagnostic counts. With multivariate adjustment for PPPI use, dysphagia to solids and weight loss in adults remained significant.

**CONCLUSIONS:** Dysphagia to solids positively predicted, and weight loss negatively predictive diagnostic counts in all ages, but after adjustment for PPPI use only remained significant in adults. Further prospective study is needed for additional confirmation.

**366** Innate Immune Responses Differ In Patients With Food Protein Induced Enterocolitis Syndrome (fpies) Who Respond Well To The Restrictive Diet And Those Who Do Not

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**RATIONALE:** FPIES is a benign condition with an excellent response to the avoidance of the offending food, i.e., restrictive diet (RD). This study hypothesized that FPIES patients with poor responses to the RD (FPIES-PR) have innate immune abnormalities compared to those with good responses to the RD (FPIES-GR).

**METHODS:** This study include FPIES-PR (N=18, 1.1-5.1 yr), FPIES-GR (N=18, 0.6-4.8 yr) and normal control (N=16, 1.0-5.5 yr) children. In addition to detailed clinical features and routine food allergy (FA) workup, we examined responses to agonists of Toll Like Receptors (TLRs) and representative luminal antigens (Ags) (soy, cow’s milk, and wheat proteins, and candida Ag) by measuring production of proinflammatory and counter-regulatory cytokines by peripheral blood mononuclear cells (PBMCs) when their GI symptoms were under control following implementation of the RD. Results: FPIES-PR children revealed more severe clinical features as evidenced by higher frequency of failure to thrive (FTT), requirement for the use of free amino acid (FAA) formulas, intolerance to probiotics, and severe anaphylaxis like reaction to rice than FPIES-GR children (all p<0.005). FPIES-PR PBMCs revealed lower production of counter-regulatory cytokine (sTNFRII and IL-10) in the absence of stimulus and with TLR 5, 7/8, and 9 agonists (sTNFRII) and with TLR2/6, 5, and 9 agonists (IL-10) as compared to normal controls. FPIES-GR PBMCs only revealed lower IL-10 production with TLR2/6 agonist than controls.

**CONCLUSIONS:** These findings indicate that decreased production of counter-regulatory cytokines in response to stimuli of innate immunity may be associated with apparent failure of developing oral tolerance in FPIES-PR children.
Remission of Refractory Aphthous Stomatitis of Celiac Disease with Etanercept
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RATIONALE: There are no specific guidelines for treatment of aphthous stomatitis with celiac disease. Such ulcers usually clear with treatment of celiac disease including strict gluten-free diet or immunosuppressive drugs in refractory cases. Here we present a 32-year old female with persistent aphthous stomatitis responding to Etanercept, an anti-tumor necrosis factor agent, after failing other treatments.

METHODS: Patient records including clinical presentation, past history, medications and physical exam were reviewed. Work-up included celiac disease anti-bodies, genetic studies, and duodenal biopsies. Informed consent and insurance approval for Etanercept use were obtained.

RESULTS: Patient had a confirmed diagnosis of celiac disease with aphthous stomatitis involving the buccal mucosa, tongue and palate. She was instructed on strict gluten avoidance, and vitamin D, thiamine and iron were replaced. Patient failed therapy with hydroxychloroquin, cyclosporine, azathioprine and colchicine. Although repeated endoscopy showed normalization of the villous ultrastructure, the ulcers persisted. Treatment with tetracycline swish and swallow also failed. Ulcers temporarily resolved with steroids but quickly relapsed upon tapering. After starting Etanercept (25mg biweekly), the patient experienced complete remission of the oral ulcers and had dramatic improvements in the inflammatory symptoms such as arthralgia and fatigue.

CONCLUSIONS: Little is known about the immunopathogenesis of aphthous stomatitis in Celiac disease, but based on the efficacy of Etanercept, one could postulate an important role for innate immunity and cytokines such as tumor necrosis factor. Further study of the role of biological agents and their safety, efficacy and tolerability need to be conducted in such patients.

Comparison of Serum Cytokine, Chemokine, and Growth Factor Profiles in Crohn’s Disease and Food Allergy in Children
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RATIONALE: Serum immunological biomarkers have not been established for Crohn’s disease or food allergy. This study aimed to establish the cytokines, chemokines, and growth factors linked to Crohn’s disease and food allergy using serum samples.

METHODS: Sera were collected from pediatric patients between 2 and 21 years of age. Patients were categorized as control (C, n = 15), Crohn’s disease (CD, n = 17), and food allergy (FA, n = 24). Levels of 30 analytes, including cytokines, chemokines, and growth factors, were determined, using a multiplex assay. Data were analyzed by one way ANOVA on Ranks followed by Dunn’s method for multiple comparisons.

RESULTS: Sixteen cytokines in these three groups were analyzed. Of those, 5 cytokines differed significantly between groups (p<0.05). CD subjects had significantly higher IFN-γ, IL-5, IL-12 and IL-2R and significantly lower IL-15 levels as compared to the control and FA subjects. Ten chemokines in these three groups were analyzed. Of those, 3 chemokines differed significantly between groups (p<0.05). IL-8, and monokine induced by interferon-γ(MIG) were significantly higher in CD subjects compared to control and FA subjects. However, monocyte chemotactic protein-1(MCP-1) was elevated in FA subjects compared to CD and control subjects. Four growth factors in these groups were analyzed. Only 1 growth factor differed significantly between groups (p<0.05): hepatocyte growth factor (HGF) was significantly elevated in CD and FA subjects compared to controls.

CONCLUSIONS: Serum cytokine, chemokine, and growth factor levels differ significantly between Crohn’s disease and food allergy and therefore might be useful biomarkers for diagnosis and treatment.

The Role of Exhaled Nitric Oxide (FENO) in Eosinophilic Esophagitis: A Pilot Study to Evaluate the Correlation of Exhaled Nitric Oxide and Esophageal Eosinophils
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RATIONALE: Eosinophilic Esophagitis (EoE) is a chronic disease caused by infiltration of eosinophils into the esophagus. Fractional exhaled nitric oxide (FENO) measurements reflect degree of eosinophilic inflammation in exhaled breath. We hypothesize that FENO levels will correlate with quantity of esophageal eosinophilia, enabling potential use of FENO in management of EoE.

METHODS: Subjects aged 6-17 years undergoing scheduled esophageal biopsy for suspected EoE were recruited in our prospective study. After obtaining informed consent, FENO measurements were obtained in duplicate using a NIOX Mino machine (Aerocine, Inc.) prior to endoscopy. Subjects were classified based on number of eosinophils/HPF (study group >15; control <14). Exclusion criteria included biopsy proven gastric/duodenal eosinophilic infiltration, steroid use, and persistent asthma. Average FENO levels were correlated with eosinophil counts on esophageal biopsy. Proposed statistical analysis included Pearson-rank correlation to identify linear/non-linear association between FENO and quantitative eosinophilia, and models to control for atopy/asthma.

RESULTS: We recruited 3/100 study and 12/100 control group subjects. The average FENO ranged 7-46 ppb in study group (mean: 20) and 7-21 ppb in control group (mean: 14; median: 10). Esophageal eosinophils ranged 20-80/HPF in study group and 0-9/HPF in control group. One patient with the largest eosinophilia (>80) had a higher than average FENO (46 ppb).

CONCLUSIONS: Our preliminary data demonstrates feasibility of our study objective to evaluate correlation of exhaled nitric oxide levels with esophageal eosinophil numbers in subjects with EoE. With ongoing active recruitment, we believe our study will be adequately powered to determine the nature of the relationship.
370 Eosinophilic Liver Involvement In Hypereosinophilic Syndrome: Clinico-pathologic Findings In Eight Patients


RATIONALE: Hypereosinophilic syndrome (HES) is characterized by peripheral blood eosinophilia >1500/mm3 and a wide variety of clinical manifestations attributable to the eosinophil. Although hepatic abnormalities, including hepatomegaly and elevated transaminases, are relatively frequent, the clinical spectrum, frequency and prognosis of liver eosinophilia in HES remains unknown.

METHODS: Subjects with HES and eosinophilic liver abnormalities were identified by retrospective chart review. Clinical signs and symptoms, laboratory parameters, and results of imaging studies and liver histopathology were reviewed.

RESULTS: Among 246 subjects referred for evaluation of marked eosinophilia, eight were found to have biopsy-proven eosinophilic liver involvement of unknown etiology. The subjects were predominantly male (n=7), and median age at presentation was 29.5 years (range 7-58). The most common presenting symptoms were fatigue (n=6), fever (n=5), myalgia (n=4), abdominal pain (n=3), emesis (n=3), and skin rash (n=3). Median AEC was 7797/mc (range 4800-22000/mc) at the time of diagnosis. Although liver histology showed eosinophilic infiltrates in all subjects, the degree and pattern of hepatocellular damage and fibrosis varied considerably. Symptomatic flares occurred despite therapy and were associated with an increase in AEC, which correlated with AST (r=0.61, p<0.0001), alkaline phosphatase (r=0.65, p<0.0001), ALT (r=0.46, p<0.0001), and total bilirubin (r=0.48, p<0.0001). Nevertheless, hepatic synthetic function was preserved in all cases, and no patients developed cirrhosis over a median of 7 years followup (range 3-20 years).

CONCLUSIONS: Eosinophilic liver involvement is an uncommon manifestation of HES. Despite recurrent episodes of eosinophilic inflammation, prognosis appears to be good, although longer followup is needed.

371 Utility of a Website-based Database of Drug Allergy Reports and Desensitization Protocols

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RATIONALE: Drug allergy consults are challenging due to the dearth of published data. This study aims to determine the utility of a website-based database of drug allergy case reports and desensitization protocols among Canadian allergists.

METHODS: An online survey (www.surveymonkey.com) was administered to Canadian allergists, who are members of the Canadian Society of Allergy and Clinical Immunology (CSACI).

RESULTS: 59 Canadian allergists filled out the survey (39% response rate). 59 and 66% of respondents work in the community and within academic hospitals, respectively. 63% of respondents see drug allergy consults in the range of 1 to 9% of their practice. The most common drugs were antibiotics and NSAIDs. Newer agents of concern included “biologics.” The most common requests for drug desensitization were for penicillins, ASA and cephalosporins. 76% of respondents would utilize a website-based database of drug allergy case reports and desensitization protocols. 51% of respondents would be interested in submitting a case report or a desensitization protocol to the database and 24% can think of a case that would be suitable for submission at the time when they completed the survey. 84% of respondents felt that such a website would improve patient management. One respondent commented that “the greater the combined experience, the better the outcomes.”

CONCLUSIONS: Among Canadian allergists, there is support for the development of a website-based database of drug allergy case reports and desensitization protocols. Long-term goals include the publication of case series and the development of clinical trials, from information gleaned from this website.

372 Outcomes Of Adult Provocation Tests In A Singapore Allergy Centre

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RATIONALE: Without appropriate consultation and confirmatory testing a label of drug allergy may be inaccurate. The aim of this study was to examine the outcome of drug provocation tests (DPT) in confirming or rejecting a diagnosis of drug hypersensitivity.

METHODS: A retrospective chart review of all adult patients referred to a single Singapore Allergy Centre for a single or multiple suspected drug allergies who underwent a DPT from the period January 2009 till June 2011.

RESULTS: Out of a total of 77 patients, 67 patients underwent 96 DPTs (median age 37; range 22-49) and 10 patients were desensitized. Seventeen patients underwent more than 1 DPT of which 52% were women. The most common suspects were antibiotics (44.8%) of which β lactams were implicated in 65.3% of the cases. This was followed by non-steroidal anti-inflammatory drugs (NSAIDS) in 11.5% and paracetamol (8.3%). The most common indication for the use of the drug was infection (56.3%) followed by pain (25%). Rash was the most commonly reported symptom (40.6%), followed by angioedema (30.2%). DPTs were performed to antibiotics (47.9%), to NSAIDS (16.7%) and paracetamol (9.4%). Of these, 92.7% had a negative DPT and 7.3% had a positive DPT. Of the positive DPT patients only 4 patients required rescue therapy which comprised solely of oral antihistamines.

CONCLUSIONS: Suspected drug hypersensitivity is common but true drug allergy is rare. DPT remain the gold standard and need to be included as part of an investigative protocol. DPT are a safe and valuable diagnostic tool in the hands of the experienced clinician.

373 Survey of Drug Allergy Testing, Challenge, and Desensitization Practice

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RATIONALE: Lack of standardized skin test products to assess a patient’s (hyper-)sensitivity to medications has required physicians to empirically develop their own testing procedures. These approaches may include puncture and intradermal skin tests, patch tests and medication challenges. Desensitization protocols are also frequently used when skin testing is not possible or when results of skin testing are positive and the necessity of a particular medication is strong. This survey examined drug hypersensitivity testing practices, as reported in 3 allergy journals.

METHODS: This review collected reports of skin tests, challenges, and desensitization to medications, vaccines, and diagnostic agents in 3 leading allergy journals from 2004 through 2008. Studies were included in the survey if they were sufficiently detailed that the reader could reasonably be expected to reproduce the technique.

RESULTS: Data for 191 drugs were included. Multiple reports for individual drugs were common. Testing with antimicrobial agents was reported most frequently (88 reports), followed by neuromuscular blocking drugs (24), antineoplastic medications (20) and local anesthetics (15), but also newer medications [monoclonal antibodies (6), antiretroviral drugs (3), interferons (1 report, 3 drugs)], and vaccines (5). Skin tests, including patch tests, were the most frequently reported procedures; however, drug challenges (34 reports) were also included along with or in lieu of skin tests. Drug desensitization schedules were reported 65 times.

CONCLUSIONS: In addition to testing for antibiotic allergy, skin testing, challenge, and desensitization schedules are being utilized to assess sensitivity to many other classes of medications. It is important to stay abreast of current testing practice for drug hypersensitivity.
374 Analysis Of Adverse Drug Reactions In Children

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RATIONALE: The incidence of adverse drug reactions (ADRs) is increasing. However, the studies about the prevalence of ADRs in children are rare compared to reports in adults. In order to investigate the causative drugs and clinical features of ADRs in children, we performed a study in a tertiary university hospital of Korea.

METHOD: We prospectively collected ADR data by a computerized spontaneous reporting system at our university hospital. ADR data of children under the age 18 collected from January 2003 to May 2011 were analyzed.

RESULTS: 1924 ADR cases were reported in 1086 male (56%) and 838 female (44%), and mean age was 9.3 ± 5.9 years (0-18 years). Antibiotics (n=769, 45%) were the most common causative drugs, followed by analgesics (n=144, 9%), followed by penicillins and β-lactamase inhibitors (n=132, 7%), radiocontrast media (n=77, 5%), and sedatives (n=70, 4%). The most common clinical features were skin manifestations (n=961, 52%) such as urticaria, pruritus, rash and angioedema. Gastrointestinal symptoms (n=402, 22%) were the second clinical features, followed by respiratory symptoms (n=157, 9%) including mainly dyspnea. Among antibiotics, penicillin/F-lactamase inhibitors (n=80, 16%), and third-generation cephalosporins (n=78, 15%) and glycopeptides (n=66, 13%) were the most frequently reported causative drugs.

CONCLUSION: Antibiotics were reported most common causative drug of ADRs in children and the clinical features were mainly skin reactions. Close monitoring system may be useful to detect ADRs early in children.

375 A Survey of Current Physician Practice and Knowledge of Drug Allergy at a University Medical Center

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RATIONALE: Physicians are challenged with drug allergy (DA) on a regular basis. This study was performed to determine current knowledge and practice patterns regarding DA at our center.

METHODS: A survey was emailed to all faculty and resident physicians at a university medical center on their management of patients with DA, knowledge of DA, and clinical indications for skin testing (ST) and desensitization.

RESULTS: 231 of 986 surveys were completed using SurveyMonkey®. The average percent of patients seen by respondents reporting any DA was 32.2%. Respondents thought only 30.5% of patients reporting DA would react if given the suspected drug; however, only 15% would give the drug. 49% reported previously performing or ordering a consult for drug desensitization. Of the 25 knowledge questions on DA, the average score for the cohort was 16.65 correct (66.6%). Urticaria with penicillin was the most frequent correct answer as a clinical indication for ST (83.5%) and desensitization (69.3%); however, only 43.7% and 46.7% of respondents cited anaphylaxis with piperacillin as an indication for ST and desensitization, respectively. 15.6% of respondents recognized that desensitization could be performed for AERD. Although no significant differences were found regarding DA knowledge between attendings and residents, or primary care physicians and specialists, physicians with any Internal Medicine training did score significantly higher than those without such training (average correct, 17.3 vs. 16.3, p<0.01).

CONCLUSIONS: Physician’s knowledge of DA was worse than expected and could adversely affect quality of patient care. Further research should focus on outcomes of patients with drug allergies.

376 Pediatric Patients with a History of Penicillin Allergy and a Positive Penicillin Skin Test May Not Be at an Increased Risk for Multiple Drug Allergies

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RATIONALE: Recent studies have suggested that patients with a history of a hypersensitivity drug reaction to sulfonamides or penicillin (PCN) may be at increased risk for reactions to other drugs. However, to our best knowledge, these studies have not been in the pediatric population. We conducted a study to determine if patients with a history of PCN allergy and a positive PCN skin test (PST) were at an increased risk for multiple drug allergies in the pediatric population.

METHODS: 778 children (less than 18 years) with a history of PCN allergy were evaluated for PCN allergy by PST. Charts were reviewed for basic demographic data, PST results, and whether there were more than 1 drug allergy listed in the medical record. Using the Fisher’s exact test, we compared the differences in the proportion of children with a positive and negative PST and multiple drug allergies. P value < 0.05 was considered statistically significant. The IRB approved the study and all subjects signed a written informed consent.

RESULTS: 778 children underwent PST and 367 (47.1%) were females. 712 (92%) of 778 patients had a negative PST. 66% (8%) had a positive PST. Among the patients with a positive PST, 14 (21%) listed multiple drug allergies; while, among negative PST patient’s, 167 (23%) listed multiple drug allergies (p = 0.7).

CONCLUSIONS: PCN allergic children may not be at an increased risk for multiple drug allergies.

377 Multiple Drug Intolerance Syndrome: Prevalence, Clinical Characteristics, And Management

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RATIONALE: Population-based data on the demographics and clinical characteristics of patients with multiple unrelated drug class “allergies” is lacking.

METHODS: Data was extracted from the electronic medical records of 2,375,424 Kaiser Permanente Southern California healthplan members who had at least 11 months of healthcare coverage in and at least one healthcare visit during 2009. Population-based drug “allergy” prevalence and incidence rates were determined for 23 classes of medications. The prevalence of multiple drug intolerance syndrome (MDIS), defined by ≥ three drug class “allergies,” was determined. The demographics, healthcare utilization, medication usage, new drug “allergy” incidence, and clinical diagnoses given to individuals with MDIS were determined.

RESULTS: On 1-1-2009, there were 478,283 (20.1%) healthplan members with at least one drug “allergy”. Individuals with a history of at least one drug “allergy” and females, in general, reported higher new population-based drug “allergy” annual incidence rates. MDIS was present in 49,582 (2.1%). MDIS cases were significantly older, 62.4 ± 16.1 years; heavier, body mass index 29.3 ± 7.1; and likely to be female, 84.9%, than average healthplan members. They had higher rates of healthcare utilization, medication usage, and new drug “allergy” incidence. They sought medical attention for common non-morbid conditions.

CONCLUSIONS: MDIS is in part iatrogenic. MDIS is associated with overweight elderly females who have high rates of healthcare and medication usage. Urticarial syndromes only explain a small fraction of MDIS cases. MDIS is associated with anxiety, but not predominately with IgE-mediated allergy or life-threatening illness. MDIS can be managed by medication avoidance and judicious re-challenge.
Characterization of Multiple Antibiotic Allergies in Adult and Pediatric Patients

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RATIONALE: Hypersensitivity to multiple structurally unrelated antibiotics is poorly studied and difficult to manage. We sought to characterize features of multiple antibiotic allergies in pediatric and adult populations.

METHODS: After IRB approval, a retrospective chart review was conducted. Inpatient and outpatient medical records from January 2011 through August 2011 were studied to evaluate for self-reported or physician-diagnosed antibiotic allergy, as recorded in the electronic medical record. Demographic data, including age, sex, and implicated drug allergy were collected.

RESULTS: We identified 7060 antibiotic-allergic patients. Multiple antibiotic allergies to two or more different drug classes were reported in 1106 (15.7%) patients. Among these patients, 78.5% reported allergy to 2 antibiotic classes, 14.5% to 3 classes, 5% to 4 classes, 1.4% to 5 classes, 0.4% to 6 classes, and 0.3% to 7 or more classes. The frequency of multiple antibiotic allergies varied by sex according to age, with a female:male ratio of 1.3 in pediatric patients and a ratio of 4.3 in patients over 20 years of age. Numbers of patients with multiple allergies increased with age in both sexes, with the greatest number seen in ages 50-69. Fifty-five percent of all multiple antibiotic allergies involved sulfas drugs, 39.3% macrolides, 38.8% penicillins, 33.8% quinolones, 17.2% cephalosporins, and 16.4% tetracyclines.

CONCLUSIONS: A significant portion of antibiotic-allergic patients have sensitivity to multiple antibiotics. Reports of reactions to macrolides and quinolones were common in these patients, in addition to reactions to sulfonamides and penicillins. There is a strong female predominance among multiple antibiotic-allergic adult patients, but not pediatric patients.

Immediate Hypersensitivity To Quinolones: Drug Photodegradation Influences The Specific Basophil Activation

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RATIONALE: Basophil activation test (BAT) can be useful for evaluating immediate hypersensitivity to fluoroquinolones with higher percentage of positive responders with ciprofloxacin than with moxifloxacin. As fluoroquinolones are photoreactive, whether laboratory light exposure can influence in the induction of photoproducts and therefore modify BAT results is unknown. To analyze the effect of laboratory light in ciprofloxacin and moxifloxacin degradation, drug-protein conjugate formation and therefore in basophil activation in patients with immediate hypersensitivity reactions to fluoroquinolones.

METHODS: Patients with confirmed immediate hypersensitivity to ciprofloxacin (N=15) or moxifloxacin (N=13) and quinolones tolerant controls (N=20) were included. BAT was done in light and dark conditions. To analyze fluoroquinolones degradation absorption and emission measurements were performed in aqueous solution and in whole blood. In the latter supernatant was divided in high (>3000Da) and low (<3000Da) molecular weight fractions before analysis.

RESULTS: BAT positivity was higher in dark conditions (57.1%) than in light (46.4%) mainly due to an increase in moxifloxacin results from 17.9% to 35.7% with no changes in ciprofloxacin results (46.4% in both conditions). There was an important decrease in the emission fluorescence intensity under light conditions for high (>3000Da) and low (<3000Da) molecular weight fractions for moxifloxacin without changes for ciprofloxacin.

CONCLUSIONS: Laboratory light conditions induce photodegradation of fluoroquinolones, especially moxifloxacin, this influences the level of quinolones free and bound to proteins being critical factor in the basophil activation in patients with hypersensitivity reactions to quinolones.
Skin Testing and Graded Challenges for Meropenem Hypersensitivity in Penicillin Allergic Subjects


RATIONALE: There are limited data suggesting lack of clinical cross-reactivity between carbapenems and penicillin (PCN). The 2010 AAAAA ACAAI, JCAAI Drug Allergy Practice Parameters state that PCN skin test negative patients can receive carbapenems. We explored whether meropenem skin testing in addition to PCN major and minor determinants identified at risk PCN allergic patients requiring meropenem.

METHODS: From inpatients (Northwestern Memorial Hospital) and outpatients (Northwestern Medical Faculty Foundation) with PCN allergy, skin testing utilized Pre-Pen, (AllerQuest, Plainville, CT), K PCN G (6000 u/mL), Na Benzylpenicilloate (0.01mol/L), Benzylpenicillloyl-n-propylamine (0.01mol/L), meropenem (1mg/mL) histamine, and saline. A positive skin test was a wheal ≥4mm with surrounding erythema within 20 minutes for prick and intradermal tests. Prior to 12/2009, patients were not tested with Pre-Pen. Most graded challenges with meropenem were 3 mg and 99mg intravenously.

RESULTS: From 8/2008 to 12/2009, skin tests with exclusively PCN minor determinants were + in 0/181 adults; from 12/2009 to 9/2011, skin tests utilizing Pre-Pen and minor determinants were + in 10/260 adults (3.8%). PCN skin tests were negative in all 85 patients who received meropenem. Prick and intradermal skin tests to meropenem were negative in 32 patients, and all patients tolerated meropenem by graded challenge. Without meropenem skin testing, 53 other patients received meropenem eventufully.

CONCLUSIONS: 1. Meropenem skin testing was negative in all 32 PCN skin test negative patients. 2. The 2-step graded challenge approach with meropenem was tolerated in all 85 patients. 3. The 3.8% incidence of + PCN skin tests is lower than 10.5% from 1984-2004.

The Potential of Using ELISPOT to Diagnose Cephalosporin-induced Maculopapular Exanthems

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RATIONALE: This study aimed to evaluate the potential role of enzyme-linked immunosport assay (ELISPOT) in the diagnosis of cephalosporin-induced maculopapular exanthems (MPE) as compared to skin testing.

METHODS: Intradermal test with delay reading and patch test were performed in 25 patients with a history of cephalosporin-induced MPE (20 ceftriaxone, 5 cefazidime). The numbers of interferon-γ (IFN-γ), interleukin-5 (IL-5), and interleukin-10 (IL-10) spot forming cells (SFC)/10^6 peripheral blood mononuclear cells (PBMC) were measured after stimulating with the culprit drug and compared to 20 non-allergic controls. The frequencies of the drug-specific cytokine responses were analyzed and spot numbers ≥ means+2 SD of the values in non-allergic controls were considered diagnostic.

RESULTS: ELISPOT was positive in 10 patients and skin testing was positive in only 2 patients (40% versus 6%, P value =0.006). Each IFN-γ and IL-5 ELISPOT was positive in 24% of patients and positive in 40% of patients if both assays were combined. ELISPOT sensitivity increased to 57.1% when the test was performed in patients with a recent history of cephalosporin-induced MPE (last reaction ≥ 2 years). The frequencies of ceftriaxone-specific IFN-γ and IL-5 secreting cells were 25.8±8.8 and 25.8±17.9 SFC/10^6 PBMC in patients with a recent history of ceftriaxone-induced MPE compared to 7.0±2.7 and 0.6±0.4 SFC/10^6 PBMC in non-allergic individuals (P values =0.027 and 0.036, respectively).

CONCLUSIONS: ELISPOT is a promising tool for confirming the diagnosis of cephalosporin-induced MPE. The test should be performed within two years of the last reaction with the combination of IFN-γ and IL-5 assays.

Epidemiology of Betalactams Hypersensitivity in a Drug Allergy Unit

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RATIONALE: Allergy to betalactams is the most frequent cause of immunomediated mediated drug reactions. We report a 10 year (1999-2009) retrospective study.

METHODS: Overall 5825 patients with suspected of drug allergy in our department, 2892 with suspected betalactam hypersensitivity reactions were evaluated. We perform skin tests with: penicilloyl polylysine (PLL), minor determinants mixture (MDM) and benzylpenicillin (penicillin G), amoxicillin, amoxicillin/clavulanic acid, ampicillin, and any other suspected betalactam. Serum-specific IgE assays were measured (CAP-FEIA Phadia, Uppsala, Sweden). Single-blind challenge with the suspected betalactam was carried out. When patient’s reaction was more than 6 months ago, all study was repeated after 4 weeks. (Retest)

RESULTS: We diagnosed 391 patients of hypersensitivity to betalactams (mean age 44.16 ± 14.8; 166 man and 226 women), 82% were immediate reactions (IR) and 18% were nonimmediate reactions (NIR). Clinical pattern was urticaria with or without angioedema 73.2% and anaphylaxis in 26.5%. 94.7% were sensitized to penicilins, 5.3% to betalactams (penicillins and cefalosporins) and 3.8% to cefalosporins. Positive skin tests to different penicillin determinants 76.7% (89.9% IR and 33.8% NIR). Specific-IgE was positive in 20.7%. Drug challenge was necessary in 23.3% (15.2% IR and 67.7% NIR) of the patients. Patients diagnosed in second work-up were 11.4% (68% IR and 31% NIR).

CONCLUSIONS: Skin test was the most useful test for betalactam allergy diagnosis 76.7%. Nevertheless, challenge test and retest were necessary for a correct diagnosis in 20.7% and 11.4% of the patients respectively.

For nonimmediate reactions skin test was less sensitive and challenge test was more necessary.

Value Of Skin Testing Solely With Penicillin G In Children With a History Of Penicillin Allergy

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RATIONALE: For most part of the last decade, PrePen® has been unavailable commercially. This situation has compromised the evaluation of many patients with a history of penicillin allergy. During this period, evaluation of children with a history of penicillin allergy continued in our center using penicillin G as the sole reagent for skin testing. We aimed to determine the negative predictive value and safety of this approach.

METHODS: We reviewed the files of all patients evaluated for a history of penicillin allergy between December 2006 and December 2009 at the CHU Sainte-Justine Allergy Clinic. Evaluation consisted of skin testing with penicillin G 10,000 IU/mL followed if negative by a graded challenge to the culprit penicillin.

RESULTS: We evaluated 563 patients during this period. Among these, 185 (32.9%) had a positive skin test to penicillin G and 378 (67.1%) had a negative skin test. A reaction occurred in 4.8% (18/375) of patients following challenge. Three patients were not challenged. All reactions were mild and generally limited to the skin. The negative predictive value of skin testing solely with penicillin G was calculated at 95.2% (92.5-97.1%).

CONCLUSIONS: This study raises questions about the relevance of skin testing children with a history of penicillin allergy with a large array of skin test reagents as the negative predictive value of penicillin G alone approximates that of the recommended approach. Also, it offers support for a simpler and safer alternative to skin testing in these children using penicillin G as the sole skin test reagent.
Cross-Reactivity between Penicillins and Third-Generation Cephalosporins


RATIONALE: Reports of cross-reactivity between penicillins and third-generation cephalosporins still provide controversial data. Published rates vary from 8% to 50%.

METHODS: Records of 1520 patients attending our drug allergy unit from September 2009 to August 2011 were reviewed. All subjects diagnosed with immediate allergy to penicillin and subsequently tested with third-generation cephalosporins were included. Two groups were formed. Group 1 (G1): The diagnosis of penicillin allergy was based on the anamnesis. After the first cephalosporin challenge, a retest was led if the time from the reaction to the study was over 6 months. Group 2 (G2): diagnosis was based on anamnesis plus tests: skin tests (54.5%), sIgE (27.3%), challenge (18.2%).

RESULTS: Beta-lactam allergy was suspected in 493 patients, 114 were diagnosed with penicillin allergy. Forty patients were challenged with third-generation cephalosporins [ceftiraxone (26), cefixime (15), cefditoren (6)]. G1 included 18 patients, mean age 71.9±9.8 years, 67% women, 22% anaphylaxis, and mean time from the reaction 177.4±199 months. G2 included 22 patients, mean age 42.9±12.2 years, 54.5% women, 50% anaphylaxis, mean time from the reaction 68.5±135 months.

The involved penicillins were: Semi-synthetic penicillins (35), benzylpenicillin (1), unknown (4). All patients in G1 tolerated the challenge and the retest with third-generation cephalosporins. Three out of the 40 patients (7.5%), all from G2 (13.6%), diagnosed with amoxicillin allergy, reacted at least to a cephalosporin [ceftiraxone (2), cefixime (2), cefditoren (1)].

CONCLUSIONS: Cross-reactivity between penicillins and third-generation cephalosporins is low (7.5%), higher if diagnosis is based on complementary tests (13.6%).

Cephalosporin Allergy: Cross-reactivity To Penicillin In Pediatric Patients

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RATIONALE: There have been few studies looking at penicillin cross-reactivity in patients with a history of cephalosporin allergy in pediatric patients. The goal of this study was to determine whether children with cephalosporin allergy are at increased risk for adverse drug reactions to penicillin.

METHODS: We conducted a retrospective study of pediatric patients (18 or under) with a history of cephalosporin allergy who underwent penicillin skin testing and this made up our patient cohort. Charts were reviewed for basic demographic and penicillin skin test results.

RESULTS: A total of 67 patients were included in this study. The mean age of patients was 5.8 years. Thirty eight patients (57%) were male and 29 (43%) were female. Six patients (9%) tested positive on the penicillin skin test and 61 patients (91%) tested negative. The most common cephalosporins implicated were ceftriaxone 21 (31%), cefalexin 12 (18%), cefdinir 12 (18%), and cefixime 8 (12%).

CONCLUSIONS: Pediatric patients with a history of cephalosporin allergy may be at increased risk of an adverse drug reaction to penicillin.

Safety and Effectiveness of Penicillin Allergy Evaluation in the Pre-Lung Transplant Patient Population

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RATIONALE: Pre-lung transplant patients reporting penicillin allergy present a unique population that would benefit from an Allergy consultation. Appropriate use of antimicrobial agents decreases the risk of developing pathogenic and opportunistic infections. Penicillin is the only beta-lactam antimicrobial agent with a standardized skin test. A detailed history, exam, skin tests and ingestion challenge can be performed safely in this vulnerable patient population.

METHODS: A retrospective chart review was performed for pre-lung transplant patients undergoing allergy consultation for a history of adverse reaction to penicillin between September 2010- September 2011. 39 adult patients were evaluated by history, exam, skin testing to Penicillin G and Pre-Pen with controls. Patients with negative skin tests, were followed by an oral ingestion challenge with 250 mg Pen VK tablet.

RESULTS: Total of 39/39 (100%) patients had negative Epicutaneous and Intradermal skin testing with Penicillin G and Prepen. 34/39 (87%) of patients consented to oral ingression challenge and 34/34 (100%) of patients tolerated oral penicillin without any adverse reactions. To date, 12/39 (31%) of these patients tolerated a subsequent penicillin antibiotic. 29/39 (75%) of patients were requiring long term supplemental oxygen therapy. Mean FEV1 in these patients was 0.65 L/min (24%), FVC was 1.81 L/min (51%) and FEV1/FVC ratio was 44%.

CONCLUSION: Penicillin allergy evaluation is warranted in pre-lung transplant patients with history of adverse reactions to penicillin antibiotics, despite their poor lung function and oxygen requirements. When performed by skilled personnel, this evaluation is safe and effectively allows use of beta-lactams in the future.

Prevalence of Penicillin Allergy and Adverse Outcomes in Geriatric Inpatients at a Tertiary Care Hospital

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RATIONALE: The prevalence of reported penicillin allergy has been documented to be as high as 20-20%, though it is estimated that 90% of these patients are not truly allergic. These patients often are treated with alternative antibiotics that may not be as effective or may have more adverse effects. We hypothesized that hospital admissions of patients with a history of penicillin allergy are associated with increased mortality and/or adverse outcomes, particularly in the geriatric population.

METHODS: After IRB approval, a retrospective chart review was performed to compare hospitalization demographics in geriatric patients (≥65 years old) admitted with pneumonia, with or without a history of penicillin allergy. Length of admission, mortality rates, and discharge disposition of all geriatric inpatients were assessed from September 2008 to August 2011.

RESULTS: Over the 36-month time period, 2,550 geriatric patients were admitted for pneumonia. The prevalence of reported penicillin allergy was 3.61% (92/2550). Patients with a history of penicillin allergy were found to have longer lengths of admission (9.40 days vs. 7.62 days, p = 0.0315) and increased mortality rates (16.30% vs. 10.90%, p = 0.1054). These patients were also less likely to be discharged to home (65.21% vs. 75.87%, p = 0.0196) as opposed to another healthcare facility.

CONCLUSIONS: Our study demonstrates that there are more adverse outcomes in hospital admissions for pneumonia in geriatric patients labeled as penicillin-allergic. Further studies are warranted to determine how penicillin allergy testing might improve hospitalization outcomes.
Carrier Molecules Displaying Dual Haptenic Presentation for in Vivo Testing to Determine IgE Antibody in Patients Allergic to Betalactams

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RATIONALE: Benzylpenicilloyl-dendrimer conjugates are recognized by IgE specific to benzylpenicillin. Herein we include other hapten, amoxicillin, and the concept of having two kinds of haptons in the same carrier. We compare the ability of different dendrimers displaying multiple presentation of only one hapten, either benzylpenicillin or amoxicillin, and mixed-dendrimer bearing both haptons, to be recognized by specific IgE to betalactams, for developing a universal in vitro allergy diagnostic test to betalactams.

METHODS: Well-defined hapten-carrier conjugates were synthesized by haptenization of 4th generation PolyAMidoAmine dendrimers with either one kind of penicillin (benzylpenicillin and amoxicillin) or both penicillins. The different conjugates were immunologically tested by RAST inhibition using sera from 4 controls and 7 patients allergic to penicillins, both selective and cross-reactive responder.

RESULTS: At the maximum conjugate concentration, all sera allergic to benzylpenicillin showed 80% of RAST inhibition to both benzylpenicilloyl-dendrimer and mixed-dendrimer, and negative to amoxicilloyl- dendrimer. All sera allergic to amoxicillin showed 90% of inhibition to both amoxicilloyl-dendrimer and mixed-dendrimer, with no inhibition to benzylpenicilloyl-dendrimer. All sera with cross-reactivity to both penicillins inhibited above 70% to the three different conjugates.

CONCLUSIONS: Results suggest that these conjugates inhibit when containing any of the haptons to which the patient is allergic to, reaching similar values with monoa aptigenic and biantigenic conjugates. Thus, IgE recognition is specific and the sensitivity does not vary from biantigenic to monoaaptigenic conjugates. This provides information about the structural chemical requirements to develop a universal in vitro test, being the described mixed-dendrimers potential candidates to progress in allergy diagnosis to betalactams.

Use of Omalizumab to Achieve Successful Desensitization After Oxaliplatin Anaphylaxis

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RATIONALE: Use of omalizumab, human anti-IgE, has been associated with improved tolerance of desensitization protocols in food allergy, insulin desensitization, venom immunotherapy, and rush immunotherapy for ragweed. Here we report the use of omalizumab therapy to achieve successful desensitization to oxaliplatin in a patient with a history of anaphylaxis upon exposure and during attempted desensitizations to oxaliplatin.

METHODS: Skin testing was performed to oxaliplatin. Two doses of omalizumab (150 mg q 2 weeks) were administered before desensitization. RESULTS: A 68 year old patient with colon cancer and prior history of severe itching, back pain, myalgias, and coughing during oxaliplatin infusion underwent prick skin testing at 5 mg/ml and 1:10 and 1:100 intradermal dilutions, which were negative. Total IgE was 151 kU/L and baseline tryptase was 4 ng/ml. A standard 12-step desensitization protocol induced severe back pain, hypoxemia, cough, and hypotension. Modified protocols times 2 were complicated by severe hypoxemia, hypotension, back pain, chills and coughing with shortness of breath, requiring epieneprine and discontinuation of this first line therapy drug. Two doses of omalizumab (150 mg q 2 weeks) were administered followed by a 16-step desensitization protocol. During and post desensitization, there was no evidence of hypersensitivity reaction. He tolerated 4 subsequent desensitizations on omalizumab with only mild reactions.

CONCLUSIONS: We report the first use of omalizumab to provide protection from life-threatening reactions to chemotherapy agents during desensitization. Anti-IgE therapy can be used as an adjuvant agent to improve the efficacy and safety of desensitization protocols.
Abstracts

394 Hypersensitivity To Docetaxel: Retrospective Study And Desensitization Protocol
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Rationale: Docetaxel is a commonly used taxane that frequently causes hypersensitivity reactions. The purpose of this study was to record the clinical characteristics of hypersensitivity to docetaxel in non-small cell lung cancer patients and to develop a desensitization protocol.

Methods: We retrospectively reviewed records of 620 non-small cell lung cancer patients treated with regimens containing docetaxel in the adjuvant or further-line setting. A total of 102 patients had exhibited hypersensitivity to docetaxel and data were analyzed according to the Common Toxicity Criteria for Adverse Events v3.0. Five patients were chosen for the desensitisation protocol and we applied the standard protocol for parenteral desensitisation to beta-lactam antibiotics. In these patients, docetaxel treatment was carried out with a series of 10-fold dilutions in sufficient volume to administer the total dose.

Results: A total of 102 patients (16.5%) were recorded as having hypersensitivity to docetaxel. Reactions were observed after approximately 2.5 (SD=± 1.0) cycles and they were more likely to happen during second- or third-line chemotherapy. Only 14 patients (14/620, 2%) developed grade 3-4 hypersensitivity. No other correlation with age, gender or atop status was observed. Five patients completed a parenteral desensitisation protocol and continued their treatment uneventfully.

Conclusions: Hypersensitivity to docetaxel responds quickly to discontinuation and re-administration may be possible with premedication and increased infusion time. We developed a desensitization protocol which provides a reliable alternative to permanent discontinuation of docetaxel.

395 Safety of Aspirin Desensitization in Patients with a Cardiac Indication for Aspirin
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Rationale: Drug desensitization carries potential risks that are undesirable in patients with cardiac disease. Patients who are allergic to aspirin may need this drug for a cardiac indication. We hypothesized that our 7 step aspirin desensitization protocol can safely be performed in this population.

Methods: Inpatient consults and outpatient clinic charts were reviewed for patients with aspirin allergy needing aspirin for a cardiac indication. Type of intervention and outcome were recorded. Patients undergoing our 7 step protocol were then identified.

Results: One hundred and fifty patients and 160 encounters were included. Of these, 19 patients underwent 22 desensitizations with our 7 step protocol. Initial allergic reaction was to aspirin in 17 of these patients and to NSAIDS in 2. These reactions included hives or rash in 12 patients, angioedema in 9, and breathing difficulties in 4 patients. The desensitization protocol was completed with no reaction in 16 patients (84.2%) and 19 desensitizations (86.3%). The three patients who had reactions, all had angioedema: lip (1), fingers (1), facial swelling (1). Desensitizations were completed in both the inpatient (6) and outpatient (16) setting. No outpatient required ER visit or hospitalization. One inpatient had his hospitalization prolonged by a few hours for observation secondary to finger swelling. Of the 18 patients successfully desensitized, 1 stopped aspirin secondary to cough and shortness of breath.

Conclusions: Our 7 step protocol can be safely administered to patients with an aspirin allergy needing aspirin for a cardiac indication. This protocol may be administered either in the inpatient or outpatient setting.

396 Value Of The Clinical History In The Diagnosis Of Nasaid Hypersensitivity Reactions Induced By Cross-intolerance
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Rationale: Multiple NSAID-induced urticaria/angioedema (MNSAID-UA) is the most common manifestation of hypersensitivity reactions to NSAIDs. Diagnostic evaluation is based on clinical history and DPT. The objective of this study was to examine the role of the clinical history in the diagnosis of MNSAID-UA by analyzing different variables, including number of drugs involved, episodes and time between drug intake and symptom onset.

Methods: We studied a group of patients with an unequivocal history of urticaria and/or angioedema. Subjects had to have had at least two episodes of cutaneous symptoms with two different COX-1 inhibitors. DPT was used in all cases as the gold standard for diagnostic confirmation. Multivariate analysis was done to identify variables and associations that could aid in the diagnosis.

Results: The risk for an allergic episode was 17 times higher in patients who developed symptoms within 60 minutes of drug intake, 13 times higher in those who experienced reactions with more than two non-chemically related NSAIDs, and 10 times higher in women.

Conclusions: A diagnosis of MNSAID-UA can be established by clinical evaluation if a number of conditions are present, thus obviating the need for DPT to confirm the diagnosis.

397 Aspirin Allergy in a Community Teaching Hospital
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Rationale: With increasing use of cardiac stents, allergists are under demand to evaluate patients with a history of Aspirin allergy.

Methods: In an IRB approved retrospective study, we report the results of 14 patients our department evaluated with reported aspirin allergy requiring stent placement. Patients were accepted for rapid desensitization using the Wong protocol if there was a history of urticaria or angioedema only, but no evidence of nasal polyps. Zyrtec was given for pretreatment. In some cases, pretreatment was added with singulair, zyflo and or prednisone.

Results: In all cases attempted, aspirin desensitization was successful before cardiac stent placement. There were no life threatening or respiratory reactions. Only 1 patient was unable to continue aspirin. Rechallenge starting at a 20mg dose was unsuccessful. Four patients had a history of respiratory complaints in the past and were pretreated with zyflo and singular in addition to zyrtec.

One patient had a history of wheezing and coughing 20 years prior on multiple occasions and was pretreated with prednisone and leukotriene blockers. He was able to attain a dosage of 81 mg .Only 1 patient was desensitized using the Scripps clinic protocol.

Conclusions: Successful aspirin desensitization can be performed in most patients allowing successful placement of cardiac stents. Many patients report aspirin allergy leading to inappropriate withholding of aspirin. With a history of cutaneous reactions alone, the Wong protocol was uniformly successful. If there is a history of respiratory reactions, addition of zyflo, prednisone and or singular may allow successful rapid desensitization.
**398** Etoricoxib: A Probable Safe Alternative For NSAID Intolerant Patients In Asia

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**RATIONALE:** NSAID intolerance is not uncommon. Etoricoxib, a specific cox-2 inhibitor NSAID, has been shown to be a safe alternative in patients with this condition. The aim of this study is to determine the rate of NSAID intolerant patients who are able to tolerate etoricoxib without adverse reactions.

**METHODS:** This study analyzed charts and electronic databases of all patients referred to the allergy clinics of the National University Hospital and Gleneagles Hospital in Singapore from 2006-2011 for oral provocation tests to etoricoxib (cumulative dose of 120mg), on the background of NSAID intolerance. Demographics, atopic comorbidities, inciting NSAID, onset and type of reaction, and provocation test outcomes were obtained.

**RESULTS:** A total of 74 patients (mean age 37; range: 16-72 years) underwent provocation tests to etoricoxib. Of these, 59% were female. Majority were Chinese (69%), followed by Malay (12%), Caucasian (8%), Indian (5%) and various other races (6%). Eighty percent of patients had a history of intolerance to 1 NSAID, while the rest (20%) had intolerance to multiple NSAIDS. Forty-one percent of patients had concomitant acetaminophen intolerance. Some of the patients had multiple symptoms on presentation, the most common of which were periorbital and facial edema (90%), breathing difficulties (26%) and urticaria (25%), with the onset of reaction occurring mostly within 30 minutes to 1 hour. Etoricoxib was tolerated in 95% of the patients. Subjects who reacted to the challenge had mild reactions which resolved with antihistamines.

**CONCLUSIONS:** Etoricoxib is a safe alternative in NSAID intolerant patients. Nevertheless, it is advisable that patients should undergo provocation tests to confirm tolerance.

**399** Desensitization to Liposomal Amphotericin B after Anaphylactic Reaction

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**RATIONALE:** Liposomal amphotericin B use has increased over the past several years due to less adverse reactions and toxicities compared to conventional amphotericin B. A few cases of anaphylaxis to liposomal amphotericin B have been reported, but no desensitization protocols have been documented in the literature.

**METHODS:** This is a case report of emergent desensitization to liposomal amphotericin B (Ambisome) in a neutropenic patient with acute myelogenous leukemia (AML) and invasive fungal sinusitis.

**RESULTS:** A 39 year old male with AML in remission was admitted with epistaxis, fever and fatigue. Bone marrow biopsy confirmed relapse of AML. He developed fever and sinus pressure a week after re-induction chemotherapy. He was neutropenic with black nasol/sinus eschar and mucormycosis was suspected. Patient was empirically started on intravenous (IV) Ambisome. Five minutes after infusion, he developed dyspnea, chest pressure, hypotension and hypoxia. The infusion was stopped, and symptoms resolved with IV diphenhydramine, fluids, albuterol and IV steroids. Ambisome desensitization was attempted without skin testing as history was convincing and there are no validated skin tests. An initial concentration of 1 ng/ml was infused at 2 ml/min rate with 3 fold increments in concentration every 30 minutes up to 1 mg/ml. There were a total of 12 increments and a target dose of 650 mg was successfully infused in 11 hours.

**CONCLUSIONS:** Desensitization protocols to liposomal amphotericin B have not been reported in literature and we report a successful IV desensitization protocol to this drug.

**400** Anaphylactic Reaction After 6-methyl-prednisolone

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**RATIONALE:** Anaphylactic reactions to systemic corticoids are uncommon, especially immediate-type reactions. Here we present a patient with an anaphylactic reaction due to 6-methyl-prednisolone.

**METHODS:** After written informed consent, we performed the following drug allergy study

**RESULTS:** A 38 year-old man received a single intravenous dose of 6-methyl-prednisolone (40 mg) to treat an asthma attack. Immediately after the dose he suffered abdominal pain, nausea, vomiting and generalized pruritus, angioedema in his lips, hands and face. After this, dizziness, tachycardia, and intense sweating appeared. He was treated with intramuscular epinephrine, intravenous saline fluid and an intravenous dose of difenhidramine (loraramine ®). He was under hospital observation until he completely recovered.

We performed a prick test with soluble 6-methyl-prednisolone (hemisuccinate ester) with a positive result (7 mm diameter). The prick and intradermal tests with oral methyl-prednisolone (succinate ester), hydrocortisone, prednisolone and fluticasone were negative.

We performed an oral challenge test with oral prednisone up to 80 mg, intravenous hydrocortisone up to 200 mg and 1 puff of inhaled fluticasone (250 mcg), all of them with good tolerance.

**CONCLUSIONS:** We have diagnosed an anaphylactic reaction caused by 6-methyl-prednisolone confirmed by a positive prick test. We have confirmed that the patient tolerated methyl-prednisolone, and so we think that he was sensitized only to hemisuccinate but not to succinate esters as in other published cases. Due to the widespread use of these drugs, it’s important to give safe corticoids alternatives to these patients performing drug challenges after negative prick or intradermal tests.

**401** Two Cases Of Riluzole-induced Lung Injury In Patients With Amyotrophic Lateral Sclerosis

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Drug-induced interstitial lung disease can be caused by an ever increasing number of medications. Riluzole has recently become the first drug proven effective in the treatment of amyotrophic lateral sclerosis (ALS). Here we report two rare cases of lung injury caused by riluzole therapy in patients with ALS. Both patients developed general fatigue, with cough or dyspnea, a few weeks after starting riluzole. Chest radiographs showed bilateral lower lobe, dorsal-dominant ground glass opacity and/or consolidation. A drug lymphocyte stimulation test of peripheral blood or of bronchoalveolar lavage cells was positive for riluzole. Histopathological examination of lung biopsy specimens revealed lung injury without fungal granuloma, vasculitis, or diffuse alveolar damage. To the best of our knowledge, this is the first report of riluzole-induced lung injury with a positive drug lymphocyte stimulation test results.
**402 Using Human Single Chain Variable Fragment (scFv) Antibodies for Identification of Potential Allergens of Neurospora crassa**

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**RATIONALE:** Neurospora crassa has been used as a platform for rapid and cost-effective vaccine production (Allgaier et al. Biologicals 2009; 37:128). The purpose of this study is to screen Neurospora crassa extract for the existence of potential allergens using human scFv antibodies.

**METHODS:** A highly complex human scFv phage library (Creative Biolabs) was panned and screened against spent soybean-based Neurospora medium. Affinity-purified soluble scFv antibodies were screened against spent medium. Antibody-binding Neurospora proteins were identified by electrophoresis and immunoblotting and, when possible, isolated using protein-L based immunoprecipitation. One such target protein was excised from a Coomassie-stained gel and identified using laser capture mass spectrometry.

**RESULTS:** Out of 65 positive clones four unique clones were identified by sequencing. All of the Neurospora-specific antibodies were affinity purified. All 4 of the antibodies recognized a specific band of about 70 kDa molecular mass by immunoblot analysis. SDS-PAGE of immunoprecipitated product also revealed protein running at the level of ~70 kDa molecular weight. Mass spectrometric analysis performed on the protein band was consistent with glucoamylase I precursor of Neurospora crassa (XP_956996). The glucoamylase shares 54% identity and 65% similarity with glucoamylase of Aspergillus niger (Q870Q8). Aspergillus-derived glucoamylase has been associated with occupational allergies (Quirce et al. Ann Allergy Asthma Immunol 2002; 89:197).

**CONCLUSIONS:** Human scFv antibodies were successfully used for identification of a potential allergen, glucoamylase I precursor, in Neurospora crassa extract.

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**403 Allergy to General Anesthetics: Evaluation of Patients Profile**

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**RATIONALE:** IgE-mediated allergic reactions during general anesthesia are estimated to occur in 1:20,000-1:350. The risk factors for having an allergy to general anesthetics are not determined whereas patients with drug allergy are usually atopic females. There are no epidemiologic studies comparing both groups.

**METHODS:** To compare the profile of patients with a history of drug allergy to those sensitized to general anesthetics identified by skin test. We retrospectively reviewed all new patients presenting to our clinic from January till December 2009, including those referred to us because of possible history or risk of allergy to general anesthetics.

**RESULTS:** The prevalence of history of drug allergy among our patients is 172/1176 (14.6%) whereas the prevalence of sensitization determined by prick skin testing to general anesthetics is 17/1176 (1.4%). Patients were most commonly sensitized to morphinic agents (16/17) followed by neuromuscular blocking agents (8/17). The patients sensitized to a general anesthetic were commonly women (12/17 or 70.6%) with a mean age of 46.4±7.4. 70.6% had a personal history of atopy. For patients with drug allergy, 98/172 or 57% were women, with a mean age of 40.8 ±2.7. 55.8% had a personal history of atopy.

When comparing both profiles, there is no significant difference for age, gender or personal history of atopy.

**CONCLUSION:** Patients sensitized to general anesthetics were mainly middle-aged women with personal history of atopy, similar to patients with other drug allergies. Moreover, these results may identify during the anesthesia pre-op consultation the patients with a significant risk for allergy to general anesthetics.

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**404 Retrospective Review of Adverse Reaction Profile of Angiotensin Converting Enzyme Inhibitors and Angiotensin Receptor Blockers**

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**RATIONALE:** Angiotensin converting enzyme inhibitors (ACE-I) and angiotensin receptor blockers (ARB) are commonly used in clinical practice. Limited and conflicting data are available regarding potential side effects. Because angioedema is a potentially fatal condition associated with these medications, this study attempts to gain further understanding of their adverse reaction profile.

**METHODS:** After IRB approval, a retrospective chart review was completed of adults admitted to an academic center with an active allergy to ACE-I or ARB between 7/1/06 and 7/1/11. Patient demographics, adverse reaction type, length of treatment before adverse event and tolerance of ARB with history of ACE-I-induced angioedema were examined.

**RESULTS:** Over the five year period, 144 patients were identified. The most commonly reported adverse effects were cough (45%) and angioedema (27%). The mean age in years of patients with cough was 60 and angioedema was 63. Seventy-seven percent of patients with cough and 66% of patients with angioedema experienced these side effects after one year of therapy. Cough and angioedema were more common in females at 57% and 62% respectively. Of the eight African American patients with reported allergy to ACE-I, 100% had angioedema. All of the patients with cough on ACE-I that were subsequently placed on an ARB tolerated it well. Out of 9 patients who reported angioedema to ACE-I, 7 were able to tolerate an ARB and 2 developed subsequent angioedema.

**CONCLUSIONS:** Cough and angioedema were the most commonly reported adverse reactions by patients taking an ACE-I or an ARB. The majority of reactions occurred after one year of therapy.

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**405 Desensitization to Epoetin Alfa in a Patient with Anaphylaxis: a Case Report**

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**RATIONALE:** Epoetin alfa is a human recombinant erythropoietin widely used to treat anemia in patients with chronic kidney disease. Anaphylaxis to epoetin alfa has been described, but desensitization to epoetin alfa in the setting of IgE-mediated reaction has not been reported. We present a case of a hemodialysis patient with anemia associated with chronic kidney disease that developed symptoms of anaphylaxis (generalized pruritus, urticaaria, oropharyngeal edema, and hypotension) after administration of epoetin alfa and eventually tolerated desensitization to the medication.

**METHODS:** Percutaneous skin testing to undiluted epoetin alfa (10,000 units/ml) and darbepoetin alfa (100 mcg/0.5 ml) from single-use, preservative-free preparations was performed, followed by intradermal testing at increasing concentrations of epoetin alfa (1 unit/ml, 10 units/ml, 100 units/ml, 1000 units/ml) and darbepoetin alfa (0.01 mcg/ml, 0.1 mcg/ml, 1 mcg/ml, 10 mcg/ml). The patient underwent a desensitization protocol to epoetin alfa over three hours to achieve a total therapeutic dose of 12,800 units. After desensitization, the patient continued a daily regimen of epoetin alfa 2,000 units subcutaneously.

**RESULTS:** Percutaneous skin testing to epoetin alfa and darbepoetin alfa was negative. Intradermal testing yielded a positive result to epoetin alfa at a concentration of 1000 units/ml, with accompanying symptom of generalized pruritus, and a positive result to darbepoetin alfa at a concentration of 10 mcg/ml. The patient successfully completed the desensitization protocol to epoetin alfa and continued to tolerate subsequent therapeutic doses.

**CONCLUSIONS:** This is the first case report to demonstrate desensitization to epoetin alfa in a patient with a history of anaphylaxis to epoetin alfa.
406 Clinical Characteristics of Severe Cutaneous Adverse Reactions in a tertiary hospital in Korea
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RATIONALE: Severe cutaneous adverse reactions (SCARs) are very rare but clinically important diseases because of their significant morbidity and mortality. Causative agents and susceptible subjects may be different according to countries. However, little has been known about SCARs in Korea.
METHODS: Medical records of patients with SCARs treated in Seoul National University Hospital from 2005 to 2010 were retrospectively reviewed.
RESULTS: During the study period, patients with Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) were 58 and those with drug reaction with eosinophilia and systemic symptoms (DRESS) were 39. Mortality rates were 6.9% and 5.1% among patients with SJS/TEN and DRESS respectively. The most frequently involved drugs were allopurinol (SJS/TEN: 11.6%, DRESS: 35.7%) and carbamazepine (SJS/TEN: 11.6%, DRESS: 16.2%). Cutaneous manifestations were found in most patients with SJS/TEN (98%) and DRESS (84%). Mucosal involvement were more prevalent in SJS/TEN (84%) compared with DRESS (30%). Lymphadenopathy was more frequently found in DRESS compared SJS/TEN (18.9% vs. 5.8%). Although eosinophilia and organ involvement were major features of DRESS (78.9% and 87.2%), considerable number of patients with SJS/TEN also had these phenotypes (34.5% and 66.1%). Seasonal patterns were observed in the development of SCARs (31.4% of SJS/TEN in winter, 47.1% of DRESS in spring). The outcome was not different according to treatment modalities (conservative management, systemic corticosteroid, and intravenous immunoglobulin).
CONCLUSIONS: Among patients with SCARs, mortality rate was 6.2% and the most common causative agents were allopurinol and carbamazepine. Overlapped features of SJS/TEN and DRESS were frequently observed.

407 Hypersensitivity Reactions To Monoclonal Antibodies: Desensitization Approach
RATIONALE: Increasing use of monoclonal antibodies (mAbs) in chronic inflammatory and oncologic disorders, leads to increasing incidence of hypersensitivity reactions (HSRs). Desensitization is a useful approach in cases without alternative therapy.
METHODS: Twelve patients were referred to our Allergy Department (May 2008-August 2011) due to immediate HSRs to mAbs. Skin-testing (ST) was performed. Two patients underwent a 12-step desensitization protocol previously described, with a total of 11 desensitizations. Dexchlorpheniramine, acetaminophen and glucocorticoids were used as premedication. Two patients were desensitized to rituximab, one of them also to infliximab.
RESULTS: mAbs involved were rituximab=6, infliximab=3, cetuximab=1, adalimumab=1, trastuzumab=2, bevacizumab=1, efalizumab=1, etanercept=1 (3 patients reacted to more than 1). HSRs were classified as mild=3, moderate=7 and severe= 2 (Brown 2004).
CONCLUSIONS: It is possible to safely use drugs with a high incidence of hypersensitivity reactions. The success of the desensitization protocol depends on the prior desensitization protocol, the reactivity of the patient and the time between the desensitization and the infusion.

408 C1-INH: Test Use Evaluation On In-Patient Adults
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RATIONALE: Deficiency of C1-INH suggests the diagnosis of HAE. Clinically, ACE-inhibitor induced and allergic angioedema are common in adults, HAE is not. There is controversy regarding the most appropriate initial tests for adults with angioedema.
METHODS: We conducted a retrospective chart review from June 2008-June 2011 at Beaumont Hospital. Adult ER and in-patients with C1-INH tests were reviewed. Tests ordered for monitoring therapy were excluded.
RESULTS: Eighty charts met the inclusion criteria, 76 were available for analysis. Two charts were not found; two were excluded since the C1-INH test was cancelled. The average age was 57.21 years. Six patients were under the age of 30. Of the 50 patients who had angioedema without hives, 20 were currently on an ACE-I, 4 on an ARB and 1 on both. New HAE cases were not found. A total of 31 unique complement ordering patterns were noted among allergists, ER physicians and internists. Out of the 37 tests ordered by allergists, 31 included 4 or more complement tests versus 13 of 26 ordered by other physicians.
CONCLUSION: There is disagreement about appropriate tests for patients with angioedema. We found wide variability in the order pattern between ER physicians, internists and allergists. Excessive testing seems to be common. Ordering unnecessary tests is taxing on health care resources. Establishing a new diagnosis of HAE in this patient population (mostly older adults) is extremely unlikely. There is a need for better testing guidelines for adult patients presenting with angioedema.

409 Distribution of Allergic Sensitization by ISAC Microarray
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RATIONALE: Allergen-based microarrays have been applied in European centers to reveal the prevalence of IgE sensitization to specific allergen proteins. We have analyzed microarray results from a cross-section of North American allergy patients to show the distribution of sensitizations.
METHODS: Patients between age 2 months and 70 years were evaluated by Phadia ISAC103 microarray. Of 286 patients, 272 were IgE sensitized to at least one allergen (median 11). Positive results were sorted by age.
RESULTS: The most prevalent food allergen source was cat Fel d1 (53%), followed by ragweed pectate lyase Amb a1 (46%), birch PR-10 Bet v1 (40%), Alternaria Alt a1 (40%), dog Can f1 (37%), grass Phl p1 (31%), Japanese cedar pectate lyase Cry j1 (29%), Arizona cypress pectate lyase Cup a1 (24%), dust mite Der p1 (24%) and birch profilin Bet v2 (17%). The most prevalent food allergen source was peanut Ara h2 (31%), followed by egg Gal d1 (19%), soy Gly m6 (15%), milk Bos d4 (15%) and hazelnut Cor a9 (15%). Kiwi thauatin was not ranked.
CONCLUSIONS: More than half (52%) of those patient sensitized to aeroallergens were also sensitized to food allergens. Few patients were sensitized to food allergens alone (4%). Considering the progression of sensitizations, patients <4 years had a greater proportion sensitized to foods only (15%), those >12 years had a greater proportion sensitized to aeroallergens only (71%). Major allergens are represented within a few protein families. Similar proteins from different sources cross-react as with pectate lyase. Sensitization to pan-allergens PR-10 (40%) and profilin (17%) was common.
410 Patterns of Intranasal Corticosteroid Use Among Individuals Diagnosed with Allergic Rhinitis: Evidence from a Large Claims Database
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RATIONALE: Evaluate patient characteristics and patterns of intranasal corticosteroid (INS) use among individuals diagnosed with allergic rhinitis (AR) from a large, retrospective claims database.
METHODS: The i3 InvisionTM Data Mart was used from 1/1/2006 through 12/31/2010. AR patients aged ≥12 years were included if they received an aqueous formulation INS (with first such date identified as index date) and had continuous insurance coverage from 6 months before through 24 months after index date. Patients diagnosed with chronic rhinitis or nasal polyps were excluded. Data are descriptive in nature.
RESULTS: The sample consisted of 163,473 individuals with a mean age of 41 (SD = 15), 59% female, and 55% residing in the southern US. Patients were most commonly prescribed generic fluticasone propionate (44%), mometasone (34%), or triamcinolone (10%) and had a high degree of related comorbidities, including sinusitis (40%), asthma (15%), and otitis media (12%). Treatment patterns during the first year after index date: a mean of 2.1 INS prescriptions were filled (median = 1.0; SD = 1.8); adherence as measured by the medication possession ratio was averaged 18% (median = 8; SD = 16), and persistence averaged 135 days (median = 30; SD = 134). Furthermore, 7% of patients switched INS products during their first year of use, with 5% of patients who initially received generic INS switching to a branded INS product.
CONCLUSIONS: Although INS are considered the gold standard for adherence as measured by the medication possession ratio averaged 18% (median = 8; SD = 16), and persistence averaged 135 days (median = 30; SD = 134). Furthermore, 7% of patients switched INS products during their first year of use, with 5% of patients who initially received generic INS switching to a branded INS product.

411 Comparison Of Patient Adherence To Intranasal Corticosteroids By Pressurized Metered-dose Inhaler Versus Aqueous Formulations
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RATIONALE: Intranasal corticosteroids (INS) for allergic rhinitis (AR) treatment were previously available in “dry” pressurized metered-dose inhaler (pMDI) and aqueous (A-INS) formulations. While offering comparable efficacy, formulations differed by patient preference and tolerability. In 2003, following environmental concerns over propellants, pMDI were no longer marketed. New FDA-approved, environmentally-friendly pMDI formulations are anticipated. Using historical data, we assessed whether patient adherence (as proxy for patient preference and tolerability) significantly differed by INS formulation.
METHODS: Matched (age at AR diagnosis, sex, race/ethnicity, allergy- and non-allergy-related comorbidities), retrospective (7/1/1997-12/31/2001) claims of Florida Medicaid enrollees, aged ≥12 years, with newly-diagnosed AR, receiving de novo INS, and having ≥18 months of follow-up compared pMDI versus A-INS groups by time and number of refills to discontinuation; percent maintaining 12-month therapy; percent with “satisfactory compliance” (INS availability ≥70%); and refill gaps.
RESULTS: Comparisons between matched patients receiving pMDI (N=195) versus A-INS (N=390) revealed no significant differences in median time and number of refills to discontinuation or percent of patients maintaining 12-month therapy. Significantly more patients receiving pMDI than A-INS achieved satisfactory compliance (33% versus 18%, p = 0.0091). Median refill gap was significantly shorter for pMDI versus A-INS patients (73 days versus 111 days, p = 0.0003).
CONCLUSIONS: Patient preference and tolerability to INS, as measured by adherence-related outcomes, varied by formulation. Whereas no significant differences were observed in time or number of refills to discontinuation and percent of patients maintaining 12-month therapy, significantly more pMDI than A-INS patients achieved satisfactory compliance, and refill gaps were significantly shorter for pMDI than for A-INS patients.

412 Evaluation of Coverage of Allergy Concepts in Electronic Health Records
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RATIONALE: Increasing numbers of physicians are using electronic health records, as they are thought to have applications in facilitating communication, compliance with legal and financial requirements, evaluating provider performance, decision support, and data-mining in translational and clinical studies. Formal and computable representation of data and knowledge is fundamental in establishing a common vocabulary and semantics. In this study, we evaluated the coverage of the largest clinical terminology (SNOMED-CT).
METHODS: Ten (10) allergy patient encounters from the Women and Children’s Hospital of Buffalo Pediatric Allergy clinic were parsed into discrete concepts. Each concept was classified as an allergy or general medicine concept. We used the National Center for Biomedical Ontology BioPortal search tool to search for matching SNOMED-CT concepts. The main outcome measure we used was adequacy of concept matching, based on a 3-point scale (0, no match; 1, partial match; 2, complete match). Findings from all encounters were combined, from which a mean coverage score was computed.
RESULTS: After combining equivalent concepts, there were a total of 541 concepts parsed. For the combined 306 allergy concepts and 235 general medicine concepts, there were 310 complete matches, 188 partial matches, and 43 no matches (mean score = 1.49±0.64). For allergy concepts, there were 148 complete matches, 130 partial matches, and 28 no matches. The mean coverage score for allergy concepts was 1.39±0.65; while the mean score for general medicine concepts was 1.62±0.60.
CONCLUSIONS: More work is needed to improve coverage and semantics of the concepts covered in order to support primary and secondary uses of captured data.

413 Blunted Emotional Availability In Mothers Who Have Food Allergy Children :an Analysis By “I Feel Pictures” Test
Y. Machino, M. Nagao, T. Fujisawa; Mie National Hospital, Tsu-City, JAPAN.
RATIONALE: Food allergy in childhood is a significant burden not only on patients but also on their caregivers. Daily food avoidance and fear of anaphylaxis may negatively affect emotional state of the mothers and consequently impair mother-child relationship.
METHODS: Forty-four mothers with food allergy children (FA moms) who visited allergy clinic and 35 mothers with healthy infants who visited well-baby clinic (WB moms) were enrolled. They completed the Japanese version of “I FEEL Pictures” test, a standardized test to evaluate caregivers’ emotional availability in the context of mother-child relationship. Mothers described their interpretation of infant emotion in free sentences by viewing 30 pictures of a 12-month old infant with various facial expressions. Then the answers by the mothers were categorized into 18 emotions including joy, fear, anger, sadness, anxiety, etc independently by two psychologists. Disagreement in categorization by the two was later settled through their discussion. Differences in interpretation of the pictures by the mothers were compared between the groups.
RESULTS: FA moms rated significantly less number of pictures as “anger” than WB moms. Likewise, less number of pictures were rated as “sadness” and “pondering” by FA moms. There were no differences in number of pictures rated as “joy”, “fear” and “anxiety”.
CONCLUSIONS: FA mom may be less sensitive to interpret emotions, especially negative ones, expressed by infants, suggesting possible emotional blunting caused by burden of food allergy.
414 Does Allergen-Specific Immunotherapy Reduce the Risk, Reverse, and/or Mitigate Asthma in Children with Allergic Rhinitis? The Pediatric IMPROVED Access to AllerGen-Specific Immunotherapy (PEDIATRIC-IMAGINE-AIRE) Study, funded by the Joint Council of Allergy, Asthma and Immunology

C. S. Hankin, L. Cox, A. Bronstner, Z. Wang, C.1BioMedEcon, Moss Beach, CA. Νova Southeastern University College of Osteopathic Medicine, Fort Lauderdale, FL.

RATIONALE: The benefits of allergen-specific immunotherapy (SIT) in preventing, reversing, and/or mitigating childhood asthma are supported by 2 European and 1 small U.S. study. We are conducting large-scale research to: 1) examine these potential benefits among children with allergic rhinitis (AR); and 2) examine SIT treatment intensity required for such potential benefits.

METHODS: The Pediatric IMPROVED Access to AllerGen-Specific Immunotherapy Asthma Incidence Rates Among Medicaid Enrollees (PEDIATRIC-IMAGINE-AIRE) Study is a matched cohort (age AR-diagnosed, sex, allergy- and non-allergy-related comorbidities, and race/ethnicity), retrospective (7/1997-12/31/2009) claims analysis of Florida Medicaid-enrolled children (aged 5-14 years). Study 1 (preventive effects of SIT on asthma) matches newly-AR-diagnosed (ICD-9 477.X) children without asthma (ICD-9 493.X) who subsequently receive de novo SIT (per CPT codes), and have ≥3 years of follow-up to counterparts who do not receive SIT. In Studies 2 (reversal of pre-existing asthma) and 3 (moderating effects of SIT on existing asthma), patient identification parallels Study 1, except that children with newly-diagnosed AR and comorbid asthma are selected. Outcomes include the likelihood of developing asthma, rates of asthma reversal, and allergy-related healthcare utilization and costs during and following SIT by treatment intensity (frequency and duration).

RESULTS: Among all (7,524,231) enrollees, we identified 158,876 AR-diagnosed children aged 5-14. For Study 1, we matched 490 newly-AR-diagnosed children without asthma who subsequently received SIT to 2,094 counterparts who did not receive SIT. Further analyses are underway.

CONCLUSIONS: This is the first large-scale, U.S. study to examine the potential for SIT to prevent, reverse and/or moderate asthma among children with AR, and associated treatment intensity required.

415 Tolerability and Efficacy of 20% Subcutaneous Immune Globulin (ScIg) Delivered by Rapid Push


RATIONALE: ScIg replacement generally occurs once weekly. An alternative mode of delivery is to provide smaller volumes several times each week by rapid ScIg push. We report 3 Common Variable Immunodeficiency patients transitioned to rapid ScIg push using 20% immune globulin (Ig) to improve convenience and reduce weekly infusion times.

METHODS: A 54-year-old female with IgG levels maintained at approximately 1400 mg/dL on 7.4 grams of 20% Ig infused once weekly was converted to 1 gram daily by rapid ScIg push. With a 6% weekly dose reduction to accommodate for standardized vial packaging, her mean IgG levels were 1360 mg/dL over an 8 month period. A 13-year-old boy was converted from 20 grams IVIG once monthly (IgG level = 825 mg/dL) to 1 gram daily by rapid push. Subsequent IgG levels averaged 1123 mg/dL over 4 months. The third patient, a 17-year-old male, received 8 grams once weekly of 20% ScIg (IgG level = 711 mg/dL). Due to recurrent infections, the total dose was increased to 9 grams (1 gram/day for 5 days, 2 grams on remaining days). The dose increase resulted in a 16.6% increase in serum IgG levels (853 mg/dL) 3 weeks later.

RESULTS: All patients successfully administered 20% Ig daily by rapid ScIg push technique. However, after 3 weeks of therapy, the third patient transitioned back to once weekly due to preference.

CONCLUSION: Rapid push with 20% ScIg is effective in maintaining serum IgG levels. Further studies examining treatment satisfaction are needed to help identify appropriate patients for this treatment modality.

416 Reliability and Validity of the Phase V® Allergic Rhinitis Treatment Satisfaction and Preference Scale (Phase V® ARTSP) In Patients with Perennial Allergic Rhinitis (PAR)


RATIONALE: Allergic rhinitis is a collection of symptoms that makes patients feel unwell and carries functional consequences, e.g., sleep disturbance and reduced social interactions. AR treatment regimens may create barriers to adherence or product misuse resulting in prolonged symptoms and functional sequelae. This paper presents psychometric evidence for the Phase V® ARTSP, designed to quantify patient AR treatment satisfaction and preference.

METHODS: Data were collected, qualitatively, from AR patient focus groups, and quantitatively in a 2 (1 wk) period cross-over RCT. 185 PAR patients were randomized to either a nasal aerosol or aqueous nasal spray. The Phase V® ARTSP comprised 9 satisfaction scales (76 items) and 17-item comparative preference assessment. Content validity was established by qualitative analysis of focus group transcripts. Reliability was assessed by intraclass correlation. Validation evidence for PAR was demonstrated using construct and concurrent validity, and responsiveness to change.

RESULTS: Qualitative content analysis confirmed the 9-scale conceptual structure. Reliability coefficients averaged 0.888 over four assessments (0.731 [Regimen Attributes] - 0.972 [Interference]). Construct validity analysis revealed significantly higher correlations between items and assigned vs. other scales. Structural equation modeling strongly supported the conceptual structure (CMIN: 27.629, p = 0.118; CFI: 0.993; RMSEA: 0.046; PCLOSE: 0.535). Responsiveness, evaluated by known-groups analysis based on reflective total nasal symptom change scores (rTnSS), revealed predicted significant differences across the 9 scales and an overall satisfaction scale.

CONCLUSIONS: The Phase V® ARTSP psychometric evidence for conceptualization, reliability, validity and responsiveness is comprehensive and positive, and supports its use as an endpoint in comparative AR treatment studies.

417 Switching From Intravenous Immunoglobulin (IGIV) Therapy to IGSC 20%: Estimated Impact on Dosing Requirements and Cost of Therapy

R. Iyer, M. Luo, J. Li-McLeod; Baxter Healthcare Corp, Deerfield, IL.

RATIONALE: For patients initiating IGSC 20% (Hizentra, CSL), the product package insert recommends increasing the previous IGIV dose by 153% to achieve systemic IgG exposure not inferior to that of the previous IGIV treatment. The objective of this study is to estimate drug utilization and economic impact of the dose adjustment per product label.

METHODS: Annual drug utilization was calculated for a hypothetical patient weighing 65 kg based on IGIV dosing of 0.4g/kg/month. IGSC 20% dose was calculated by adjusting the required IGIV dose by the label recommended factor of 153%. Cost estimate was based on published average wholesale price. A sensitivity analysis was conducted to examine results based on a range of ± 15% of the dose adjustment factor.

RESULTS: When patients switched from IGIV to IGSC 20%, an additional 165g IG per patient per year (PPPY) was required, which resulted in an incremental cost of $25,002 per patient per year for IGSC 20% over IGIV. This amount is equivalent to treating an average PI patient with IGIV for approximately 6 months. Based on sensitivity analysis the incremental IG usage with IGSC 20% could range between 94g to 237g with IGIV for approximately 6 months. Additional 165g IG per patient per year (PPPY) was required, which resulted in an incremental cost of $25,002 per patient per year for IGSC 20% over IGIV. This amount is equivalent to treating an average PI patient with IGIV for approximately 6 months. Based on sensitivity analysis the incremental IG usage with IGSC 20% could range between 94g to 237g with IGIV for approximately 6 months.

CONCLUSION: This analysis indicates that when patients switch from IGIV to IGSC 20%, dose adjustment requirements would increase overall drug utilization and cost.
Abstracts

418 Assessment of Actual Dose Adjustment in Patients Switching From Intravenous Immunoglobulin (IGIV) Therapy to IGSC 20% M. Luo, R. Iyer, J. Li-McLeod; Baxter Healthcare Corp, Deerfield, IL.

RATIONALE: The IGSC 20% (Hizentra, CSL) package insert recommends dose adjustment to achieve the desired clinical response and serum IgG trough level when patients switch from IGIV therapy to IGSC 20%. The objective of this study is to examine if dose adjustment occurs in patients switching from IGIV to IGSC 20% in real world settings.

METHODS: Pharmacy claims for IG products dispensed to treat Primary Immunodeficiency (PI) were extracted from specialty pharmacy dispensing data from January 2009 - June 2011. Route of administration was identified by prescribed brand and dosing frequency. Patients with ≥2 claims for any IGIV who subsequently switched to IGSC 20% were included in the analysis. Dosing adjustment was calculated for each patient as the ratio of mg/30days of [IGSC 20%] / [IGIV]). Wilcoxon signed rank test was used to test the research hypothesis that the dose adjustment ratio was different from 1.

RESULTS: There were 83 patients who switched from any IGIV to IGSC 20% after applying the inclusion and exclusion criteria. Among them, 81% had a median dose adjustment of 1.41 (mean 1.46) when switching from IGIV to IGSC 20%, which was statistically significant (p<0.0001). The median number of days on IGIV and IGSC 20% therapy in this study was 342 and 163 days respectively. The median 30 day dose was 35.7g for IGIV and 44.2g for IGSC 20%.

CONCLUSION: Using real world pharmacy dispensing data, this study suggests that dose increase occurred when PI patients switched from IGIV therapy to IGSC 20%.

419 Gene-Environment Interaction between TLR4 Gene and Mold Exposure in Infancy on the Development of Allergic Rhinitis in School-Aged Children H. Kim1,2, J. Seo1,2, S. Lee1,2, M. Kang4, J. Kwon1,2, B. Kim1,2, Y. Song1,2, Y. Kim4, S. Hong1,2, T. Childhood Asthma Atyor Center, Department of Pediatrics, Asan Medical Center, University of Ulsan College of Medicine, Seoul, REPUBLIC OF KOREA, 2Research Center for Standardization of Allergic Diseases, Seoul, REPUBLIC OF KOREA, 3De- partment of Pediatrics, Hallym Sacred Heart Hospital, Hallym University College of Medicine, Anyang, REPUBLIC OF KOREA, 4Asian Institute for Life Sciences, Asan Medical Center, Seoul, REPUBLIC OF KOREA, 5Department of Pediatrics, Seoul National University Bundang Hospital, Seongnam, REPUBLIC OF KOREA, 6Department of Pediatrics, Inje University Haenundae Paik Hospital, Busan, REPUBLIC OF KOREA, 7Childhood Asthma Atyor Center, University of Ulsan College of Medicine, Seoul, REPUBLIC OF KOREA.

RATIONALE: Mold is considered as a risk factor of allergic diseases including allergic rhinitis (AR), but the relative and the overall contributions of mold exposure remain unexplored. This study was performed to identify risk factors associated with AR and to confirm the effects of gene-environment interactions on the development of AR.

METHODS: A cross-sectional study of children (n=1828) aged 9-12 years from a rural village, a rural town and an urban city in Korea was conducted. The TLR4 polymorphism (rs1927911) was genotyped by TaqMan assay. The TLR4 polymorphism (rs1927911) was genotyped by TaqMan assay. Genotypes of the TLR4 polymorphism (rs1927911) were compared between children with and without AR. Moreover, potential gene-environment interactions were explored using the TLR4 polymorphism and a history of mold exposure in infancy.

RESULTS: The prevalence of AR was as follows: lifetime AR diagnosis by questionnaire, 24.0%; treatment in the past 12 months, 18.3%; and current AR (lifetime AR diagnosis by questionnaire together with AR symptoms in the past 12 months), 17.0%. Parental history of AR and mold exposure were independent risk factors for AR in school-aged children. When children with parental history of AR were exposed to mold in infancy, the risk for current AR (aOR 5.262, 95% CI 2.572-10.766) increased. There was no significant association between current AR and TLR4 polymorphism. In children with the CT+TT genotype of TLR4 polymorphism, mold exposure in infancy increased the risk for current AR (aOR 2.669, 95% CI 1.331-5.350).

CONCLUSIONS: This study identified that mold exposure in infancy is independent risk factor for AR. Parental history of AR and mold exposure in infancy had additive effect on a high prevalence of current AR. In addition, a potential gene-environment interaction between TLR4 gene and mold exposure in infancy was identified.

420 Development of an In Vitro Fluo-4 Calcium Assay for Assessing Selective TRPV1 Agonist Responses B. P. Davis, U. Singh, L. Haar, K. W. Jones, J. A. Bernstein; University of Cincinnati, Cincinnati, OH.

RATIONALE: Intranasal capsaicin is effective for treatment of chronic rhinitis patients with a significant non-allergic component. Using patch clamping, capsaicin was found to act as a selective TRPV1 agonist. The purpose of this study was to develop and test an in vitro assay that would facilitate investigation of TRP receptor activity in the respiratory tract.

METHODS: Cath.a neuronal or murine embryonic fibroblast (MEF) cells that express TRPV1 and MEF TRPV1-/- constructs were cultured at a density of 1x10^3 cells/mL, plated on coverslips andperfused with Tyrode’s solution. Fluo-4 AM dye was then added to allow cell membrane permeability and detection of Ca2+ mobilization using a laser scanning microscope. Time series confocal images corresponding to dynamic cell changes were obtained while perfusing with different concentrations of capsaicin (0.05 ml of 0.1, 0.25, 0.5 and 1ppm). After subtracting baseline fluorescence, region of interest fluorescence as a function of dynamic changes within cells, related to calcium uptake, were plotted.

RESULTS: Using cath.a cells, mean fluorescence (±SE) after perfusing with either capsaicin 1 ppm (n=14), 0.5 ppm (n=13), 0.25 ppm (n=9), 0.1 ppm (n=7) or blank control (n=3) was 80, 55, 35 and 30 respectively. Using MEF (n=5) and MEF TRPV1-/- (n=4), the mean fluorescence for capsaicin 1 ppm was 21 and 0, respectively.

CONCLUSIONS: This report demonstrates the utility of an in vitro fluorescent cell assay for evaluating selective TRPV1 receptor activity of a pharmacologic agent that could supplant patch clamping. The magnitude of response appears to vary between cell types.

421 Differential TRPV1 Activation Responses to Rhinoconjunctivitis Pharmacologic Agents Using an In Vitro Fluo-4 Calcium Assay U. Singh, L. Haar, W. Jones, J. Bernstein; University of Cincinnati, Cincinnati, OH.

RATIONALE: In vitro fluorescent assays provide a simpler approach to measure TRP activity of different cell types. Recently this assay was standardized using capsaicin, a known TRPV1 agonist. The purpose of this study was to determine whether commonly used rhinoconjunctivitis topical agents manifested TRPV1 activity.

METHODS: Wild type murine embryonic fibroblasts (wtMEF) that express TRPV1 and MEF TRPV1-/- constructs were cultured at a density of 1x10^3 cells/mL, plated on coverslips and perfused with Tyrode’s solution. Fluo-4 AM dye was then added to allow cell membrane permeability and detection of Ca2+ mobilization using a laser scanning microscope. Time series confocal images corresponding to dynamic cell changes were obtained while perfusing with capsaicin 1ppm, bepotastine 1.5%, azelastine 1.5%, olopatidine 0.2% and fluticasone .55 ug/ul. After subtracting baseline fluorescence, region of interest fluorescence as a function of dynamic changes within cells, related to calcium uptake, were plotted.

RESULTS: For wtMEF, mean fluorescence (±SE) after perfusing with capsaicin (n=5), bepotastine (n=6), azelastine (n=5), olopatidine (n=1) and fluticasone (n=3) was 21, 35, 20, 0, 0, respectively. Using MEF TRPV1-/-, the mean fluorescence for capsaicin 1 ppm (n=4), azelastine (n=6) and bepotastine (n=6) were 0.

CONCLUSIONS: This in vitro fluorescent cell assay makes it feasible to test for selective TRPV1 receptor activity in response to a spectrum of rhinoconjunctivitis topical pharmacologic agents. These results may have significant ramifications regarding an alternative mechanism of action that could explain therapeutic responses observed in patients with non-allergic rhinoconjunctivitis which warrants further investigation.
422 Specific Nasal Provocation Test With Predator Mites
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RATIONALE: Predator Cheyletidae mites are frequently found in house dust samples in our subtropical area (Canary Islands, Spain). The potential allergenicity of this mites family is still unknown in humans.
METHODS: We selected 14 non consecutive adult patients (5 males/9 females; mean age: 34.6 y.o.) with persistent rhinoconjunctivitis and sensitized to a Cheyletus eruditus extract i.e. showing a positive skin prick test with a wheel of at least 3 mm. Six adult subjects with non allergic persistent rhinitis were selected as control group. The overall measurement of the clinical response was assessed by specific nasal provocation test (NPT) with Cheyletus eruditus. The NPT was considered positive with a decrease above 25% in the MCA and/or VOL 2-5.5 cm3 measured by acoustic rhinometry or a clinical score above 5 points.
RESULTS: All 14 sensitized subjects showed a positive NPT to Cheyletus eruditus. The six patients of the control group had a negative NPT. As 12 out of the 14 Cheyletus e.-sensitized were also allergic to Pyroglyphidae mites different patterns of dust mite sensitization have been found.
CONCLUSIONS: This is, to our knowledge, the first study to show a specific nasal response to the predator mite Cheyletus eruditus in a selected population with persistent respiratory symptoms. Further work is still needed to evaluate both clinical relevance and the degree of immunologic cross-reactivity with other mites, specially Dermatophagoides spp.

423 Effects of Head-of-Bed Elevation in Supine Nasopharyngeal Reflux
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RATIONALE: Nasopharyngeal reflux (NPR) of gastric contents can contribute to perennial nasopharyngitis, cough and asthma. However, unlike gastroesophageal reflux disease (GERD), effective treatment strategies for NPR remain inadequately defined. We hypothesized that head-of-bed elevation would improve supine NPR as it does in GERD when measured by overnight nasopharyngeal pH monitoring.
METHODS: A retrospective chart review was performed of patients seen at Scripps Health, Division of Allergy, Asthma and Immunology who had undergone overnight nasopharyngeal pH monitoring with a commercially available nasopharyngeal pH monitoring device. Dx-pH Measurement System from Restech, San Diego, CA. Patients were included if they had documented solely supine NPR and then underwent sequential pH monitoring before and after elevating the head of bed six inches. We performed a descriptive analysis of observed data.
RESULTS:Sequential overnight nasopharyngeal pH monitoring before and after head-of-bed elevation was obtained in 13 individuals. Ten subjects demonstrated significant improvement of supine NPR, 8 of which demonstrated complete resolution of supine NPR with six inches of head-of-bed elevation.
CONCLUSIONS: This study provides new evidence that six inches of head-of-bed elevation can be an effective means of treating supine NPR.

424 Seasonal Local Allergic Rhinitis in Areas with High Exposure to Grass Pollen
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RATIONALE: Several studies have demonstrated the existence of local allergic rhinitis (LAR) in patients previously diagnosed with non-allergic rhinitis (NAR). The aim was to replicate these observations in two areas of high grass pollen exposure located in central Spain (Madrid and Ciudad Real).
METHODS: A group of 70 seasonal non-allergic rhinitis (NAR) patients was included. A clinical questionnaire, serum total IgE, and nasal allergen provocation test with phleum (NAPT-Phl) were performed. Response to NAPT-Phl was monitored by nasal symptoms, acoustic rhinometry and determination of nasal specific IgE (sIgE), tryptase and ECP in nasal lavage at different times (baseline, 15 minutes and 1 hour after challenge).
RESULTS: Seasonal LAR was detected in 37/61 patients (61%). Nine patients presented nasal hyperreactivity to saline challenge and were excluded. LAR patients had a mean age of 37 years, 5% were children, and 14% reported a disease onset in childhood. The majority showed a predominance of women, family history of atopy, non-smokers/ex-smokers and referred persistent-moderate seasonal rhinitis. Conjunctivitis (97%) and asthma (35%) were the most frequent co-morbidities. Up to 40% of subjects reported a worsening of rhinitis in the last 2 years. Hyposmia was referred by 28% of patients.
CONCLUSIONS: Seasonal LAR to the grass pollen phleum was detected in a high percentage of NAR patients from two areas with high exposure to grass pollen. Childhood population may be affected. Conjunctivitis and asthma were frequently detected. Further multicentre studies including children and adults populations and a wide panel of aeroallergens are needed.

425 Evidence of Local Allergic Rhinitis in Areas with High and Permanent Aeroallergens Exposure
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RATIONALE: Local allergic rhinitis (LAR) is a new phenotype of rhinitis characterized by a localized allergic response in absence of systemic atopy. The purpose was to verify if this entity occurs in areas of high and perennial allergens exposure such as the Canary Islands.
METHODS: Forty-eight asymptomatic patients with perennial-persistent non-allergic rhinitis (NAR) were included. Clinical questionnaire, SPT, serum-sIgE, bilaterally NAPT with saline and D. pteronyssinus (NAPT-Dp), and nasal lavage were performed. The NAPT-Dp was monitored by score of nasal symptoms and acoustic rhinometry at baseline, 15 minutes and 1 hour after challenge. Samples of nasal lavage were obtained for determination of nasal-sIgE, tryptase and ECP.
RESULTS: From the total number of patients included, 39 patients were studied; 9 were excluded because of nasal hyperreactivity. LAR was detected in 64% by NAPT-Dp with a median age of 42 year (range:10-57). The 32% of them had their first symptoms in childhood. A predominance of women, non-smokers, and no family history of atopy was detected. The majority of patients (71%) had persistent and moderate symptoms (modified ARIA criteria). Hyposmia was present in 61% of cases and conjunctivitis (68%) was the most frequent co-morbidity reported.
CONCLUSIONS: A high percentage of LAR to DP was detected in NAR patients from the Canary Islands, a geographical area with high environmental dust mite load. In more than 30% of cases disease onset was in childhood. Multicenter epidemiological studies in children and adults are needed to establish the prevalence of this new entity.
Anti-Allergic Effects of So-Cheong-Ryong-Tang, A Traditional Korean Herbal Medicine, in An Allergic Rhinitis Mouse Model

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RATIONALE: The herbal medicine, So-Cheong-Ryong-Tang (SCRT) has been empirically used for the treatment of allergic rhinitis for hundreds of years; however, its in-vivo effects on allergic rhinitis have been rarely elucidated. We aimed to evaluate the anti-allergic effects of SCRT in an allergic rhinitis mouse model and to examine the underlying mechanism(s) of its anti-allergic effects.

METHODS: BALB/c mice were sensitized with ovalbumin (OVA) and alum and then challenged intranasally with OVA. SCRT (1 g/kg) was given to the treatment group, and multiple parameters of allergic responses were evaluated to determine the effects of SCRT on allergic rhinitis. All animal experiments in this study followed the guidelines for Institutional Animal Care.

RESULTS: SCRT reduced allergic symptoms, such as rubbing and sneezing, and eosinophil infiltration into the nasal mucosa. It also suppressed serum total IgE, OVA-specific IgE, and OVA-specific IgG1 levels and increased OVA-specific IgG2a level. SCRT significantly reduced expression of the Th2 cytokine, IL-4, however, the expression of IL-5, IFN-γ, and IL-10 was unchanged in the nasal mucosa of the treatment group (by real-time RT-PCR). In splenocyte culture, levels of both IL-4 and IL-5 decreased, and IFN-γ level increased in the treatment group; however, levels of IL-10 and TGF-β were unaffected by administration of SCRT.

CONCLUSIONS: This study shows that SCRT induced anti-allergic effects by decreasing, locally and systemically, the Th2 cytokine IL-4, and suppressed serum total IgE, OV A-specific IgE, and OVA-specific IgG1 levels and increased OVA-specific IgG2a level. SCRT significantly reduced expression of the Th2 cytokine, IL-4; however, the expression of IL-5, IFN-γ, and IL-10 was unchanged in the nasal mucosa of the treatment group (by real-time RT-PCR). In splenocyte culture, levels of both IL-4 and IL-5 decreased, and IFN-γ level increased in the treatment group; however, levels of IL-10 and TGF-β were unaffected by administration of SCRT.

RATIONALE: Continuous positive airway pressure (CPAP) is successfully used in treatment of obstructive sleep apnea (OSAS). The use of nasal interface (nasal mask (NM) or nasal pillow (NP)) in patients with nasal congestion can worsen the symptoms of stuffiness and lead to higher rates of non-compliance. Our hypothesis is that patients with nasal congestion may prefer face mask (FM) as the interface for delivery of CPAP as compared to nasal interface.

METHODS: After IRB exemption, data was collected from retrospective chart analysis on patients who underwent polysomnograms during a 10 month period (January - October 2010). History of nasal congestion and allergic rhinitis was obtained from a self-administered pre-sleep study questionnaire. Patients with OSAS who underwent CPAP trial were offered FM, NM and NP at the time of trial and their final choice was recorded. Association of the above predictors with final mask choice was analyzed using chi-squared testing for dichotomous outcomes.

RESULTS: A total of 226 patients underwent CPAP titration. Nasal congestion was reported in 179 (76%) patients. Sixty patients (33.5%) with nasal congestion selected facemask as a final interface. We did not find statistically significant association with facemask choice in patients with nasal congestion or underlying allergies (p>0.05).

CONCLUSIONS: Presence of nasal congestion or rhinitis does not influence patient’s selection of delivery interface for CPAP used during sleep.

Clinical Validation of Controlled Grass Pollen Challenge in the Environmental Exposure Unit (EEU)

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RATIONALE: The Environmental Exposure Unit (EEU), a controlled allergen exposure model of allergic rhinitis (AR), has traditionally utilized ragweed pollen. We sought to clinically validate the use of grass pollen in the EEU.

METHODS: Healthy volunteers with a history of AR symptoms during grass pollen season and supportive skin test responses attended the EEU for 3 hrs of rye grass pollen exposure (Lolium perenne). Non-atopic controls were also recruited. Subjects assessed individual rhinoconjunctivitis symptoms using the Aptar BiDose system.

RESULTS: 78 subjects were screened, of whom 39 were eligible and attended the 2x3h EEU visits, plus 8 non-atopic controls. Mean TSS, TNSS and PNIF values among participants in the higher pollen concentration group (target 3500 grains/m3) after the first 3 hour exposure were 18.9, 9.7 and 68, respectively. In comparison, mean TSS, TNSS and PNIF values in the lower pollen concentration (2500 grains/m3) group were only 13.3, 7.6, and 82, respectively. The subsequent day of pollen exposure did not appreciably alter the maximal TSS/TNSS, but rather resulted in a more rapid onset of symptomatology, with higher mean scores at the 30min, 60min and 90min timepoints. The non-atopic controls remained asymptomatic.

CONCLUSIONS: This study provides clinical validation of the ability to generate allergic rhinoconjunctivitis symptoms amongst grass-allergic individuals in the EEU.

Does Presence of Nasal Congestion Influence Patient’s Choice of Delivery Interface for Continuous Positive Airway Pressure Therapy?

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RATIONALE: Continuous positive airway pressure (CPAP) is successfully used in treatment of obstructive sleep apnea (OSAS). The use of nasal interface (nasal mask (NM) or nasal pillow (NP)) in patients with nasal congestion can worsen the symptoms of stuffiness and lead to higher rates of non-compliance. Our hypothesis is that patients with nasal congestion may prefer face mask (FM) as the interface for delivery of CPAP as compared to nasal interface.

METHODS: After IRB exemption, data was collected from retrospective chart analysis on patients who underwent polysomnograms during a 10 month period (January - October 2010). History of nasal congestion and allergic rhinitis was obtained from a self-administered pre-sleep study questionnaire. Patients with OSAS who underwent CPAP trial were offered FM, NM and NP at the time of trial and their final choice was recorded. Association of the above predictors with final mask choice was analyzed using chi-squared testing for dichotomous outcomes.

RESULTS: A total of 226 patients underwent CPAP titration. Nasal congestion was reported in 179 (76%) patients. Sixty patients (33.5%) with nasal congestion selected facemask as a final interface. We did not find statistically significant association with facemask choice in patients with nasal congestion or underlying allergies (p>0.05).

CONCLUSIONS: Presence of nasal congestion or rhinitis does not influence patient’s selection of delivery interface for CPAP used during sleep.

Nasal Symptoms Induced By A Standardized Nasal Challenge Test With Aqueous Phleum pratense Reflects Seasonal Nasal Symptom In Grassic Allergic Patients

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RATIONALE: Nasal challenge may be used to assess the severity of allergic rhinitis in grass allergic patients, and to monitor the clinical effect of allergen-specific immunotherapy (SIT). However, highly standardized procedures for the allergen provocation and well defined readouts for the clinical symptoms assessment have to be established, before nasal challenge can be used in future clinical development programs for SIT.

METHODS: 40 patients with hayfever and a skin prick test verified grass pollen allergy (Phleum pratense, Timothy grass) without current asthma and airway hyper responsiveness, were challenged with Phleum pratense pollen extract using the Aptar BiDose system. Exactly 100 microl, were applied with the BiDose system through each nostril, aiming at the medial lacrimal caruncle away from the septum. After nasal challenge participants were asked to complete a Total Nasal Symptom Score (TNSS) assessment form 30 minutes post challenge. For four weeks during the peak of the grass pollen season, participants were asked to complete a Rhinitis Quality of Life Questionnaire (miniRQLQ), in order to obtain an average score of their symptoms for the past weeks.

RESULTS: Modest (r values = 0.344-0.475), but significant correlations (p values = 0.03-0.003) were found comparing scores for runny nose, nasal itch, nasal congestion, and sneeze between pre-seasonal challenge and seasonal exposure to grass allergens.

CONCLUSIONS: Symptom scores obtained through this standardized grass allergen challenge with grass allergen extract reflect the nasal symptoms experienced by patients during seasonal exposure. This suggests that controlled challenges may be used to monitor the effect of allergen-specific immunotherapy.
430 Using Objective Tools to Evaluate Pediatric Patients with Chronic Rhinitis
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RATIONALE: Rhinitis is a constellation of symptoms including rhinorrhea, nasal congestion, sneezing, and itching; it affects up to 40% of the pediatric population; and is often complicated by asthma. There are no validated objective measures to evaluate symptoms in pediatric patients.
METHODS: Patients were recruited from a combined Allergy/Otolaryngology clinic where they were evaluated by a board certified allergist and a pediatric otolaryngologist. The 22-item Sino-Nasal Outcome Test (SNOT-22) was administered, and skin testing and acoustic rhinometry were performed.
RESULTS: Patients ranged in age from 6 to 18 years (median 7.6). In our group, 36% had been diagnosed with asthma and 18% had at least one allergic sensitivity. The most severe symptoms reported were congested/ blocked nose (mean 3.9 (maximum 5)), waking up tired (mean 3.5), and need to blow nose (mean 3.2). Total symptom scores were not significantly different among patients with allergy compared with no allergy (p < 0.05) or asthma compared with no asthma (p = 0.5). Overall the mean absolute minimum cross-sectional area (MCA) was 0.34 cm² and occurred at the inferior turbinate in 64% of patients. The MCA was not different among allergic and nonallergic patients (p = 0.3) and patients with and without asthma (p = 0.2). Symptoms scores did not correlate with “bother” scores (p = 0.1) or acoustic rhinometry results (p = 0.1).
CONCLUSIONS: Elevated symptom scores for waking up tired and congestion suggest severity of pediatric symptoms. Total symptom scores do not distinguish patient groups (allergic, nonallergic, or asthma).
Symptom scores, including congestion, and rhinometry are not related. Both tests detect independent parameters and are useful in patient evaluation.

431 In Mice Sensitized to Milk, Epicutaneous Immunotherapy Prevents Further Sensitization to Peanut or House Dust Mite
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RATIONALE: Epicutaneous immunotherapy (EPIT) to milk was proved safe and efficacious in children with milk allergy (Dupont al, JAllergyClinImmuno 2010). This study evaluated in a model of milk-sensitized mice if milk EPIT might prevent from further sensitization to other allergens.
METHODS: After oral sensitization, BALB/c mice were treated with EPIT (milk-Viaskin or sham-Viaskin) and then further sensitized to peanut or HDM. After further sensitization to peanut, mice were exposed to a sustained peanut regimen, known to induce eosinophilic eosinophilic infiltration correlated to Th2 cytokines mRNA expression. After further sensitization to HDM, mice were challenged by aerosol and tested by BAL fluid content and plethysmography. Specific antibodies to milk, peanut and HDM were monitored. Naive mice were included.
RESULTS: Milk EPIT was efficient, with a significant increase of sIgG2a, 1.25±0.35v.0.05±0.02µg/ml (EPIT vs Sham, p < 0.05) and a significant decrease of Th2 cytokines secreted by milk-reactivated splenocytes (vs Sham, respectively, IL-4: 17 pg/ml vs 86 pg/ml, p < 0.05; IL-5: 14 pg/ml vs 153 pg/ml, p < 0.05; IL-13: 11 pg/ml vs 159 pg/ml, ns). After further sensitization, only the milk-EPIT group showed significant increase of sIgG2a to PPE or HDM and decrease of sIgE for PPE. Also, the milk-EPIT group did not exhibit any increase in 1/ eosinophilic eosinophilic infiltration after peanut exposure, 3 eosinophils/mm² vs 27 eosinophils/mm² (sham, p < 0.01), and vs 4 eosinophils/mm² (naive, ns), 2/Pen values after HDM aerosol challenge, AUC=121±4.9 vs 204±19.6 (sham, p < 0.01) and vs 117±4.0 (naive, ns).
CONCLUSIONS: In mice sensitized to milk, EPIT suppressed the immune response to further sensitizations by other allergens.
Protection from Oral Peanut-Induced Esophageal Lesions in Sensitized Mice treated by Epicutaneous Immunotherapy is Mediated by CD25+CD4+ Tregs

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RATIONALE: In a previous study in mice sensitized to peanut, we have established that EPIT decreased eosinophile infiltration of the esophasus after oral provocation with peanut, increased expression of Foxp3 in the esophageal mucosa, and increased CD4+ CD25+ FoxP3+ number in spleen, suggesting a role for T regulatory cells (Tregs). The aim of this study was to confirm the role of Tregs in EPIT mechanism.

METHODS: First, mice sensitized to peanut were treated by EPIT during 8 weeks. Some also received injection of anti-mouse CD25 antibody. EPIT+CD25 was then compared to EPIT alone, sensitized Sham and naive mice after oral PPE. Second, CD25+CD4+ Tregs from EPIT or Sham treated mice were transferred to PPE-sensitized mice which then received oral PPE. Eosinophilic infiltration of the esophagus after intensive oral exposure to peanut was then measured.

RESULTS: Compared to sham, EPIT decreased the PPE-induced eosinophile infiltration (13.30 ± 4.07 vs 36.39 ± 5.61 eosinophils per mm2 (E/ mm2)), eotaxin expression (1.17 ± 0.15 vs 2.11 ± 0.29 eotaxin relative mRNA, p < 0.05), and increased Foxp3 expression (2.04 ± 0.49 vs 0.62 eotaxin mRNA and 0.71 ± 0.18 FoxP3 mRNA; p < 0.05 vs EPIT). Transferring Tregs from EPIT treated mice prevented eosinophil infiltration and eotaxin expression, and induced Foxp3 in esophagus whereas transfer of cell from Sham treated mice demonstrated no effect.

CONCLUSIONS: Induction of Tregs by EPIT is able to prevent eosinophile infiltration in mice sensitized to peanut confirming the Treg mediated mechanism of EPIT.

Bioavailability of IgG Administered by the Subcutaneous Route

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RATIONALE: The US FDA requires dose adjustments to assure non-inferior area under the curve (AUC) of serum IgG vs time on subcutaneous IgG (SCIG) vs IVIG. We compared bioavailabilities of different SCIG preparations to determine if different dose adjustments are needed with different products.

METHODS: Published data on different SCIG products were compared, and serum IgG levels were measured in patients switching from one product to another at the same dose.

RESULTS: Using the formula: percent bioavailability = AUC(SCIG) / AUC(IVIG)x100/dose ratio; bioavailabilities of different SCIGs are: Gamunex 64.9%, Hizentra 65.5%, Gammagard 67.0%, Vivaglobin 69.4%. In an EU study (N=19) the mean serum IgG levels on the same doses of other SCIG products and Hizentra were 843 ± 138 mg/dl and 833 ± 125 mg/dl, respectively (p = NS). In a US study (N=19), the mean serum IgG level on 149.3 ± 57.5 mg/kg/wk of Vivaglobin was 1139 ± 249 mg/dl and the mean serum IgG level on 155.1 ± 58.3 mg/kg/wk of Hizentra was 1158 ± 311 (p = NS). The lack of any change in levels when switching from other SCIGs to Hizentra at the same dose suggests that there is no difference in the bioavailabilities of the different products.

CONCLUSIONS: Bioavailabilities of different SCIGs are all within the range 66.7 ± 2.7% as compared to IVIG. Decreased bioavailability of SCIG appears to be a basic property of IgG and not a result of any manufacturing process or product concentration. Since serum levels do not vary when patients switch from one product to another at the same dose, different dose adjustments are not necessary with different SCIG products.
437 Anti-Inflammatory Activity Of Sublingual Immunoglobulins (SLIG) In A Preclinical Model Of Allergic Asthma
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RATIONALE: Intravenous immunoglobulins (IVIG) display anti-inflammatory activities in many diseases. Subcutaneous administration of anti-IgE in humans provides benefit in severe persistent uncontrolled allergic asthma. Given the well established efficacy of sublingual allergen immunotherapy in respiratory type I allergies, we investigated the therapeutical potential of sublingual immunoglobulins (SLIG), especially SLIG anti-IgE, in a murine model of allergic asthma.
METHODS: BALB/c mice sensitized with ovalbumin (OVA) were treated sublingually with rat monoclonal IgG1 or IgG2a, directed to either mouse IgE or with no reported specificity. Airway hyperresponsiveness (AHR) was assessed by whole body plethysmography and cellular infiltrates were characterized in bronchial alveolar lavages (BAL).
RESULTS: AHR and BAL eosinophil infiltrates were substantially decreased in mice treated with particulate OVA (positive control) as well as in animals receiving various rat IgG1, irrespective of their specificity for murine IgE. In contrast, no improvement was observed in mice treated with PBS (negative control) or various rat IgG2a. Mass spectrometry analysis indicated that the differential efficacy of rat IgG1 and IgG2a is not related to the presence of specific glycan moieties, such as sialic acid, which might have influenced their interaction with lectins borne by oral immune cells.
CONCLUSIONS: In a murine model of allergic asthma, SLIG has an anti-inflammatory activity irrespective of the immunoglobulin specificity and in the absence of allergen. SLIG can be applied to other inflammatory diseases beyond allergic asthma.

438 The Effect of Allergen Immunotherapy on Exhaled Nitric Oxide in Adult Subjects with Allergic Rhinitis and Asthma
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RATIONALE: Fractional Exhaled Nitric Oxide (FENO) is reduced in asthmatic children by treatment with omalizumab, but not allergen immunotherapy. We studied the effect of allergen immunotherapy on FENO in adult subjects with allergic rhinitis and asthma.
METHODS: Seventeen subjects with both seasonal and perennial allergic rhinitis, age 34.9±12.1 (mean±SD) were treated with allergen immunotherapy and followed for one year with visits at baseline and 4, 8, 12, 24, and 52 weeks later. 11 were female, 9 had asthma, Body Mass Index (BMI) was 24.8±3.8. At baseline, 1 subject was on inhaled steroids and 5 on nasal steroids. Allergic Rhinoconjunctivitis Symptoms (ARCS) and Asthma Control Test (ACT) scores at baseline were 6.4±4.2 and 22.7±4.4, respectively, FEV1 was 103.2±12.1%. Analyses were undertaken with STATA release 10 and significance accepted at alpha<0.05.
RESULTS: FENO (measured in triplicate) was increased in our study subjects at baseline (36.2±23.4 ppb, 8-88), but did not significantly change throughout the follow-up period (p=0.39 between first and last study visit).
No association was found between FENO and age, sex, BMI, number of positive skin tests, asthma (p=0.39), FEV1, or other spirometric values at any visit. However, a significant correlation was found between FENO and ARCS and ACT at the final visit (p=0.02).
All but two study subjects judged the immunotherapy clinically effective at the end of the study.
CONCLUSIONS: In adult subjects clinically effective allergen immunotherapy did not affect FENO. Validation of this finding should be undertaken in additional patient cohorts.

439 Inhibition Of Human IgE-Allergen Interaction By Antibodies Of Rabbits Immunized With Depigmented-polymerized Allergen Extracts
RATIONALE: Successful allergen immunotherapy has been linked to an increase of blocking antibodies, mainly specific IgG. Allergoids have, in comparison with native allergens, a reduced allergenicity, whereas immunogenicity is preserved. Our objective was to compare the induction of blocking-IgG antibodies by a native and a depigmented-polymerized (Dpg-Pol) birch pollen extract.
METHODS: Sera from New Zealand rabbits immunized (130 μg protein) of native (n=2) and Dpg-Pol (n=2) extract of Betula alba were obtained. Inhibition of human-IgE binding to B. alba extract by rabbit IgG was tested by ELISA . Microplates were coated with native B. alba extract (20 μg/ml) and rBet v 1 (1 ug/ml), and incubated with native and Dpg-Pol rabbit-antiserum and their preimmune. After, plates were incubated with a pool of sera (1/10 dilution) from birch-allergic patients, and with anti-human-IgE peroxidase.
Percentage of inhibition was calculated from the OD difference between inhibition with rabbit’s immunized sera and their corresponding preimmune.
RESULTS: Rabbit IgG antibodies induced by native and Dpg-Pol extracts can inhibit the binding of allergic patient’s IgE to B. alba extract and to rBet v 1. Percentage of IgE-inhibition to B.alba extract was similar (i.e. at 1/8 dilution: 79% and 77%) for native and Dpg-Pol, respectively, whereas inhibition IgE to Bet v 1 was higher for Dpg-Pol (68% vs 81%).
CONCLUSIONS: Dpg-Pol extracts induce IgG- antibodies, that are able to block the Ig-E-binding of birch-pollen allergic patients, at least at the same degree than native extracts. These results show that immunogenicity is maintained after deagglutination and chemical modification of allergen extracts.

440 Immunological Changes During Subcutaneous And Sublingual Grass Pollen Immunotherapy
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RATIONALE: Modified T-cell responses and induction of regulatory T-cells (Treg) might be pivotal for the effect of allergen-specific immunotherapy. We intend to investigate changes in T-cell responses during grass pollen subcutaneous immunotherapy (SCIT) and sublingual immunotherapy (SLIT) and study the effect of CD4+CD25+CD127low T-cells on T-cell response to Phleum pratense allergens.
METHODS: Grass allergic patients were treated with grass SCIT (Alutard grass; n=15), grass allergy immunotherapy tablets (Grazax; n=15), or left untreated (n=10). Treatment will be continued for 16-18 month. The current interim analysis compares average responses of the individual treatment groups after 4 months of treatment (end of SCIT-up-dosing). Grass-allergic specific T-cell proliferation was determined by thymidine incorporation. Percentages of CD4+CD25+CD127low Foxp3+ Treg cells in peripheral blood were determined by flow cytometry.
RESULTS: Differences in T-cell proliferation between immunotherapy treated patients and the control group were observed at some time points, but this was mainly caused by increased T-cell proliferation in the control group and was not affected by blocking Treg cells activity with IL-10R, TGF-b, CTLA-4, or GITR specific antibodies.
At baseline, the average percentages of CD4+CD25+CD127low and CD4+CD25+CD127low Foxp3+ cells were 2.8% (+/-1.0) and 2.4% (+/-1.0) respectively. However, average percentages of CD25+Foxp3 and CD127low Foxp3 were higher, 4.8% (+/-1.7) and 4.4% (+/-1.8), respectively (n=20).
CONCLUSIONS: The average Treg percentages differ considerably depending on the FACS markers used to define this cell population.
No consistent changes in T-cell proliferation were observed between treatment and control groups after 4 month of treatment. Subsequent analyses will investigate the number, phenotype and effect of CD4+CD25+CD127low Treg cells in these patient groups.
**441** Induced-Tolerogenic Dendritic Cells That Promote Tolerance And De Novo Differentiation Of Regulatory T Cells

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**RATIONALE:** We hypothesized that incubation of Dendritic Cells (DC) with tolerogenic factors may induce tolerogenic function artificially in order to use these cells as research and therapeutic tools.

**METHODS:** several combinations of compounds were tested for their effect on the tolerogenic function of DC. The capacity of these cells to induce deletion of antigen-specific cells and induce the differentiation of naïve CD4+ T cells (Tn) into Treg was surveyed in vitro as well as their capacity to ameliorate autoimmune conditions in vivo.

**RESULTS:** mouse splenic and bone marrow-derived DC and human monocyte-derived DC treated in vitro with rapamycin and TGFβ induce Treg differentiation. The resulting adaptive Treg (aTreg) are phenotypically and functionally equivalent to natural-occurring Treg (nTreg) in vivo. These induced-tolerogenic DC (iTDC) cotreated with toll-like receptor (TLR) agonists remain tolerogenic, required direct iTDC-Tn contact, TCR stimulation, but no cell division or IL-6, IL-10, TGFβ1. IDO1, FasL, or EB13 to stimulate Treg differentiation. Further, a marked reduction in the numbers of Tn in the first two days could be observed previous to Treg differentiation. Using the Experimental Autoimmune Encephalomyelitis model (EAE) we demonstrate that infusions of iTDC blocked the progress of the disease while significantly diminishing the number of effector T cells and increasing Treg.

**CONCLUSIONS:** iTDC can shape the repertoire of antigen specific T cells through the elimination of antigen specific cells and/or the induction of aTreg that suppress autoimmune responses in vivo.

**442** Modulation of Eosinophil Responsiveness to TSLP-Mediated Degranulation

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**RATIONALE:** Eosinophils and TSLP are increased during allergic inflammation in the epithelium of the respiratory tract, skin, and on the ocular surface. Our previous studies demonstrated that eosinophils respond to high concentrations of TSLP with degranulation. The purpose of these studies was to examine whether priming with TNFα and IL-3 (previously shown to upregulate eosinophil TSLP receptor expression) or co-incubation with primary human conjunctival epithelial cells could affect the responsiveness of eosinophils to TSLP.

**METHODS:** Eosinophils were stimulated with various concentrations of TSLP and evaluated for release of eosinophil derived neurotoxin by ELISA. For priming experiments, degranulation was evaluated in the presence and absence of pre-treatment with TNFα and IL-3. For co-incubation experiments, primary human conjunctival epithelial cells were cultured on transwell filters in 24 well plates. Eosinophils were added either apically or basally (and to epithelial cell free, control wells) and challenged with TSLP.

**RESULTS:** Eosinophils pre-treated with TNFα and IL-3 had enhanced sensitivity to TSLP-mediated degranulation with approximately 80-fold less TSLP concentration needed to achieve comparable degranulation. Co-incubation of eosinophils with conjunctival epithelial cells reduced TSLP-mediated degranulation in both apical and basal compartments, suggesting that the response was not contact dependent.

**CONCLUSIONS:** Eosinophils exhibit enhanced responsiveness to TSLP when exposed to cytokines present in allergic inflammation. Conversely, epithelial cells may provide a stabilizing influence on TSLP-mediated eosinophil degranulation.
444 Aspirin Activation of Eosinophils: Relevance to Aspirin-Exacerbated Respiratory Disease (AERD)

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RATIONALE: Aspirin hypersensitivity reactions are triggered when constraints upon eosinophils, normally supplied by PgE2, are released secondary to COX inhibition. However, the underlying mechanisms driving cellular activation are unknown. We investigated the capacity of aspirin to directly activate eosinophils.

METHODS: Eosinophils were enriched from control and AERD subjects via magnetic affinity columns. Eosinophils were activated for 30 mins with lysine ASA (LysASA). To address the importance of COX inhibition to this process, parallel samples were activated with the non-COX inhibiting NSAI NaSalicylate (NaSal) and the non-salicylate NSAI ketorolac. Activation was evaluated as Ca+2 flux and secretion of cysteinyl leukotrienes (CysLT) and EDN. Parallel samples were exposed to PGE2 prior to activation.

RESULTS: LysASA and NaSal both induced EDN and CysLT secretion while no activation was observed with ketorolac. LysASA, but neither NaSal nor ketorolac, induced Ca+2 fluxes. EDN secretion was inhibited in the presence of PGE2. There was no difference in sensitivity or extent of release between AERD and control subjects.

CONCLUSIONS: Aspirin directly induces eosinophil activation irrespective of aspirin hypersensitivity status and this is inhibitable in the presence of PGE2. This does not reflect COX inhibition as NaSal shares this effect and ketorolac does not. More than one activation pathway is involved as only LysASA induced Ca+2 fluxes. In vivo, activation with NaSal does not occur either because this agent does not reduce ambient PGE2 or Ca+2 activation is required. These studies identify novel aspirin activation pathway(s), but do not establish the basis for the unique sensitivity of AERD subjects to aspirin.

445 Eosinophils Promote Epithelial To Mesenchymal Transition Of Bronchial Epithelial Cells

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RATIONALE: Eosinophilic inflammation and structural changes of the airways including subepithelial fibrosis and myofibroblast hyperplasia are the predominant and characteristic pathological findings of bronchial asthma. A strong association of epithelial to mesenchymal transition (EMT) with the increased number of myofibroblasts in the asthmatic lung was reported. Increased levels of eosinophil-derived TGF-beta1, a key factor in the mechanism of EMT, have been reported in the bronchoalveolar lavage fluid (BALF) and bronchial tissue specimens of patients with asthma.

METHODS: For the in vivo study, eosinophils were generated from mouse bone marrow progenitors and injected intratracheally in wild type mice. Then, BALF and lung tissue were collected from mice and the degree of fibrosis was evaluated. For the in vitro study, we co-cultured the human bronchial epithelial cell line BEAS-2B with eosinophils purified from healthy individuals and EMT was evaluated by quantitative PCR, immunofluorescence microscopy, ELISA and immunoblot.

RESULTS: Intratracheal administration of eosinophils triggered inflammatory cell infiltration, fibrosis, and increased expression of type I collagen in the lung. Eosinophils induced morphological changes towards a more spindle shaped fibroblast-like cells in BEAS-2B cells. Moreover, eosinophils released E-cadherin and increased alpha-smooth muscle actin expression in BEAS-2B cells. EMT occurred only when eosinophils contacted directly with the epithelial cells and was dependent on the activation of eosinophils. Anti-TGF-beta1 mAb treatment blocked EMT of BEAS-2B cells in the co-culture system.

CONCLUSIONS: EMT occurred in bronchial epithelial cells after stimulation by eosinophils, suggesting that eosinophils may contribute to the pathogenesis of airway remodeling in chronic asthma.
447 Analysis of Eosinophil, Mast Cell, and Basophil Siglec-8 Expression on Human Cell Lines and Hematologic Malignancies

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METHODS: Siglec-8 was evaluated on blood eosinophils and basophils from patients with hypereosinophilic syndrome (HES), chronic myelogenous leukemia (CML), and chronic eosinophilic leukemia (CEL), as well as bone marrow mast cells in indolent mastocytosis.

RESULTS: Siglec-8 was consistently detected on human eosinophils and basophils from subjects with HES, CML, and CEL (>95% positive, n=2-4). It was weakly detected on HMC-1.1 (10-20% positive), but not on EOL-1 or KU812 cells (<5% positive) (n=1-5). Siglec-8 was bright and consistently expressed on HMC-1.2 (>90% positive, n=5) and LUMA cells (>70% positive, n=3) as well as bone marrow mast cells in indolent mastocytosis (>95% positive, n=3).

CONCLUSIONS: Siglec-8 is expressed on normal and neoplastic human eosinophils, basophils and mast cells, including certain mast cell lines. Siglec-8-based therapies should target mature and malignant eosinophils, mast cells, and basophils.

448 Cord Blood (CB) Eosinophil/Basophil (Eo/B) Progenitors Predict Respiratory Outcomes Until The Age Of Two

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RATIONALE: Cloning of the human CB Eo/B progenitor cell recruitment.

METHODS: In a subcohort of 40 children of the LINA study (Lifestyle and environmental factors and their Influence on Newborns Allergy risk) frozen cord blood PBMCs were used for methylcellulose assays to assess Eo/B differentiation by colony formation (CFU) in the presence of IL-3, IL-5 or GM-CSF. Standardized questionnaires were recorded during 34th week of pregnancy and annually thereafter till the age of two. Ex vivo stimulated CB cytokines were measured using the cytokometric bead array (CBA).

RESULTS: For the CB Th2 cytokines IL-4 and IL-13 a positive correlation was seen with the number of IL-5 responsive Eo/B CFUs (p<0.05). Enhanced CB IL-5- (but not IL-3- or GM-CSF-) responsive Eo/B CFU numbers predicted the occurrence of bronchitis and treated wheezing within the first 12 or 24 months (p<0.05).

CONCLUSIONS: Our data confirm the hypotheses that a modified Th2 milieu at birth may contribute to the recruitment and differentiation of Eo/B progenitors. We could further show that the predictive value of CB EoB progenitors in terms of respiratory illnesses is not restricted to high-risk children.

449 Mechanisms Of Tlr-mediated Cord Blood Cd34+ Progenitor Cell Eosinophil Differentiation: Signaling And Autocrine Pathways

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RATIONALE: Eosinophils are multi-functional leukocytes that play a role in allergic inflammation, and their lineage commitment has been shown to be dependent on hematopoietic cytokine signaling and the activation of STAT5 and MAPK signaling pathways. We have previously reported that LPS can induce eosinophil-basophil (Eo/B) differentiation of cord blood (CB) progenitors; however, the mechanism for this was unclear.

METHODS: CB CD34+ cells were stimulated with LPS and assessed for Eo/B colony forming units (CFU) by methylcellulose cultures, cytokine production by luminex and phosphorylation of STAT5 and MAPK proteins by phospho-flow cytometry. The importance of LPS induced hematopoietic cytokines and signaling molecules in Eo/B CFU formation was determined by neutralizing Abs and pharmacological inhibitors respectively.

RESULTS: Stimulation with LPS increased the formation of IL-3-, and GM-CSF-responsive Eo/B CFU compared to hematopoietic cytokine stimulation alone (p<0.017). Overnight stimulation of CB CD34+ cells resulted in the secretion of IL-3 and GM-CSF which stimulated Eo/B CFU production ex vivo, since Ab blockade of these cytokines significantly reduced Eo/B CFU formation (p<0.016). Likewise, LPS induced the phosphorylation of p38 MAPK, STAT5, and ERK 1/2 in a time-dependent manner (P<0.043); blocking these proteins resulted in suppression of LPS mediated Eo/B CFU formation (p<0.025).

CONCLUSIONS: We show for the first time that LPS stimulation of human CB CD34+ cells can influence Eo/B differentiation directly through STAT5 and MAPK signaling pathways and hematopoietic cytokine secretion. Since LPS-mediated immuno-modulation of CB CD34+ progenitors can shape neonatal immunity, understanding microbial influences on eosinophilopoiesis may aid the development of therapies for eosinophil-related allergic disorders.

450 Differential Effect of TGF-β1 and Eotaxin on Novel CLC3 Ion-Channel Variants in Human Peripheral Blood Eosinophils

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RATIONALE: Chloride channels are implicated in differentiation, proliferation, apoptosis and migration of cells. Previously, we reported the involvement of CLC3 in TGF-β1-induced migration, presence of CLC3b and CLC3e transcript variants and membrane expression of the CLC3 in human peripheral blood eosinophils. In this study, we examined the effect of eotaxin in CLC3 expression in eosinophils.

METHODS: Eosinophils were isolated and purified (>99% pure >98% viable) from venous blood of healthy donors with negative selection. Cells were incubated with either TGF-β1 (10ng/ml) or eotaxin (10ng/ml) for 24 hours. Total RNA was isolated using mirVana kit and 500ng RNA was reverse transcribed using Impront RT II. The qPCR data was analyzed using GAPDH as the reference gene with 2^-ΔΔCt as fold increase.

RESULTS: There was a 2-fold increase in CLC3 mRNA transcripts (exon 10-exon 11) with TGF-β1 (n=4, p<0.05) relative to GAPDH. Eotaxin has no statistically significant effect on CLC3 mRNA transcripts (exon 10-exon 11). CLC3b mRNA (exon 12-exon 14) was increased 3-4-fold with TGF-β1 (n=4, p<0.05) compared to 2-fold increase with eotaxin. Eotaxin increased the mRNA transcripts CLC3e (exon 13-exon 14) 3-4-fold (n=4, p<0.05) compared to 2.2-5-fold increase with TGF-β1. However, CLC3e mRNA transcripts decreased in combination of TGF-β1 and eotaxin.

CONCLUSIONS: mRNA transcript levels of CLC3 with different primer sets suggest the presence of more transcript variants in human blood eosinophils than the known variants, CLC3b and CLC3e. The significant increase in the transcript level of CLC3e with eotaxin suggests the role of CLC3e ion-channel in eotaxin-induced migration of eosinophils in allergic asthma.
Identification of Suitable qPCR Reference Genes during IL-5 Induced Cord Blood Eosinophilopoiesis

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RATIONALE: Quantitative time polymerase chain reaction (qPCR) has become a widely used and often preferred tool to study gene expression in a variety of applications. However, in order to use this sensitive technique reliably, a set of unaffected reference genes is necessary for each evaluation. Of increasing interest to researchers is the study of umbilical cord blood for potential biomarkers of future atopy; our laboratory in particular is interested in mRNA expression of genes important to eosinophil-lineage commitment. We therefore aimed to identify reference genes amongst a panel of twelve that would be optimal to study gene expression during IL-5 induced eosinophilopoiesis in umbilical cord blood.

METHODS: Mono-nuclear cells (MNCs), isolated from random human cord blood cord samples, were stimulated with recombinant human Interleukin-5 (rhIL-5; 1ng/mL) ex vivo to induce eosinophilopoiesis. Cells were collected at 0, 24, 48 and 72 hours post stimulation to isolate RNA and reverse-transcribed. A panel of twelve human reference genes (GAPDH, TUBB, PP1A, ACTB, YWHAz, RN185S, B2M, UBC, TBP, RPLP0, GUSB and HPRT1) were investigated via qPCR. Primer pairs for the above listed genes were obtained from tataabiocenter.

RESULTS: Utilizing the statistic software ‘GenEx’ we identified that a combination of HRTPI and UBC as reference genes were most suitable for this application.

CONCLUSIONS: HRTPI and UBC are optimal reference genes for the evaluation of human cord blood eosinophilopoietic evaluations. These results will ensure accurate and optimal findings from future gene expression analyses involving IL-5 induced eosinophilopoiesis from umbilical cord blood samples.

Selective Glucocorticoid Receptor Modulator CpdA Overcomes GM-CSF-induced Resistance of Eosinophil to Glucocorticoid Treatment

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RATIONALE: The benefits of glucocorticoids (GC) for treatment of eosinophil-associated inflammatory diseases such as asthma are limited by their side effects and the development of GC resistance. Most effects of GC are mediated through the glucocorticoid receptor (GR), which enhances the transcription of GR responsive genes (transactivation) or suppresses proinflammatory transcription factors (transrepression). Since GC-responsive genes may induce side effects, novel selective compounds with limited transactivating activity have been developed to optimize anti-inflammatory benefits of GC treatment. In the present study we tested the effect of Compound A (CpdA), a dissociating GR ligand with selective trans-repression activity on eosinophil viability and activation.

METHODS: Calcein AM/ethidium homodimer-1 assay for cell viability and phosphorylation of GCR were tested on eosinophils isolated from peripheral blood of healthy donors. Eosinophils were stimulated with GM-CSF and treated with CpdA, Dexamethasone (Dex) or RU486 at three different concentrations for 72 h.

RESULTS: Both Dex and CpdA induced apoptosis in non-activated eosinophils. GM-CSF inhibited both constitutive and Dex-induced eosinophil apoptosis rendering eosinophils resistant to Dex treatment. On the other hand, CpdA potently inhibited GM-CSF-induced eosinophils survival. Inducible phosphorylation of GCR on Ser211 and p38 MAP kinase in response to Dex revealed further differences between the nonselective (Dex) and selective (CpdA) GR modulators.

CONCLUSIONS: We report here for the first time that selective GR modulator CpdA with limited transactivating properties inhibits viability of activated eosinophil. Our findings also suggest that the transactivating effect of Dex may sustain GM-CSF-induced prolongation of eosinophil survival providing possible mechanism of GC resistance in chronic asthma.

Failure Of Hepoxilin A-3 To Chemoattract Eosinophils In An in-vitro Gradient Barrier System

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RATIONALE: Hepoxilin A-3 (HXA3) is a nonclassic eicosanoid that is known to drive migration of neutrophils (PMNs) across the epithelial barrier in response to apical infection of lung epithelial monolayers with Pseudomonas aeruginosa (PA). Eosinophilic eosinophagitis is characterized by superficial eosinophilia and transcriptional upregulation of HXA-3 synthetic genes (phospholipase A2 and 12-lipoxygenase (12-LO) isoforms) has been reported from affected tissue. We hypothesized that HXA-3 might also play a role in eosinophil transepithelial migration.

METHODS: Eosinophils were obtained by density gradient and negative magnetic bead selection (StemCell). The PMN fraction served as control. The granulocytes were placed on one side of a lung epithelial monolayer transwell culture system. Control chemoattractant gradients were established in parallel with gradients of HXA-3. HXA-3 was from three sources: apical infection of H292 monolayers with PA; lipid extractions from conditioned supernatants of separate cultures of PA infected lung epithelial cells; or purified commercial HXA-3. Migration was quantified by peroxidase activity.

RESULTS: Eosinophils but not PMNs migrated in response to an eotaxin-3 gradient as expected. They also responded to fMLP as did PMNs, but were nonresponsive to IL-8. Eosinophils failed to respond to HXA-3 gradients, while PMNs responded as expected. Both eosinophils and PMNs migrated to gradients of LTBP4.

CONCLUSIONS: Human eosinophils under these in-vitro conditions failed to migrate in response to gradients of the 12-LO product HXA-3 suggesting that hepxolins do not play a direct role in eosinophil chemotaxis. The 5-LO product, LTBP4, did induce migration of both human eosinophils and PMNs.
**455** Membrane Cholesterol Removal from Human Eosinophils Disrupts Cholesterol Rich Membrane Microdomains Resulting in Down Regulated MAP Kinase Signaling but not JAK/STAT Signaling

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**RATIONALE:** Eosinophils contribute to allergic asthma exacerbation and can undergo excessive recruitment to the lungs where their activation leads to tissue damage and fibrosis. Interleukin-5 (IL-5) family cytokine receptors, which are critical for eosinophil recruitment/activation, are proposed to exist in membrane microdomains. Because cholesterol-rich microdomains are linked to signal regulation in diverse receptor systems, and because hypercholesterolemia is an asthma risk-factor, we tested the hypothesis that cholesterol-rich plasma membrane microdomains are central to IL-5-family cytokine action in eosinophils.

**METHODS:** Purified human blood eosinophils were incubated (1 hr) with the cholesterol-chelating agent methyl-β-cyclodextrin (MβCD) or soluble cholesterol (MβCD pre-loaded with cholesterol), followed by cholesterol/membrane microdomain analyses via flow cytometry and confocal microscopy or stimulation with IL-5. Eosinophil activation was determined via immunoblotting for activated p38 MAPK, activated transcriptional regulator STAT5, cyclin D3 (MAPK-dependent), or Pim1 (STAT-dependent).

**RESULTS:** MβCD decreases, and soluble cholesterol increases, membrane cholesterol content in a dose-dependent manner as assessed by flow cytometry. Likewise, confocal microscopy confirmed MβCD disrupts membrane microdomains. Furthermore, MβCD attenuates IL-5-induced p38 phosphorylation compared to control (p<0.001, N=10), whereas soluble cholesterol restores IL-5-induced p38 phosphorylation and significantly elevates basal phosphorylation (p<0.05, N=10). Cyclin D3 up-regulation is blocked by MβCD treatment but unaffected by soluble cholesterol addition (N=3). Neither MβCD nor soluble cholesterol appears to alter IL-5-induced STAT5 phosphorylation (N=3) or Pim1 up-regulation, suggesting a selective action of these agents (N=2).

**CONCLUSIONS:** These studies reveal that disturbances in eosinophil membrane cholesterol content selectively affect eosinophil signaling, suggesting that in vivo cholesterol levels may direct eosinophilic function and inflammatory capacity.

**456** Inhibition of Eosinophil Differentiation in vitro by a PPARγ Agonist

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**RATIONALE:** Peroxisome proliferator-activated receptor (PPAR) agonists have been observed to have an inhibitory effect on differentiation of several cell types, including erythroid cells. However, no study has examined the effects of PPAR agonists on IL-5 induced eosinophil differentiation.

**METHODS:** Peripheral blood was drawn from forty atopics donors. Non-adherent mononuclear cells (NAMNCs) or CD34+ cells were grown for 2 weeks in Methocult® cultures stimulated with 10ng/mL IL-5 plus/minus 25ng/mL IL-3, in the presence of 1-1000nM PPARγ agonist (GW9578), PPARβ/δ agonist (GW501516), PPARγ agonist (rosiglitazone) or diluent. The number of eosinophil/basophil colony forming units (Eo/Bo CFU) was quantified using an inverted microscope at 40x magnification. The data were analyzed using a repeated measures ANOVA.

**RESULTS:** The number of Eo/Bo CFU grown from NAMNC and CD34+ cells increased significantly in response to IL-5 and IL-5+IL-3 stimulation (p=0.0001 and p<0.0001 respectively). Incubation of NAMNC and CD34+ cells with 10-1000nM rosiglitazone significantly inhibited the number of IL-5-induced Eo/Bo CFU (p=0.0011 and p=0.0078 respectively) and the number of IL-5+IL-3-induced Eo/Bo CFU (p=0.0144) with no effect of GW9578 (P=0.3) or GW501516 (P=0.7).

**CONCLUSION:** We observed an inhibitory effect of rosiglitazone on eosinophil differentiation in vitro, suggesting a mechanism whereby PPARγ agonist can regulate the development of eosinophils.

**457** Mast Cell Phenotypes and Distribution in Nasal Polyps with Allergic Airway

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**RATIONALE:** Nasal polyps (NP's) is a multi-factorial disease, however, Four histological types were described; edematous, fibro-inflammatory, glandular and that with atypical stroma. We studied mast cell (MC) phenotypes and distribution in NP's of allergic patients.

**METHODS:** Based on history, clinical examination, Para nasal C-T, high serum IgE and +ve sIgE Ala top screening test, 31 allergic adults with NP's who underwent polypectomy were included. Paraffin sections of specimens from NPs and nasal turbinate mucosa were subjected to: staining with H&E,PAS and Masson trichrome to classify polyps and Trypstat and chymase immunohistochemical staining for MC's phenotypes.

**RESULTS:** NP's were reclassified into: Edematous, fibrotic, MC tryptase (MCþ) dominated the glandular type (11.33 ± 0.95) while the tryptase - Chymase (MC−) dominated the fibrotic (12.96 ± 11.06) Phenotype distribution showed dominant MCþ at the stroma and at sub epithelium of glandular (11.33 ± 0.95) and mixed polyps (4.38 ± 3.28) respectively. While, MC− dominated the stroma and sub epithelium of fibrotic(12.96 ± 11.06) and edematous polyps (4.17 ± 3.53) respectively. No MCs subset were found in epithelium of NP's except the fibrotic type((MCþ) (1.06±0.55). MC− concentration expressed similarity in both; the polyps and nasal mucosa. While MCþ are preferentially increased in the edematous (2.54 ± 1.31) and fibrotic polyps (12.96 ± 11.06) than mucosa.

**CONCLUSIONS:** Phenotypes and distribution of MCs suggests that allergy is one determining factor in NP's formation. MCþ is responsible for glandular hyperplasia and MC− for polyp fibrosis.

**458** A Role for Mast Cell Chymase in Regulating Levels of Immunoglobulin E

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**RATIONALE:** The His45Arg single nucleotide polymorphism (SNP) in the gene for mast cell chymase (CMA1) is associated with atopy and serum IgE levels in asthmatics. We have hypothesized that chymase is involved in IgE production, and that this SNP encodes an inactive form of the enzyme.

**METHODS:** The cDNA for pre-pro-chymase was ligated into the baculovirus transfer vector BacPAK8 and transfected into SP9 cells. Pro-chymase was purified from supernatants by sequential application of heparin agarose and 5-200 Sephacryl chromatography and subjected to N-terminal sequencing, SDS-PAGE and immunoblotting with chymase-specific antibodies. The recombinant pro-enzyme was activated by dipetidyl peptidase I. The ability to cleave of sac-AAF-pNA and RETF-4NA was measured spectrophotometrically, and hydrolysis of angiotensin I, but the variant was without any activity. Incubation of B cells with wild-type chymase (0.25-10ng/ml) stimulated an increase in supernatant IgE concentrations of more than five-fold after 5 days. However, at high concentrations of chymase (100-1000ng/ml) there was a reduction in IgE levels as a consequence of IgE cleavage. The variant form of chymase or the heat-inactivated wild-type failed to provoke increased IgE secretion or cleavage of this immunoglobulin.

**CONCLUSIONS:** Chymase may play key roles in the regulation of IgE levels. This may explain why expression of the inactive variant can offer protection from atopy in asthmatics.
Role of the Aryl-hydrocarbon Receptor (AhR)-ligand Axis in Mast Cells

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RATIONALE: The significance of AhR signaling in immune regulation has been suggested, but its role in mast cells remains unknown. We hypothesized that AhR and its ligands may affect mast cells function and play an important role in the regulation of allergic diseases.

METHODS: Mouse bone marrow-derived mast cells (BMMCs) were treated with several known AhR ligands including TCDD and FICZ. The levels of cytokines and mediators release in activated mast cells by IgE/Ag treated with AhR ligands could be reversed by the addition of an antioxidant, N-acetylcysteine, and calcium channel blockers. In addition, when BMMCs from AhR-null mice were analyzed, significant reductions in the numbers of BMMCs and in the levels of IL-3-induced proliferation were noted, as compared to those seen in wild-type mice. Mast cell deficiency was confirmed in various tissues and peritoneal cavity of AhR-null mice.

RESULTS: Exposure of BMMCs to low-doses (< 10 nM) of AhR ligands significantly increased the levels of IL-13, degranulation, LTC4, and PCA response. Mechanistically, exposure of the cells to AhR ligands resulted in significantly increased levels of intracellular calcium and ROS, concomitant with enhanced activation of several signaling pathways involving ERK and PKC. Notably, the functional effects of AhR ligands could be reversed by the addition of an antioxidant, N-acetylcysteine, and calcium channel blockers. In addition, when BMMCs from AhR-null mice were analyzed, significant reductions in the numbers of BMMCs and in the levels of IL-3-induced proliferation were noted, as compared to those seen in wild-type mice. Mast cell deficiency was confirmed in various tissues and peritoneal cavity of AhR-null mice.

CONCLUSIONS: These results thus provide evidence supporting critical roles of AhR signaling in maintaining the homeostasis, maturation and optimal activation of mast cells.

Abnormal Mast Cell Migration in the Wv/+ Piebald Mouse


RATIONALE: KIT, the receptor for stem cell factor, plays a critical role in mast cell biology. It helps regulate mast cell proliferation, differentiation, and survival. In mastocytosis, one KIT allele expresses a mutation that increases function; and the result is increased proliferation and activation. In contrast, disruption of the activity of both alleles results in mast cell deficiency as observed in W/Wv mice. Less is understood concerning the consequences of the presence of one normal allele and one allele which has lost some functional activity.

METHODS: To explore the consequences of such a situation on mast cell behavior, we cultured bone marrow derived mast cells from the Wv/+ (mutation at Y568) mouse and examined mast cell proliferation to IL3, mediator release, and chemotaxis. In addition we checked both passive systemic and passive cutaneous anaphylaxis in our mouse model to determine if tissue mast cell burden was affected.

RESULTS: On a cellular basis, only chemotaxis was abnormal. There was no difference in mast cell degranulation by IgE/Ag cross-linkage. Initial studies on signal transduction indicate that signaling pathways that do not involve Src family kinases are decreased. We next examined mast cell responses in vivo. In the Wv/+ mouse, there was less response to passive cutaneous anaphylaxis, there was no difference in passive systemic anaphylaxis as measured though temperature decrease.

CONCLUSIONS: Thus, Wv/+ mouse mast cells with one deficient KIT allele exhibit a defect in chemotaxis, suggesting disturbances in pigmentation may not be due only to disordered melanocyte migration.

A Potential IL-33 Autocrine Loop in Mast Cells

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RATIONALE: Interleukin-33 (IL-33) appears to play a crucial role in the pathophysiology of allergic diseases, but its cell source and the regulatory mechanisms remain to be fully elucidated. Mast cells, one of the major effector cell populations, express high levels of IL-33 receptor, ST2, and have been shown to express IL-33 transcripts. In this study, we aimed to examine the secretion of IL-33 in mast cells and their response to IL-33.

METHODS: The level of the IL-33 gene expression was analyzed by real time PCR. The expression of ST2, cytokolic IL-33 protein and its levels of secretion in the supernatants from mouse bone marrow derived mast cells (BMMCs) as a model were measured by the use of Flow cytometry, Western blotting and cell-based ELISA, respectively. BMMCs were treated with rm IL-33, and the resulting production levels of IL-6 and IL-13 were examined by ELISA.

RESULTS: Exposure of BMMCs to IL-33 significantly increased the levels of IL-13 and IL-6 expression, concomitant with enhanced activation of ERK. Activation of BMMCs by cross-linkage of an antigen (ovalbumin, OVA) and OVA-specific IgE MAbs significantly induced the expression of IL-33 gene expression, cytokolic and secreted IL-33.

CONCLUSIONS: These results suggest that mouse BMMCs are capable of producing IL-33, and that IL-33 plays an important role in regulating mast cell functions.

Effects of the KIT K509I Extracellular Activating Mutation on Human Mast Cell Homeostasis

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RATIONALE: KIT is a tyrosine kinase receptor which binds stem cell factor (SCF) and is critical to human mast cell (HuMC) development and survival. Recently we identified a patient with systemic mastocytosis harboring a rare heterozygous germline KIT K509I mutation. K509I is located in the 5th immunoglobulin region and such mutations are thought to enhance KIT homodimerization and activation. With this in mind, the effect of the K509I mutation on HuMC development was investigated.

METHODS: Primary HuMC’s were derived from CD34+ peripheral blood progenitors collected by apheresis. Cells were cultured in IL-3 (week 1) and IL-6 +/- SCF for 8 weeks. HuMC growth and survival was analyzed by MTT and Annexin V apoptosis assays. HuMC morphology was assessed by electron and light microscopy. Multiparameter FACS analysis, β-hexosaminidase release and total cell histamine assays were performed.

RESULTS: K509I progenitors cultured in SCF demonstrated a ten-fold expansion compared to progenitors from healthy controls; and developed into morphologically appearing mature HuMCs. K509I HuMC expansion continued for up to 20 weeks, exceeding normal survival (10-12 weeks); and K509I HuMCs displayed decreased apoptosis upon SCF withdrawal compared to normal HuMCs. A two-fold increase in FceRI surface expression, cell histamine content and antigen mediated degranulation was observed in K509I HuMCs. K509I progenitors cultured in the absence of SCF also survived and developed into hypogranular appearing HuMCs.

CONCLUSIONS: KIT K509I progenitors develop into mature appearing HuMCs with enhanced proliferation, survival and activation. K509I progenitors provide a unique opportunity to study the impact of a KIT germline mutation on HuMC homeostasis.
**463** Functional Differences in Bronchoalveolar Lavage (BAL) Mast Cells (MCs) in Severe and Mild Asthma

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**RATIONALE:** BAL mast cells (MCs) respond to anti-human IgE by degranulating and generating eicosanoids. (Flint, 1985; Pearce, 1987) We recently reported increased epithelial and luminal MCs in severe compared to milder asthma. However, little is understood regarding their function. We hypothesized that severe asthma (SA) MCs would generate greater tryptase than mild asthma (MA) MCs in response to anti-IgE.

**METHODS:** BAL cells from SA (n = 11) and MA (n = 7) from the Severe Asthma Research Program were suspended in media, then left unstimulated or stimulated with anti-human IgE for 20 minutes (T1) and 18 hours (T2). Supernatant tryptase was measured by enzyme immunoassay and cellular tryptase mRNA measured by qPCR. Due to distribution, data was analyzed nonparametrically.

**RESULTS:** Basal [1.4(0.1-3.2) vs. 2.2(0.3-4.2) ng/mL] and anti-IgE stimulated [2.8(2.3-4.7) vs. 7.6(1.2-19.5) ng/mL] tryptase did not differ between MA and SA at T1 despite higher baseline tryptase mRNA in SA [2.3(0.4-3.3) vs. 8.2(3.0-8.2) ng/mL, p = 0.03]. By 18 hours, there was a tendency for higher tryptase protein from unstimulated [0.5(0.1-3.6) vs. 4.6(0.4-7.5) ng/mL, p = 0.1] and stimulated cells [3.5(0.8-4.4) vs. 10.2(1.3-20.6) ng/mL, p = 0.1] in SA. Unstimulated tryptase protein increased from T1 to T2 in SA (39% vs. no change in MA, p = 0.048). While tryptase protein increased following anti-IgE at both time points, the percent increase was not different between SA and MA.

**CONCLUSIONS:** Although tryptase mRNA levels support more luminal MCs in SA, spontaneous and anti-IgE stimulated release of tryptase protein did not differ between groups at 20 minutes. However, SA MCs spontaneously released more tryptase at 18 hours, suggesting a higher and more sustained basal activity.

**464** Mast Cell Tryptase as a Stimulus for Upregulation of Adhesion Molecule Expression and Cytokine Release from Endothelial Cells

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**RATIONALE:** Mast cell tryptase can be released in substantial quantities at sites of allergic inflammation. Pro-inflammatory actions mediated through protease activated receptor 2 (PAR-2) have been proposed, but the precise contribution of tryptase to disease processes remains unclear. We have investigated alterations in the whole genome expression profile of endothelial cells in response to tryptase.

**METHODS:** Human umbilical vein endothelial cells (HUVECs) were isolated from umbilical vein tissue and grown to confluence. Following addition of tryptase or other agents, quantitative polymerase chain reactions (qPCR) followed by whole genome microarray analysis were performed. Translation of certain genes was investigated by immunocytochemistry or specific ELISA. In parallel studies, calcium flux was measured using a fluorescence based microplate procedure.

**RESULTS:** Microarray analysis indicated that the genes whose expression was most up-regulated following tryptase treatment of endothelial cells were those for the adhesion molecules ICAM-1, VCAM-1, EPCAM and ITGAL, and the cytokines IL-2, IL-3, IL-6 and CXCL10. Increased expression was seen also for genes for the cell signalling molecules TNP1P3, TLL1 and SMAD2. None of these were up-regulated in response to a peptide agonist (SLIGKV-NH2) of PAR-2. Microarray data was confirmed by separate qPCR experiments, immunocytochemistry and by the measurement of cytokines in cell supernatants. The actions of tryptase were inhibited using selective protease inhibitors indicating a requirement for an intact catalytic site. Addition of tryptase to endothelial cells stimulated concentration-dependent increases in intracellular calcium.

**CONCLUSIONS:** The pro-inflammatory actions of tryptase may be mediated through increased expression of adhesion molecules and production of inflammatory cytokines in endothelial cells.

**465** Effects Of Rupatadine On Platelet Activating Factor (PAF)-induced Human Mast Cell Degranulation Compared With Desloratadine And Levocetirizine

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**RATIONALE:** Platelet activating factor (PAF) is a lipid mediator that appears to be involved in the amplification of mast cell (MC) activation in anaphylaxis. Rupatadine is an antihistamine with demonstrated anti-PAF effect but its capacity to inhibit PAF-induced MC degranulation has not already been evaluated. Our objective was to investigate the ability of rupatadine to inhibit PAF-induced MC degranulation compared with desloratadine and levocetirizine to confirm that rupatadine effects are mediated only by anti-PAF activity.

**METHODS:** The human MC line LAD-2 was used. MC mediators release was evaluated by β-hexosaminidase release assay. A PAF dose-response curve was performed to define the optimal dose to induce mediators release without MC mortality. Rupatadine (H1 antagonist + PAF receptor antagonist) 5, 10 and 25 μM were compared with desloratadine (H1 antagonist) 1 and 10 μM and levocetirizine (H1 antagonist) 1, 10, 100 μM.

**RESULTS:** PAF’s optimal dose for MC degranulation was 10 μM. Rupatadine 5, 10 and 25 μM inhibited degranulation 40%, 53% and 46% respectively (p<0.01). Levocetirizine 1 μM and desloratadine 1 μM showed only 3% and 3.5% inhibition respectively (without statistical significance). Levocetirizine 10, 100 μM and desloratadine 10 μM did not show any inhibitory effects.

**CONCLUSIONS:** We report for the first time that rupatadine inhibits PAF-induced MC degranulation in vitro, confirming its anti-PAF activity. In contrast, neither levocetirizine nor desloratadine show any anti-PAF effect. These results suggest that rupatadine could be more effective than other antihistamine drugs in anaphylaxis treatment.
466 Obesity is Not Linked with Increased Whole-Body Mast Cell Burden in Children
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RATIONALE: Recent studies demonstrated a role for mast cells in the development of obesity in mice and suggested similar roles for mast cells in human obesity. We hypothesized that despite evidence of increased numbers of mast cells in white adipose tissue from obese individuals, obesity is not associated with an increased serum tryptase level.
METHODS: Serum samples and obesity-related clinical data were collected from 184 children with and without obesity. Total tryptase levels were measured from serum samples. Patients were grouped based on BMI and BMI percentile (with and without impaired glucose tolerance), and tryptase levels were compared between groups. Multiple linear regression analysis was performed to determine the relationship between multiple clinical parameters and serum tryptase.
RESULTS: No statistically significant differences were found in serum tryptase levels between lean, overweight, and obese individuals, as defined by BMI percentile (P = 0.068) or BMI alone (P = 0.449). Examining only patients with normal glucose tolerance (NGT), no statistical difference was found between groups defined by BMI percentile (P = 0.138) or by BMI alone (P = 0.386). Multiple linear regression analysis showed no association between multiple obesity-related parameters and serum tryptase.
CONCLUSIONS: Children with increased body fat do not have increased serum tryptase levels, contrary to the implications made in recent studies. While mast cells may be involved in the development of obesity in mice, additional studies are needed to establish this link in humans.

467 Human Basophil Activation Is Associated With Expression Of Co-stimulatory Molecules And Ige Dependent Antigen Capture
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RATIONALE: In addition to their role as allergic effector cells, human basophils may act to immunomodulate secondary immune responses. Our objective was to demonstrate the capacity of human basophils to upregulate immunomodulatory signals and to capture antigen in an IgE-specific fashion.
METHODS: Basophils in human peripheral blood mononuclear cells were sensitized with anti-Derp2 IgE and stimulated with recombinant Derp2 for detection of co-stimulatory molecules. IgE-specific antigen capture was assessed after anti-NP IgE sensitization and stimulation with NP-BSA-conjugated fluorescent beads. Assessment of basophil co-stimulatory molecules and antigen capture was performed using flow cytometry.
RESULTS: The frequency of IL-4 positive basophils increased 2.3 fold at maximal antigen stimulation compared to unstimulated basophils (median 10.7% vs. 26.0%, p = 0.002). CD40L positive basophils increased 12.4 fold at maximal antigen stimulation from medium (median 0.14% vs. 2.56%, p = 0.05). HLA-DR positive basophils increased 1.5 fold at maximal antigen stimulation from medium (median 3.61% vs. 5.35%, p = 0.02). Surface FcεRI expression decreased 1.4 fold in CD63hi versus CD63dim basophils (53000 vs 73700 FcεRI receptors, p = 0.005). Capture of antigen-coated beads was IgE-dependent and significantly higher in activated basophils than non-activated basophils (83.3% vs. 16.7%, p = 0.04).
CONCLUSIONS: Allergen-stimulated human basophils can upregulate CD63, IL-4, CD40L, and HLA-DR expression. Upregulation of CD63 in basophils is associated with decreased surface FcεRI expression and increased antigen capture, suggesting IgE-mediated antigen capture. Hence, human basophils may function as allergen-driven immunomodulators in secondary allergic immune responses.

468 Antigen-induced Anergy In Human Basophils Is Not Antigen Specific
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RATIONALE: Allergen-specific immunotherapy induces suppression of basophil activation through an IgE-mediated pathway. Using an in vitro model of basophil anergy, we sought to investigate whether allergen-induced basophil anergy is non-antigen specific.
METHODS: Human peripheral blood mononuclear cells isolated from blood donors (n=5) were sensitized with anti-Derp2 IgE and anti-NP IgE, and were anergized in calcium-free media with either recombinant Derp2 (rDerp2) or NP-BSA. A second stimulation with either Derp2 or NP-BSA in calcium-present media was used to assess anergy. Markers of basophil activation, CD63 and CD203c, were measured using flow cytometry.
RESULTS: Antigen specific suppression of basophil activation was seen in anergized versus non-anergized cells, with rDerp2 (9% vs. 37% CD63hi basophils, p=0.003) and NP-BSA (3% vs. 15% CD63hi basophils, p=0.002). NP-BSA-induced basophil activation in rDerp2 anergized basophils was suppressed in comparison to baseline NP-BSA stimulation (7% vs. 15% CD63hi basophils, p=0.02). rDerp2-induced basophil activation in NP-BSA anergized basophils was suppressed in comparison to baseline rDerp2 stimulation (15% vs. 37%, p=0.02). CD203c followed a similar pattern to CD63 expression.
CONCLUSION: Allergen-induced basophil anergy can cross-suppress basophil activation to a second antigen in vitro. The cross-modulation of IgE-mediated allergen activation suggests that allergen-specific immunotherapy could exert a therapeutic non-specific effect on other IgE-mediated allergic disease through suppression of basophil activation.

469 Anti-CD40 antibody or 8-oxo-dG reduces Migratin or Inactivation of Mast Cells via Chemokines and Fospx+ Regulatory T Cells In Experimental Allergic Encephalomyelitis
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Mast cells contribute to the pathogenesis in multiple sclerosis (MS). Regulatory T (Treg) cells regulate autoimmune immune responses. However, the relationship between mast cells and Treg in MS has not been elucidated yet. This aimed to investigate how anti-CD40 Ab or 8-oxo-dG reduces mast cell population in and attenuates the development of experimental autoimmune encephalomyelitis (EAE). C57BL/6 mice received i.c. injection of myelin oligodendrocyte glycoprotein peptide (MOG35-55) and pertussis toxin. One day after pertussis toxin injection, anti-CD40 Ab, 8-oxo-dG or both was injected i.p. for 5 days. Mast cells were stained by May-Grünwald-Giemsa. Treg cells were isolated from brain cells by positive selection kit, mast cell surface markers and Treg, Fospx3, CCL2/CCR2, and Act1 by western blot, mRNA expressions of Fospx3 and cytokines by RT-PCR, co-localization of mast cells and Treg cells by immunofluorescence. Anti-CD40 Ab or 8-oxo-dG reduced EAE score, population of mast cells, expressions of OX40L and Act1, levels of TNF-α, LTs and cAMP, inflammatory cytokines, all of which are increased in EAE brain tissues. Anti-CD40 Ab or 8-oxo-dG enhanced Fospx3+Treg cell population, Act1 and cAMP levels, Treg cells-related cytokines (IL-10, TGF-β, IL-2, IL-35 and IL17), and adjacent localization of mast cells and Treg cells, all of which are increased in brain tissues or Treg of EAE model.
The data suggest that anti-CD40 Ab or 8-oxo-dG reduces mast cell migration into brain tissues through CCL2/CCR2 axis, and Treg cells induced with each treatment also counteract the activation of mast cells via OX40/OX40L in the development of EAE.
**470 IgE Expression on Murine Lung Conventional Dendritic Cells During a Paramyxoviral Infection**

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**RATIONALE:** We have previously documented that respiratory infection with Sendai virus (SeV) drives expression of the high-affinity IgE receptor, FcεRI on murine lung conventional dendritic cells (cDC). FcεRI is expressed by day 3 post-inoculation (p.i.) and is required for development of post-viral atopic disease. Interestingly, anti-viral specific IgE appears between day 5 and 7 p.i.; which is after FcεRI expression. We undertook this study to determine the relationship between receptor and IgE in this model.

**METHODS:** C57BL6 mice were inoculated intranasally with 2 x 10^7 pfu SeV. At various time points p.i. mice were euthanized, lungs removed, and single cell suspensions made. Using flow cytometry, IgE on CD11c+ cDC was then determined and compared to FcεRI expression.

**RESULTS:** No IgE was detected on naïve mouse cDC (0.97 +/- 0.15 fold MFI; n=2). IgE was not bound on day 3 or 5 PI (1.2 +/- 0.12 and 1.1 +/- 0.15, respectively; n=3). IgE was, however, detected by day 7 (1.5 +/- 0.08; n=3, p<0.05) and remained through at least day 10 (1.5; n=1). This demonstrates a delay, as FcεRI is expressed by day 3 p.i. and remains expressed past day 10 p.i., but does parallel serum anti-SeV IgE levels.

**CONCLUSIONS:** IgE binds FcεRI on murine lung cDC between day 5 and 7 p.i. This expression parallels the SeV specific IgE levels in the serum, and is delayed compared with expression of FcεRI on cDC. Further studies are necessary to assess the functional significance of anti-viral IgE on lung cDC.

**471 Incidence of Clinical Cross-Reactivity Between Shrimp, Dust Mite, and Cockroach Allergy at an Inner City University-Based Outpatient Setting**

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**RATIONALE:** Shrimp is one of the most common food allergies seen in the United States. The protein that commonly causes shrimp allergy, tropomyosin, is also found in dust mite (DM) and cockroach. We investigated the prevalence of shrimp with concurrent DM or cockroach allergy to determine the clinical cross reactivity to that seen in vitro and in vivo with skin tests.

**METHODS:** A retrospective chart review of patients 5 to 80 years old (n=1043) who receive outpatient care at our allergy clinic was performed (January 1995-April 2011). Data was collected on shrimp, DM, and cockroach sensitization based on IgE prick skin (Dermapik®) or in vitro (immunoCAP®) testing and presence of asthma, allergic rhinitis (AR), and atopic dermatitis (AD). Odds ratios were determined to assess the likelihood of concurrence and 2-tailed Fisher’s exact test.

**RESULTS:** 62(6%) of patients reported a clinical shrimp allergy. Of these patients, 28(45.9%) had shrimp allergy confirmed by skin or in-vitro testing. Patients with reported shrimp allergy had a 12.5 fold risk of being sensitized to cockroach (p=0.0003,CI 2.98-52.3) and 4.3 fold risk of being sensitized to DM (p=0.03,CI 1.18-15.4). Of the reported shrimp allergic subjects, 47(77%) had AR, 22(36.1%) had asthma, and 12(19.7%) had AD.

**CONCLUSION:** Shrimp allergic subjects have a high concurrence of sensitization to cockroach and DM, which in turn contribute to allergic disease.

**472 The Effect of Epigallocatechin Gallate on IgE Levels in HIV-1-infected Subjects**

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**RATIONALE:** HIV-1-infected patients have increased IgE levels and a higher prevalence of atopy. Epigallocatechin gallate (EGCG), green tea catechin, has many properties including antiviral effects, shown previously in our laboratory to inhibit HIV-1 gp120 binding to CD4. Recent studies demonstrated anti-allergic effects of EGCG by suppression of IgE responses in vitro, however, no studies have been performed in vivo.

**METHODS:** HIV-1-infected individuals 18-65 years of age, not on antiretroviral therapy with CD4 counts > 250 cells/mm³ were recruited for a randomized, double-blind, placebo-controlled, phase I study. Subjects were given Polyphenon® E containing EGCG 800 mg twice daily or placebo for 14 days. Plasma IgE and IL-4 were measured on days 1, 8, 14 and 21 by ELISA (GenWay; sensitivity range 3.125-200 ng/mL; Biologic, respectively).

**RESULTS:** HIV-1-infected subjects (n=5; 24 total to be enrolled) were found to have a baseline IgE level of 23-2066 ng/mL (mean 456.9 ng/mL). Interim results revealed that IgE levels decreased on days 8, 14, and 21 by an average of 10.1%, 3.3%, and 10.6%, respectively, with the greatest decrease observed in subject 32 at 29.1%, 13.6%, and 20.4%, respectively (blinded treatment code maintained). IL-4 levels were not measurable.

**CONCLUSIONS:** Suppressed IgE levels in HIV-1-infected subjects were sustained 7 days after cessation of treatment in an EGCG versus placebo study. Furthermore, upon unblinding it may be possible to determine if EGCG may be a potential alternative therapy for allergic diseases in addition to its antiviral effects.

**473 Determination of Mast Cell/Basophil Inhibitory Activities of Traditional Chinese Herbal Medicines**

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**RATIONALE:** IgE-stimulated mast cells degranulate and release several potent mediators in response to specific antigen exposure, and are key effector cells in food allergy. Traditional Chinese medicines have showed potent potential for treating allergies. The aim of this study was to identify TCM herbal medicines which inhibit mast cells degranulation.

**METHODS:** Dried water extracts of 296 Chinese herbal medicines (CHM), previously reported to have anti-inflammation and allergy effects, were labeled with code numbers. These CHM were dissolved into PBS before in vitro experiment. Effects of 100 μg/mL and 500 μg/mL of each extract on RBL-2H3 cells were determined. The cells were sensitized with anti-DNP IgE and challenged with DNP-BSA. β-hexosaminidase released into the culture media was measured. Potential toxicity was analyzed by MTT assays. The 3 most effective herbs on β-hexosaminidase release were also tested on human basophile activation using peripheral blood samples from patients with food allergy by flow cytometry.

**RESULTS:** Among 296 herbal extracts tested 43 inhibited the release of β-hexosaminidase by more than 50% and 16 inhibited more than 80% at 500 μg/mL. 18 CHMs inhibited more than 50% at 100 μg/mL, 3 of which by more than 80%. These 3 CMHs (code 0173, 0070 and 0249) also inhibited human basophil activation by 50-100% in vitro to peanut stimulation.

**CONCLUSIONS:** This study found several CMHs that significantly inhibited mast cell β-hexosaminidase release in vitro and peripheral blood basophil activation in a non-toxic manner. Further investigation of these herbal medicines might lead to the development of novel mast cell/basophil inhibitors for allergic treatment.
Maternal IgE Levels Are Associated With IgE Levels At Ages 10 And 18 In Girls But Not In Boys, Isle Of Wight Birth Cohort A. Sadeghnajad 1, W. Karmous 2, S. H. Arshad 3; 1Department of Medicine, Capital Health System, Trenton, NJ, 2University of South Carolina, Columbia, SC, 3The Asthma and Allergy Centre, Isle of Wight and University of Southampton, UNITED KINGDOM.

RATIONALE: We have previously shown that parental history of asthma exerts a higher risk on asthma in the same gender offspring, “parent of origin effect”. To investigate this concept, IgE was utilized as an objective measure of atopy. We conducted gender-specific analyses for the relationships between maternal IgE and IgE levels at ages 10 and 18.

METHODS: Of 1456 subjects in this birth cohort, maternal IgE was available for 1057 (73%) at 10 years and for 560 (38%) at 18 years. To evaluate the associations between maternal and adolescence IgE we used linear and logistic regressions. IgE levels were either logtransformed (linear regression) or dichotomized (logistic regression). We adjusted the effect of maternal IgE for pet exposure, smoking and birth weight. Raised IgE levels were defined as levels above median.

RESULTS: In linear regressions, maternal IgE was associated with IgE levels at ages 10 (p = 0.0005) and 18 (p = 0.02) in girls but not in boys. In logistic regressions, the odds ratios (OR) for the association of raised maternal IgE levels on raised IgE levels at ages 10 and 18 were larger in girls (OR = 2.07, p = 0.001 and OR = 1.72, p = 0.07) compared to boys (OR = 1.93, p = 0.004 and OR = 1.04, p = 0.90).

CONCLUSIONS: This is the first study investigating the association of maternal IgE and adolescence IgE. Maternal serum IgE seems to have stronger relationship with IgE levels at ages 10 and 18 years in girls compared to boys. These findings suggest that there is a gender-specific imprinting of the maternal atopy status.

An Unusual Case of Mast Cell Activation Syndrome R. Patel 1, J. Celestin 1, M. Friel 1, 1 Nassau University Medical Center, East Meadow, NY.

RATIONALE: Mast Cell Activation Syndrome (MCAS) can be difficult to diagnose. This is due in part to common multiple organ involvement and overlap with other disorders. Most recently, criteria to diagnose this entity have been published. It can be primary, secondary due to chronic autoimmune urticaria (CAU), or idiopathic.

CASE PRESENTATION: We describe a 74 year old Caucasian male who is being treated for Neuroendocrine Antral Carcinoid since 2008, with Sandostatin. Since 2003, he has been having intermittent episodes of urticaria, sometimes associated with flushing and diarrhea. His current medications are H1 and H2 blockers, ketotifen, and opioids for spinal dermatitis, asthma, and allergic rhinitis. Both required frequent oral corticosteroids and had heights less than the third percentile.

RESULTS: Despite markedly elevated IgE levels, both were treated with omalizumab and within 4 months following initiation of treatment had rapid increases in linear growth. Patient A is now at the fifth percentile for age and patient B is at the fifteenth percentile for height.

CONCLUSION: Omalizumab is thought to decrease the release of mast cell mediators, which may reduce the amount of PAF released, enhancing PGE2 production and allow accelerated linear skeletal growth to occur, as was observed. While decreased therapeutic need for oral glucocorticoids and/or serendipitous growth spurts in these patients cannot be ruled out, the rapid growth observed pursuant to omalizumab therapy suggests that clinical trials of omalizumab in severely atopic children with short stature are indicated.
478 Prolonged Exposure to Sublingual Immunotherapy Improves Safety of Oral Immunotherapy

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RATIONALE: We recently compared sublingual (SLIT) to oral immunotherapy (OIT) following a short SLIT escalation for treatment of cow’s milk (CM)-allergy and found that while SLIT was safer than OIT, it was less efficacious. This analysis sought to determine if a more prolonged period on SLIT could improve safety of subsequent OIT.

METHODS: 30 children with IgE-mediated-CM-allergy were randomized to either SLIT (goal 7mg daily, N=10) or 4 weekly SLIT escalations to a dose of 3.7mg followed by OIT (goal 1000 or 2000mg daily, N=20). After 60 weeks of maintenance, SLIT subjects who reacted to less than 4gm CM-protein on food challenge crossed-over to OIT. Dose escalation started at less than ¼ the tolerated food challenge dose and escalated to 2000mg daily for one year. The rates of adverse events across dosing regimens were compared using negative binomial analysis with generalized estimating equations.

RESULTS: 8 SLIT subjects crossed over to OIT. Symptoms occurred with 24.4% of 2251 doses (oral 23.1%, skin 0.84%, GI 0.89%, lower respiratory 0.27% and upper respiratory 0.13%). One subject withdrew due to persistent GI symptoms. Antihistamines and inhaled beta-agonists were given for 1.3% and 0.04% of doses. Although the overall rates of reactions with OIT following brief versus prolonged SLIT were similar (p=0.976), lower and upper respiratory reactions were significantly less common (p=0.02 and p=0.006, respectively) and antihistamines and inhaled beta-agonists used less frequently (p=0.002 and p=0.001, respectively) in the prolonged SLIT group.

CONCLUSION: Prolonged SLIT before OIT dosing appeared to improve safety, but did not eliminate all symptoms.

479 Open Clinic-Based Peanut Food Challenges are a Viable and Safe Method of Evaluating Clinical Sensitivity and Tolerance

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RATIONALE: Oral food challenges (OFC) help determine presence of food allergy or tolerance. Indications for peanut (OFC) are not standardized. Decision to challenge is based on history, percutaneous skin testing (PST) and serum specific IgE (sIgE). We compared published OFC criteria for sIgE levels and PST wheal size to those used in a tertiary academic allergy practice. Prior published data recommend sIgE or PST which is <3.5 kUA/L or PST size <8mm.

METHODS: Retrospective chart review was performed for 26 peanut OFC (23m - 74 yrs, 46% male) performed January 2011-July 2011. sIgE and PST wheal size in our study population were compared to published values.

RESULTS: 25 patients met criteria for OFC and one exceeded both challenge criteria. 15 had pre-OFC sIgE testing levels below 0.88kAU/L, representing <25% of 3.5kAU/L. 3 of 20 with PST had wheal size ≥4mm (range 0-40mm). The patient not meeting criteria, and 4 patients who met criteria failed OFC with development of urticaria and puritis. All subjects who met criteria had a history of no accidental exposures; urticaria on first exposure; and exceeded either sIgE or PST for peanut OFC.

CONCLUSION: Peanut OFC can safely be preformed by following published guidelines. In our experience, patients who met criteria for peanut OFC but failed challenges had urticaria on first exposure, were without a history of accidental exposure, and exceeded one criteria for OFC.
**482 Body Mass and Corticosteroid Response in Childhood Asthma**


**RATIONALE:** To determine if body mass index (BMI) affects the corticosteroid (CS) response in children with asthma, we studied PBMC and bronchoalveolar lavage (BAL) cells from asthmatic children who underwent clinically indicated bronchoscopies.  

**METHODS:** Children were characterized by spirometry (age ≥ 5 y), BMI percentile (%) to adjust for age, plasma leptin (ELISA), daily inhaled CS dose (mcg budesonide equivalent [BE]), and in *vitro* response to CS. Mitogen-activated protein kinase phosphatase-1 (MKP-1) induction by 10^(-6) M dexamethasone (DEX) was evaluated by PCR in PBMC and BAL cells. Pearson correlation coefficients (r) were calculated.  

**RESULTS:** Sixty-one children with asthma (median [range] FEV1 80%-predicted [52-121%], FEV1/FVC 80% [55-99%], FEV1 change by bronchodilator 8% [-11-47%]), age 2 to 18 y (median 1 y) were studied. Fifty-six children used ICS. Median ICS dose was 1100 mcg BE (range 0 - 4500 mcg). BMI% correlated with plasma leptin (r=0.5, p=0.0001), daily ICS dose (r=0.37; p=0.0035), and DEX-induced MKP-1 fold induction in PBMC (r=-0.45, p=0.0009) and BAL cells (r=-0.42, p=0.0205). MKP-1 induction correlated between PBMC and BAL cells (r=0.46; p=0.0176). A negative correlation was found between MKP-1 induction and daily ICS requirement (r=-0.396, p=0.0303).  

**CONCLUSIONS:** Children with asthma demonstrate a decreased *in vitro* CS response with increasing BMI% that correlated with a higher daily ICS requirement. Further studies are needed to determine if obese asthmatics will have enhanced CS responses after their BMI is normalized.

**483 Steroid Requirements and Immune Associations With Vitamin D Are Stronger In Children Than Adults With Asthma**

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**RATIONALE:** The associations of serum vitamin D status on atopy, steroid requirement and functional responsiveness to corticosteroids in children vs. adults with asthma have not been studied systematically.  

**METHODS:** Serum vitamin D levels were examined in a prospective study of adults and children with asthma (n=103). Peripheral blood mononuclear cells (PBMC) were cultured for 3h +/-100nM dexamethasone (DEX), expression of corticosteroid-regulated genes was detected by real time PCR. Serum IgE levels were measured; information about asthmatics’ steroid requirement was collected.  

**RESULTS:** 47.6% of asthmatics had deficient serum vitamin D levels (<20ng/ml) with meanSD of 20.79±8.9ng/ml. In multivariate regression models, a significant positive correlation between serum vitamin D and the expression of vitamin D regulated targets - cyp24a by PBMC (p=0.0084, pediatric asthma group only) and serum IL-37 levels (p=0.0006, p=0.0067 for the pediatric and adult asthma groups, respectively) was found. An inverse association between serum IgE levels and vitamin D was observed only in the pediatric asthma group (p=0.006). In the pediatric asthma group serum vitamin D (p=0.05) and PBMC expression of cyp24a (p=0.0312) demonstrated significant inverse relationship with patients daily ICS dose. In vitro DEX suppression of TNFalpha (p=0.05) and IL-13 (p=0.0094) expression by PBMC positively correlated with PBMC cyp24a expression of the pediatric asthma group.  

**CONCLUSIONS:** The study demonstrated significant associations between serum vitamin D status and IgE, steroid requirement and in vitro responsiveness to corticosteroids in children but not adults with asthma. The study suggests that future vitamin D oral supplementation studies should compare vitamin D response in children vs. adults.

**484 Ganoderic Acid C Isolated From *Ganoderma Lucidum* Suppress Lps-induced Macrophage Tnf-α Production By Down-regulating Mapk, Nf-kappab And Ap-1 Signaling Pathways**

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**RATIONALE:** ASHMI (Antiasthma Simplified Herbal Medicine Intervention) has therapeutic effects in an animal model of asthma and in a controlled clinical trial. *Ganoderma lucidum* (Ling-Zhi) one of the three herbs in ASHMI, inhibits TNF-α production as does ASHMI. We performed bioactivity activity-guided isolation procedures to identify the bio-active compounds in Ling-Zhi that inhibit TNF-α production, and investigated the molecular mechanisms underlying these effects.  

**METHODS:** Liquid-liquid extraction, silica gel chromatography, sephadex LH-20 chromatography and preparative HPLC were used to obtain pure compounds from Ling-Zhi. The effect of these isolated compounds on TNF-α production by RAW 264.7 macrophages was examined at different concentrations (5, 10, 20 and 40 μg/ml). Alterations in levels of proteins involved in the MAPK, NF-kappab and AP-1 signaling pathways were determined by Western Blot assays.  

**RESULTS:** 6 triterpenes (ganoderic acids A, C, D, J, K, and L) were isolated from Ling-Zhi and identified based on LC-MS and NMR spectra. Ganoderic acid C was more effective than other compounds and showed dose- dependently inhibition on macrophage TNF-α production (IC50=24.5ig/ ml). Pretreatment with ganoderic acid C inhibited JNK activation (JNK phosphorylation) by LPS stimulation in macrophages. Phosphorylated c-Jun, a downstream signal of the JNK pathway, was also significantly down-regulated by pretreatment of macrophages with ganoderic acid C at 20 μg/ml. LPS induced ERK activation was blocked by ganoderic acid C pretreatment. As expected, nuclear translocation of p65 following LPS stimulation was also down-regulated by pretreatment with ganoderic acid C.  

**CONCLUSIONS:** Ganoderic acid C suppresses LPS-induced TNF-α production by down-regulating MAPK, NF-kappab and AP-1 signaling pathways in macrophages.

**485 Methoxyphenols Exhibit Anti-inflammatory Effects on Airway Epithelial Cells by Acting Post-transcriptionally**

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**RATIONALE:** The respiratory epithelium plays a central role in the inflammatory response in asthma and is a prime therapeutic target. Methoxyphenolic compounds are purported to be effective anti-inflammatory agents, and we characterized the effects of multiple 4-substituted methoxyphenols on human airway epithelial cells.  

**METHODS:** Human airway cells (A549, BEAS2B, and normal primary bronchial epithelial cells) were stimulated with TNF-α in the presence or absence of 4-substituted methoxyphenols. Expression of various cytokines and Reactive oxygen species (ROS) production was measured with a reactive fluorescent probe (3'6'-diamidino-2'7'-dichlorofluorescein). Activation of NF-kB was measured by nuclear translocation and phosphorylation. Post-transcriptional regulation was measured by actinomycin D assays and biotin pulldown affinity binding.  

**RESULTS:** Multiple inflammatory mediators were inhibited by methoxyphenols, including: CCL2, CCL5, IL-6, IL-8, ICAM-1, MIF, CXCL1, CXCL10, and Serpin E1. IC50 values were obtained for each compound that showed anti-inflammatory activity: diacapin (20.3 μM), resveratrol (42.7 μM), 2-methoxyhydroquinone (64.3 μM), apocynin (146.6 μM), and 4-amino-2-methoxyphenol (410 μM). The compounds had no effect on reactive oxygen species production or NF-kB activation, as previously reported in leukocytes. However, methoxyphenols acted post-transcriptionally to affect mRNA stability and translation, in part by inhibiting binding of HuR to transcripts.  

**CONCLUSIONS:** Methoxyphenols are a novel class of anti-inflammatory molecules that exert effects on the airway epithelium post-transcriptionally.
**486 Asthma Control And Disordered Microbial Communities In The Lower Airways Of Patients With Poorly Controlled Asthma**

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**RATIONALE:** The role of lower airway bacterial communities in asthma is poorly understood.

**METHODS:** BAL samples were obtained from 23 adults with chronic asthma (FEV1 73.6 ± 13.1%, PC2O 1.4 ± 2.3 mg/ml) and 20 normal control subjects. Bacterial DNA was extracted using QiaGen EZ1 system and sequenced using 454 FLX chemistry. Taxonomic identification was performed with RDP classifier and BLAST. Asthma control was measured by asthma control questionnaires (ACQ).

**RESULTS:** Based on microbiome data, asthmatics were subdivided into three groups: group A with commensal flora only (n=6); group B with expansions (>10% of 16s RNA sequences) in organisms also seen in normal controls (n=9); group C with expansions (>10%) of unique organisms not seen in the normal microbiome (n=8) (Comamonas testosteroni (n=1), Simonsiella muelleri (n=1), Asticcacaulis (n=4), Streptococcus pseudopneumoniae (n=2)). A progressive reduction in the number of sequences for Genera Prevotella (Rsq = 0.65, p < 0.0001) and Veillonella (Rsq= 0.53, p = 0.0006), considered to be major airway commensal flora, was observed in groups B and C. The lowest number of commensal sequences was observed in group C. There was progressively lower asthma control from groups A to C (ACQ test score 0.86 versus 4.00), considered to be major airway commensal flora and increased richness/bacterial diversity. The loss of asthma control was the greatest with expansion of unique organisms.

**CONCLUSIONS:** Expansion of selected groups of bacteria in the lower airway of asthmatics was associated with reduced numbers of commensal flora organisms and increased richness/bacterial diversity. The loss of asthma control was the greatest with expansion of unique organisms.

**487 TH IL-17 Immunophenotyping Reference for Children**

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**RATIONALE:** Hyper-IgE Syndrome (HIES) is a complex primary immunodeficiency characterized by gene mutations in STAT3 and Dock8, which lead to impaired T helper IL-17 (Th17) development. Currently, there are no published studies documenting the normal values for IL-17 secreting CD4 T cells in children.

**METHODS:** Blood samples from forty six subjects ages 0-18 years (yrs) were collected, peripheral blood mononuclear cells on each subject were isolated; stimulated overnight with PMA/Ionomycin in presence of Brefeldin A; surface stained for CD3, CD8; followed by intracellular isolation; CD154+IL4+ and CD154+IFNy+ T cells were detected in both allergic and non-allergic donors, respectively, as expected. Allergen-induced CD4+IL10+ responses were significantly greater in non-allergic donors compared to allergic donors. Furthermore, in non-allergic donors, a close correlation was observed between memory Th2 and CD4+IL10+ responses. This correlation was absent in allergic participants. The phenotype of CD154+ cells was largely central memory in both donor groups, but the IL4 response in non-allergic donors was dominated with T cells expressing multiple surface markers consistent with a naive phenotype. In contrast, allergic individuals exhibited largely memory IL4 responses.

**CONCLUSIONS:** CD154 expression following allergen stimulation may be useful for the ex vivo detection of allergen-specific T cells. The findings demonstrate a fine balance between Th1, Th2 and IL-10 responses in health, such that Th1 responses are counterbalanced by Th2 IL-10 responses. Naive T cells may produce IL4 in response to allergens which may represent a source of IL4 during Th2 priming.

**488 Ex Vivo Identification and Phenotyping of Allergen-Specific T Helper Cells In Human Peripheral Blood based on CD154 Expression**

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**RATIONALE:** This research utilised multiparametric flow cytometry to identify and phenotype allergen-specific T helper lymphocytes ex vivo based on CD154 expression.

**METHODS:** Peripheral blood mononuclear cells isolated from non-allergic and allergic donors were stimulated with relevant native allergens (cat dander, birch pollen, grass pollen) for 16 hours in the presence of Brefeldin A. Responding cells were identified and phenotyped by multiparametric flow cytometry.

**RESULTS:** Allergen-induced CD154 expression was observed universally and did not differ in magnitude between allergic and non-allergic donors. CD154+IL4+ and CD154+INFγ+ T cells were detected in both groups, with highly Th2 and Th1 polarised responses in allergic and non-allergic donors, respectively, as expected. Allergen-induced CD4+IL10+ responses were significantly greater in non-allergic donors compared to allergic donors. Furthermore, in non-allergic donors, a close correlation was observed between memory Th2 and CD4+IL10+ responses. This correlation was absent in allergic participants. The phenotype of CD154+ cells was largely central memory in both donor groups, but the IL4 response in non-allergic donors was dominated with T cells expressing multiple surface markers consistent with a naive phenotype. In contrast, allergic individuals exhibited largely memory IL4 responses.

**CONCLUSIONS:** CD154 expression following allergen stimulation may be useful for the ex vivo detection of allergen-specific T cells. The findings demonstrate a fine balance between Th1, Th2 and IL-10 responses in health, such that Th1 responses are counterbalanced by Th2 IL-10 responses. Naive T cells may produce IL4 in response to allergens which may represent a source of IL4 during Th2 priming.

**489 Calculated Globulin (CG): A Viable Tool for the Identification of Patients with Potential Antibody Deficiency**

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**RATIONALE:** Calculated globulin (CG), often performed as part of liver function testing (LFT), is defined as the plasma total protein minus albumin and is linearly associated with IgG content. While there are many causes of a low CG, including antibody deficiency, this information is often missed. The current study was conducted to examine the utility of CG as a tool for the identification of patients with antibody deficiency.**

**METHODS:** Serum aliquots from routine LFT samples with a CG of <18g/L were collected over a period of 6 months from 4 NHS clinical laboratories in Wales. These originated from a wide range of specialties including general practice, haematology, oncology, nephrology, intensive care and rheumatology. Immunoglobulin levels (IgG, IgA and IgM), serum electrophoresis and, in a subset, immunofixation were performed.

**RESULTS:** A total of 700 patients with a CG of <18g/L (range 4-17g/L) were identified. Of these, 352 patients (50%) had an IgG level <4.0g/L (range 1.41 to 3.99g/L). In addition seventeen paraproteins with immunoparesis were identified.

**CONCLUSIONS:** CG assessment identified a considerable number of patients with antibody deficiency due to a range of causes who would not otherwise have been further investigated. These results suggest a potential utility of CG as a screening tool for antibody deficiency.
A Central B Cell Tolerance Defect In Group Ia CVID Patients
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RATIONAL: Common variable immune deficiency patients with expanded CD21do B cell populations (CVID group Ia) are more likely to develop non-infectious complications including autoimmune disease. The presence of a central B cell tolerance defect may mediate this risk by allowing an increased frequency of self-reactive B cells to enter a patient’s peripheral blood. Central B cell tolerance in CVID group Ia patients has not yet been well described.

METHODS: We determined the Igk repertoire from 1226 single CD19+CD10+IgMhiCD27- new emigrant/transitional B cells from 49 individuals including 21 healthy controls, 11 CVID group Ia patients and 17 CVID non-group Ia patients utilizing single cell sorting, RT-PCR and DNA sequencing. We also expressed recombinant antibodies from the new emigrant/transitional B cells of 3 CVID group Ia patients and 8 healthy donors. The reactivity of the recombinant antibodies to LPS, Insulin and dsDNA was determined by ELISA to evaluate for defects in central B cell tolerance.

CONCLUSIONS: The unique Igk repertoire of CVID group Ia patients demonstrates diminished secondary recombination events and suggests a central defect in B cell receptor editing. Evidence of this central defect is reflected by an increased frequency of autoreactive new emigrant B cells in the peripheral blood of CVID group Ia patients.

Enhanced Organic Dust Induced Airway Inflammation in Protein Kinase C Epsilon Deficient Mice is Associated with Dysregulation of Nitric Oxide
J. A. Poole, A. Gleason, C. Bauer, W. W. West, D. J. Romberger, T. A. Wyatt, UNMC, Omaha, NE.
RATIONALE: Chronic organic dust exposure in the agricultural industry results in significant airway disease. Prior studies found that swine facility organic dust extract (DE)-induced pro-inflammatory cytokine production was greatly diminished when epithelial cell protein kinase C epsilon (PKCε) activity was reduced. However, the role of PKCε in modulating airway inflammation to DE in vivo is not known.

METHODS: Using an established murine model, PKCε knockout (KO) and wild type (WT) mice were intranasally challenged with DE or saline once or daily for 3 weeks (repetitive exposure). Outcome measurements included bronchoalveolar lavage to quantitate leukocyte influx and cytokine/chemokine and nitrate levels. Lung tissues were collected for histology. Isolated lung macrophages from WT and KO mice were also ex vivo stimulated with DE.

RESULTS: Macrophage and neutrophil influx increased by greater than 2-fold in PKCε KO mice following single and repetitive DE exposure. Lung pathology revealed that bronchiolar and alveolar inflammation and lymphoid aggregates were significantly increased in PKCε KO mice as compared to WT. There were no significant differences in cytokine/chemokine levels between animals. However, DE-induced nitric oxide production differed in that DE exposure increased nitrate levels in WT animals, but not in PKCε KO mice. Moreover, ex vivo stimulation of lung macrophages from KO mice with DE failed to upregulate NO production.

CONCLUSIONS: PKCε deficient mice were hypersensitive to organic dust-induced exposure. These data suggest that a normative lung inflammatory response to DE is mediated by PKCε. The response observed in PKCε KO mice may be due to dysregulation of the nitric oxide pathway.

492 Enhanced Organic Dust Induced Airway Inflammation in Protein Kinase C Epsilon Deficient Mice is Associated with Dysregulation of Nitric Oxide
J. A. Poole, A. Gleason, C. Bauer, W. W. West, D. J. Romberger, T. A. Wyatt, UNMC, Omaha, NE. J ALLERGY CLIN IMMUNOL VOLUME 129, NUMBER 2 Abstracts AB129

SUNDAY
493 Cigarette Smoke Exposure and its Effect on Bronchial Responsiveness and Response to Fluticasone Propionate, Salmeterol and their Combination

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RATIONALE: Cigarette smoke adversely affects asthma and COPD. Inhaled corticosteroids (ICS) and beta adrenergic agonists are the commonly used therapeutic treatments for asthma. This study is to investigate the effect of cigarette smoke exposure (CSE) on bronchial hyperresponsiveness (BHR) to methacholine (MCh) and on bronchoprotective effects of steroid and beta-agonist agonist.

METHODS: whole body cigarette smoke exposure in C57Bl/6j mice (6-8 wk, f) was done for 3 weeks (3 h/day, 5 days/week). Salmeterol (100 ug/mL), fluticasone (100 ug/mL) and their combination (FS, 100 ug/mL each) was administered by nebulization using ultrasonic nebulizer. Then mice were challenged with 3.125, 6.25, 12.5 and 25 mg/mL of MCh and BHR was measured using whole body plethysmography. The percent change over baseline Penh was used to analyze BHR. IL-13, TNF-α, IL-1β and GM-CSF in bronchial alveolar lavage (BAL) fluid were measured by murine ELISA kit (eBioscience).

RESULTS: The percent change over baseline Penh at 25 mg/mL of MCh of smoked mice (632.8±107.03; n=9) was significantly higher (p=0.017) compared to non smoked mice (325.7±54.12; n=10). The percent change over baseline Penh at 25 mg/mL of MCh of salmeterol, fluticasone and FS treated smoked mice was 515.62±76.64 (n=10), 458.35±117.47 (n=10), and 465.86±57.22 (n=10) respectively where as for non smoked mice was 293.97±64.02 (n=10), 274.6±50.73 (n=10) and 271.13±42.99 (n=9) respectively.

CONCLUSION: Smoking significantly increased the degree of BHR as measured by the response to methacholine in smoked mice compared to non-smoked mice. Fluticasone, salmeterol and their combination did not protect significantly against bronchial hyperresponsiveness to methacholine after cigarette smoke exposure compared to non-smoked mice.

494 The Prevalence of Asthma, Hay Fever and Allergic Sensitization in Amish Children

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RATIONALE: The Amish population uses very traditional farming practices and has large family size. The Amish immigrated from Switzerland to the United States in the 1800’s. We sought to contrast the prevalence of asthma, hay fever and allergic sensitization in Swiss and Amish populations of the United States in the 1800’s. Although urban individuals maintain their customs, adoption of new practices and has large family size. The Amish immigration from Switzerland to the United States in the 1800’s. Since the 70’s have seen significant differences in the prevalence of atopic diseases between rural and urban areas. We hypothesized that diet and lifestyle are associated with increased prevalence of atopy.

METHODS: Two-hundred Colombian African-descent children with similar genetic background were selected, 100 of them living in rural area and remaining in urban environment. Atopy was determined by skin sensitization to 10 common allergen extracts. Dietary and lifestyle pattern were assessed with appropriate questionnaires. We measured Total-IgE and Specific-IgE to Blomia tropicalis and Ascaris lumbricoides extracts.

RESULTS: The overall prevalence of atopy was 15.5%. B. tropicalis was the most prevalent allergen (80.6%). Urban vs. rural prevalence of atopy were 24% and 7% respectively [p=<0.001]. Kitchen inside the house, sewing area and not burning trash were associated with atopy in urban population [p=0.0019, p=0.099, p=0.02]. Fast food consumption was a risk factor for atopy in urban population [OR=2.9, 95%CI (1.31 - 6.43), p=0.008]. High raw milk consume was detected as protective factor in rural population [OR=0.11, 95%CI (0.11 - 0.87), p=0.037]. Total-IgE levels were significantly higher in rural subjects [2450.7±757.5 IU/mL vs 777.5±3 IU/mL, (p<0.0001)]. No difference in IgE-levels to A. lumbricoides and B. tropicalis was found between the two environments, but specific-IgE to these extracts were significantly correlated [R spearman=0.646, p=0.0000].

CONCLUSIONS: Atopy was different between the two environments. Although urban individuals maintain their customs, adoption of new dietary foods and new lifestyle can influence the development of atopy.

495 Influence of Rural and Urban Dietary and Lifestyle Patterns in Atopic Diseases in a Colombian African-descent Population

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RATIONALE: Since the 70’s have seen significant differences in the prevalence of atopic diseases between rural and urban areas. We hypothesized that diet and lifestyle are associated with increased prevalence of atopy.

METHODS: Two-hundred Colombian African-descent children with similar genetic background were selected, 100 of them living in rural area and remaining in urban environment. Atopy was determined by skin sensitization to 10 common allergen extracts. Dietary and lifestyle pattern were assessed with appropriate questionnaires. We measured Total-IgE and Specific-IgE to Blomia tropicalis and Ascaris lumbricoides extracts.

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CONCLUSIONS: Atopy was different between the two environments. Although urban individuals maintain their customs, adoption of new dietary foods and new lifestyle can influence the development of atopy.

496 Vitamin E Reduces Airway Granulocyte Recruitment after Inhaled Endotoxin Challenge in Normal Volunteers

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RATIONALE: Epidemiologic studies suggest that dietary vitamin E is an important candidate intervention for asthma. Our group has shown that daily consumption of a gamma tocopherol-enriched supplement (γT) has anti-inflammatory actions on peripheral blood mononuclear cells and decreases nitrosative stress. The objective of this study was to test the effect of γT supplementation on airway neutrophil recruitment after inhaled endotoxin challenge in normal volunteers.

METHODS: Thirty nonasthmatic subjects completed a double-blinded, placebo controlled crossover study where they consumed either a γT-enriched capsule vs. a sunflower oil placebo (SO) capsule. After 7 days of daily supplementation, they underwent an inhalation challenge to 20,000 endotoxin units of Clinical Center Reference Endotoxin (CCRE). Peripheral blood and induced sputum were obtained 6 hours after inhaled CCRE challenge. Serum levels of γT, its primary metabolite 2, 7, 8-trimethyl-2S-(3-hydroxychromane (γCEHC), and a marker of nitrosative stress, 5-nitroT were compared during the placebo vs. active periods. The effect of γT compared to placebo on airway granulocyte recruitment after inhaled endotoxin challenge was compared using a repeated measures analysis of variance (ANOVA).

RESULTS: Compared to placebo, supplementation with γT significantly increased serum levels of γT and γCEHC (p<0.001) and significantly decreased levels of 5-nitroT (p=0.01). After CCRE challenge, γT treatment significantly decreased induced sputum neutrophils (p=0.03) and eosinophils (p=0.02) compared to placebo.

CONCLUSIONS: Supplementation with γT reduces systemic nitrosative stress, and reduces CCRE-induced sputum neutrophil and eosinophil recruitment. These results suggest that γT is an excellent antioxidant candidate to test in preventing and/or treating environmentally-induced asthma and allergic disease in larger studies.
Maternal Low Dose Peanut and Cholera Toxin Subunit B Induces Oral Tolerance Associated with An Increased Number of T Regulatory Cells

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RATIONALE: Food allergy, including peanut allergy (PNA), has become as a major public health issue in western countries. Maternal allergen exposure may affect the development of offspring allergy. We previously found maternal feeding of peanut plus cholera toxin (CT) as adjuvant suppressed peanut-IgE responses in sensitized offspring from peanut allergic mothers. The aim of this study was to investigate how maternal peanut (PN) plus non-toxic cholera toxin subunit B (CTB) consumption induces peanut tolerance in offspring.

METHODS: PN mothers received low dose PN plus CTB (PN+CTB) or water (Sham) during pregnancy and lactation. Offspring from these mothers were sensitized with PN for 8 weeks and challenged intragastri- cally with PN. Anaphylactic reactions, plasma histamine levels, peanut-specific serum antibodies and cytokine levels were determined. CD4⁺CD25⁺ FoxP3⁺ T regulatory cells from splenocytes were analyzed by flow cytometry.

RESULTS: PN sensitized offspring of PN+CTB mother (O/PN+CTB) showed no anaphylactic symptom following oral PN challenge whereas 100% of sensitized offspring of sham-treated mothers (O/Sham) developed anaphylactic reactions. This protection in O/PN+CTB was associated with significantly reduced plasma histamine levels, PN-IgE levels, decreased IL-4 and IL-17 and increased IL-10 production by cultured splenocytes (SPCs) compared to offspring of sham mothers (all p < 0.05). Increased number of CD4⁺CD25⁺ FoxP3⁺ T regulatory cells in SPCs from offspring of PN+CTB mother were also increased (O/PN+CTB vs. O/Sham: 0.73 % vs. 1.98%).

CONCLUSIONS: Maternal consumption of low dose PN plus CTB induces immune tolerance to active peanut sensitization in offspring of peanut allergic mothers. Tolerance is associated with increased number of CD4⁺CD25⁺ FoxP3⁺ T regulatory cells.

Development of a Dendrimeric Structure containing ole e 1 and CpG to Modulate an Allergic Response in an Experimental Model

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RATIONALE: Polymers containing immunogenic peptides bonded to dendrimeric structures have been developed to have been used for vaccines in cancer, infectious diseases, and allergy. Our aim is to design a dendrimeric structure containing Ole e 1 and CpG, in order to modulate an allergic immune response towards Th1, in an experimental model of anaphylaxis.

METHODS: C56BL/6 mice were sensitized by intranasal administration of olive extract+cholera toxin B, for 6 weeks. Then, mice received immunotherapy (IT) treatment by subcutaneous (sc) injection of dendrimer-Ole1+CpG, for 8 weeks. Seven days after the IT, mice were challenged with 100 μg of olive extract (ip). Severity of anaphylaxis was measured by drop in body temperature and the humoral response by ELISA.

RESULTS: Olive sensitized mice treated with the dendrimer-Ole1 without CpG developed a drop in body temperature similar to anaphylactic mice (35.02 ± 1.39 vs. 34.48 ± 0.82, respectively), indicating that Ole e 1 within a dendrimeric structure is recognized in vivo. On the other hand, 8 weeks after immunotherapy, mice receiving the dendrimer-Ole1+CpG were significantly protected from the development of systemic anaphylaxis (37.18 ± 1.58 vs. 34.48 ± 0.82, p < 0.05, respectively). IgE and IgG2a levels decreased after 1-2 weeks of treatment and remained stable over the time; however IgG1 were normal.

CONCLUSION: Our results indicate that the dendrimer-Ole1 is recognized in vivo. Furthermore, we show that administration of dendrimer-Ole1+CpG protects sensitized mice from the onset of anaphylaxis, and the decrease in IgE levels during the IT treatment may be responsible for that protection.
Anaphylaxis in America - Results from a National Telephone Survey
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Rationale: To delineate triggers of anaphylaxis and compare awareness, knowledge and behaviors among patients at-risk for anaphylaxis.

Methods: A nationwide, cross-sectional random-digit-dial telephone survey was conducted using a standardized questionnaire. Household members were screened for allergic reactions to foods, insect stings, latex, medications, and other allergens, and for idiopathic reactions. When multiple household members had allergies, the person with the most severe allergic reaction was chosen for the interview. Participants were asked over 100 questions about anaphylaxis awareness, triggers, symptoms, treatments, knowledge, perceptions, behaviors, and quality of life.

Results: Over 20,000 phone calls were made to identify and interview a nationally representative sample of 1,000 persons who had experienced anaphylactic reactions within the past ten years. The survey found that 18% of persons with these types of allergies had experienced at least one likely anaphylactic reaction. Among those reporting anaphylactic reactions, 42% occurred within 15 minutes of exposure and the most common triggers were medications (33%), followed by foods (28%), insect stings (21%), other (15%), unknown (7%), and latex (3%). Also among those reporting anaphylaxis, 38% sought emergency room care, 28% self-treated with antihistamines, 13% went to a doctor’s office, and 13% self-administered epinephrine. Although 57% reported two or more lifetime episodes, only 18% of the individuals reporting anaphylaxis currently carry epinephrine.

Conclusions: Severe allergic reactions consistent with anaphylaxis are common among persons reporting allergic reactions in the general population. This comprehensive national survey on anaphylaxis, including its triggers and treatment, supports the need for public health initiatives to improve anaphylaxis practices and education.

Food Allergy and Increased Asthma Morbidity in a School Inner-city Asthma Study
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Rationale: Children with asthma have increased prevalence of coexisting food allergies. While food allergy has been shown to be an independent risk factor for increased asthma morbidity, this has not been examined within an urban inner-city asthma school-aged cohort. The School Inner-City Asthma (SICAS) is an NIH-funded prospective study evaluating specific risk factors and asthma morbidity among urban children. We aimed to determine the relationship between food allergy and asthma morbidity.

Methods: We prospectively surveyed children from 20 urban, inner-city schools with a diagnosis of asthma, followed by full clinical evaluation and pulmonary function testing. Food allergy symptoms were reported including symptoms experienced within one hour of food ingestion. Asthma morbidity, pulmonary function, and resource utilization were compared between children with food allergies and without. Significance was tested using Wilcoxon rank-sum tests.

Results: Fifty-five (24%) of the 228 asthmatic children surveyed had food allergies. Asthmatic children with food allergies had significantly more hospitalizations than those without food allergies (OR: 2.15, 95% CI: 1.16-4.00, p = 0.01). Percent-predicted FEV1 scores were significantly lower in the food allergy group (median: 93.8, IQR 82.9-111.5) compared to the non-allergic group (median 101.5, IQR: 91.4-112.9, p = 0.04).

Children with food allergies were more likely to have been prescribed an asthma controller medication (OR: 1.73, 95% CI 0.90-3.34, p = 0.10) and have escalated asthma therapy in the last 12 months (p = 0.07).

Conclusions: School-aged children with asthma and coexisting food allergies have increased asthma morbidity, decreased lung function, and increased healthcare utilization.
504 Bullying and Teasing In Children With Food Allergy: A Survey of Pediatric Patients In Urban Jackson, Mississippi Outpatient Allergy and Immunology Clinics

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RATIONALE: A previous study found bullying to be common among food allergic patients attending Food Allergy and Anaphylaxis Network meetings. We are evaluating whether bullying children with food allergies occurs in our patient population, and if so, the characteristics of the bullying.

METHODS: Validated questionnaires are completed anonymously by food allergic children or their parents. Questionnaires assess characteristics of bullying, perpetrators, and resources to mitigate bullying. Subjects are from a primarily lower socioeconomic population of our University-based clinics.

RESULTS: Eighteen surveys have been completed. Those surveyed are between ages 6-12. A minority (6/18; 33.3%) have experienced bullying related to food allergies. Most episodes occurred at school and were perpetrated by classmates, although teachers, a teammate, and siblings were also implicated. Most bullying occurred in the form of verbal acts. Three of the respondents also reported physical acts of the allergen waved in their face, chased with the allergen, and being pushed, tripped, hit, or kicked. Half of those (3/6) bullied due to food allergies also experienced bullying for reasons cited such as size for age and having eczema. Eczema was also a source of bullying in two food allergic patients that denied bullying due to food allergies. Half of those bullied reported that their schools had programs to prevent bullying.

CONCLUSIONS: Although bullying in children with food allergies was not the predominant response in this initial sample, it creates emotional distress and possible physical risks for those experiencing bullying.

505 Novel Use of a Social Network for Families with Food Allergic Children

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RATIONALE: Little is known about the impact of online social networks as peer support for families of children with food allergies. We investigated whether an online social network intervention would be acceptable to families of children with food allergies.

METHODS: Twenty families with 27 food allergic children (average age = 7.3 years, 56% male, 93% white) were recruited from participants at a food allergy retreat. Baseline assessments included media/technology use and the Food Allergy Quality of Life Questionnaire (FAQLQ). The families were invited to a secure, private, moderated Facebook group page for the purpose of peer support. Families were invited to participate in weekly interactive activities to encourage communication for 4 weeks. Family user satisfaction and FAQLQ-PF were subsequently assessed.

RESULTS: The children had an average of 4.7 food allergies with the most common being peanut, tree nuts, and milk. 70% of parents logged onto an online social network at least once daily. Sixteen families with 21 children enrolled in the Facebook group. Eight families with 12 children participated in at least one activity or discussion. Seven families had multiple interactions with the Facebook group. 86% of the families stated they enjoyed the group and would participate again and 71% stated it enhanced the effect of the food allergy retreat. There was no change in the food allergy quality of life after the intervention (p=0.6).

CONCLUSIONS: An online social network to promote peer support had moderate participation rates but was well accepted by families with food allergic children.

506 Food Allergy Attitudes and Beliefs among School Nurses in an Urban Public School District

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RATIONALE: Attitudes and beliefs regarding food allergy have been studied among primary care physicians, but not among school nurses. Since these perceptions may influence prevention and management of school-based reactions, we evaluated them among nurses in an urban school district.

METHODS: District of Columbia public school nurses were asked to anonymously complete a 10-item food allergy attitude questionnaire. Responses were tabulated across a 5-point Likert scale, ranging from strongly disagree to strongly agree. Chi-squared tests were used to test associations between nurse characteristics and item responses.

RESULTS: A total of 162/196 (83%) eligible nurses completed the questionnaire. Most (94%) felt food allergy is a serious health problem for children. Although 94% agreed schools should have food allergy guidelines, fewer believed that nut-free schools (82%) and allergen-free tables (44%) should be implemented. Agreement with allergen-free tables was significantly associated with prior food allergy education (p=0.01) and a belief that it is hard for food-allergic students to eat at school (p=0.02). Regarding quality of life, 40% felt students with food allergy worry about their condition and only 32% believed students were teased or bullied about their food allergy. Negative perceptions of parents were identified as 55% agreed parents of food-allergic children are overprotective and 15% felt they make unreasonable requests of schools.

CONCLUSIONS: While most nurses agreed food allergy is a serious problem that schools should address, negative attitudes exist regarding food allergy avoidance policies, impact on affected children, and parents. Food allergy education of school nurses should address not only knowledge, but also these negative perceptions.

507 Comparing the Effect of Intranasal Fluticasone Furoate (FF) to Intracutaneous Olopatadine (OLO) on the Ocular Response to Nasal Allergen Challenge (NAC)

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RATIONALE: NAC leads to a nasal ocular reflex, which is inhibited by FF. We investigated whether intracutaneous OLO controlled ocular symptoms after NAC.

METHODS: We performed a randomized, double blind, double dummy, placebo controlled, 4-way crossover trial in subjects with seasonal allergic rhinitis out of season. Subjects were randomized to receive one week pretreatment with intranasal placebo and intracutaneous PL (PL/PL), intranasal PL and intracutaneous OLO (0.2% solution) (PL/OLO), intranasal FF (220 mcg/day) and intracutaneous PL (FF/PL), and the combination (FF/OLO). Subjects then underwent NAC with either grass or ragweed on 2 consecutive days. Nasal lavages were evaluated for levels of tryptase and the number of sneezes, and nasal and ocular symptoms recorded.

RESULTS: NAC after PL/PL resulted in increase in symptoms and tryptase. There was a reduction in eye symptoms (median change from diluent) on the second day of challenge from 6.0 after PL/PL to 0 after FF/PL (p=0.001), 2.5 after PL/OLO (p=0.3), and 1.5 after FF/OLO (p=0.003). Further, there was no significant difference between the response after FF/PL vs FF/OLO and a significant difference between FF/PL and PL/OLO (p=0.02). Levels of tryptase followed a similar trend with a significant reduction compared to placebo on FF/PL (p=0.002) and FF/OLO (p=0.002), no significant difference between the response after FF/PL vs FF/OLO, and a significant difference between FF/PL and PL/ OLO (p=0.003).

CONCLUSIONS: Our data confirm the existence of a naso-ocular reflex after NAC. OLO alone or the addition of OLO to FF does not impact ocular symptoms caused by the naso-ocular reflex.
**508** Long-term Safety Study of MP29-02 (Novel Intranasal Formulation of Azelastine Hydrochloride and Fluticasone Propionate) in Subjects with Chronic Allergic or Non-allergic Rhinitis

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**RATIONALE:** The objective of this study was to evaluate the long-term safety of MP29-02 in subjects with chronic allergic or non-allergic rhinitis.

**METHODS:** This was a 1-year, randomized, open-label, active-controlled, parallel-group study. Subjects were randomized in a 2:1 ratio to: (1) MP29-02 one spray/nostril twice daily (total daily doses of azelastine and fluticasone were 548 mcg and 200 mcg, respectively) or (2) fluticasone propionate two sprays/nostril once daily (total daily dose 200 mcg). Safety and tolerability assessments were made at months 1, 3, 6, 9, and 12. Efficacy was assessed by the 12-hour reflective total nasal symptom score (rTNSS) and the Rhinoconjunctivitis Quality of Life Questionnaire (RQLQ).

**RESULTS:** A total of 612 subjects were randomized. Early discontinuations were similar with MP29-02 (21.7%) and fluticasone (24.6%), mostly for nonclinical reasons. The most common treatment-related adverse events were headache (4.3%) with fluticasone and dizziness (2.5%) with MP29-02. No appreciable changes in laboratory values were observed. There were no nasal mucosal ulcerations or perforations and ocular examination findings were unremarkable. There were no significant differences between groups in fasting AM serum cortisol levels. The RQLQ score met the clinically important change of -0.5 units in both groups from Month 1 to Month 12. Based on the additional therapeutic benefit in SAR. Further studies are needed to confirm these observations and identify a mechanism.

**CONCLUSIONS:** MP29-02 was well tolerated in this study and there was no evidence of any unusual or unexpected adverse events. Based on the additional therapeutic benefit in SAR. Further studies are needed to confirm these observations and identify a mechanism.
512 Allergen Specific IL-10 Producing T-Regulatory Cells Are Upregulated in Children Who Have Acquired Tolerance to Egg A. B. Fishbein1, K. A. Erickson2, C. Szychlinski1, M. J. Kvasny2, R. L. Fuleihan1, A. M. Singh1; 1Northwestern University Children’s Memorial Hospital, Chicago, IL, 2Northwestern University, Chicago, IL.
RATIONALE: Egg is the second most common food allergy in childhood, and is often outgrown. The mechanism by which children acquire tolerance is not well understood. We hypothesized that the development of allergen specific T-regulatory cells is responsible for inducing tolerance to egg protein.
METHODS: PBMCs were stimulated with ovalbumin for 48h (0.1,10,50,100mcg/ml) in 44 children with confirmed allergy, recently acquired tolerance to egg or no egg allergy. Supernatants were collected and cytokines associated with Th1, Th2 and Treg pathways measured. In a subset of patients after stimulation, flow cytometry was performed with cells stained for surface (CD3, CD4, CD25, CD14, CD19) and intracellular markers (IL-10 and Foxp3).
RESULTS: A significant difference was noted between allergies, non allergics and tolerant with respect to Ifn-γ, IL-4, IL-5 and IL-10 after stimulation at each concentration. Tolerants had elevated IL-10:IL-4 and IL-10:IL-5 ratios as compared to allergics (p<0.05). Foxp3 was most upregulated in tolerant (n=3) with a mean MFI of 1406(1218-1507) in CD3+CD4+Foxp3+CD25+ cells, as compared to allergics: μ = 706(430-912), n=4, and controls; μ=564(478-649), n=2 (p<0.05). In response to stimulation with 100mcg/ml of Ova, tolerant patients most upregulated IL-10 in Foxp3+ cells, median change 9%(4-12-1.9), versus allergies -10%(46-3.2-4).
CONCLUSIONS: Children with recently acquired tolerance have increased IL-10 and decreased Th2 cytokine responses. T-regulatory cells producing IL-10 are upregulated after allergen stimulation in tolerant versus allergic patients. These findings suggest that allergen specific Tregs are important in active tolerance. In addition, these results may help identify markers of tolerance and develop therapeutic interventions to induce tolerance to food allergens.

513 IL-4, But Not IL-2, Induces Th2 Cells To Resist The IL-27 Counterregulation By Downregulating Stat1 Phosphorylation Z. Chen1,2, S. Wang2, C. Bai1, R. Alam2, R. Kiatla2, H. Huang2, Z. Zhongshan Hospital, Shanghai, CHINA, 2National Jewish Health, Denver, CO.
RATIONALE: Asthma is a chronic airway inflammation caused by overproduction of Th2 cytokines. IL-27 has been shown to inhibit differentiation of naïve CD4+ cells into Th2 cells in mice. However, it is not known whether human IL-27 can inhibit Th2 cell differentiation. Moreover, it is not clear whether IL-27 can inhibit Th2 cell differentiation in atopic asthmatics.
METHODS: Purify CD4+ cells from human PBMC and mice spleen were cultured under Th2 or Th2 + IL-27 conditions. IL-4 and IFN-gamma were detected by ELISA. IL-27R and p-STAT1, p-STAT2, p-STAT3, p-STAT4 were determined by qRT-PCR and Western blot respectively.
RESULTS: Human IL-27 suppressed Th2 differentiation in healthy subjects (p=0.006), but failed to do so in asthmatics (p=0.064). This finding led us to consider if memory Th2 cells in asthmatic patients are responsible for the induction of resistance to IL-27. We found that Th2-inducing conditions induced resistance to IL-27 in a dose-dependent manner. Further analysis revealed that IL-4, but not IL-2, was critical in the induction of IL-27 resistance. We demonstrated that high dose of IL-4 treatment resulted in impairment of STAT1 and STAT2 phosphorylation, but not STAT3 or STAT4 phosphorylation.
CONCLUSIONS: IL-4, but not IL-2, induces Th2 cells to resist the IL-27 counterregulation by downregulating STAT1 phosphorylation.

514 Increased Number of Regulatory T cells in Skin Draining Lymph Nodes Suppress Priming Towards New Antigens S. Mahapatra1, E. Robinson2, C. Herrick2, M. Albrecht3, A. M. Dittrich1; 1Hannover Medical School, Hannover, GERMANY, 2Yale University School of Medicine, New Haven, CT.
RATIONALE: We have previously shown that a Th2-polarized pulmonary inflammation can facilitate priming to new, unrelated antigens. We were thus interested whether Th2-polarized allergic skin inflammation can also facilitate priming towards new antigens.
METHODS: To compare epicutaneous vs. pulmonary priming towards secondary antigen, we used initially sensitized mice with ovalbumin (OVA) intraperitoneally (i.p.). OVA sensitized mice were challenged initially via the intranasal or epicutaneous route with OVA and sub-immunological doses of KLH (keyhole limpet hemocyanin) concomitantly. Two weeks later they were rechallenged with KLH alone via the intranasal or epicutaneous route. As read-outs three days after the second challenge, we analyzed antigen-specific antibody responses, proliferation, cytokine profiles and Foxp3 expression in draining lymph nodes.
RESULTS: In contrast to the lung model, we did not observe significant levels of KLH specific immunoglobulins in the mice which had received OVA and KLH epicutaneously during first challenge followed by challenge with KLH alone during the second challenge. Lymph node proliferation & cytokine secretion also did not provide evidence that priming against KLH occurs after epicutaneous exposure. We did observe continuously elevated expression of Foxp3 in the epicutaneous group compared to the lung group.
CONCLUSIONS: Our results indicate, that in contrast to the lung, an ongoing allergic skin inflammation doesn’t facilitate priming to a secondary, unrelated antigen. Increased numbers of regulatory T cells in the skin draining lymph nodes could play an important role in suppressing collateral priming in skin in comparison to the airways.

515 IgE Cross-Linking Downregulates Expression of Glycolytic Genes in Human Plasmacytoid Dendritic Cells G. Bajwa, F. Z. Chowdhury, J. D. Farrar, J. Suderth, R. J. Deberardinis, M. A. Gill; UT Southwestern Medical Center at Dallas, Dallas, TX.
RATIONALE: IgE cross-linking results in impaired viral-induced IFNα secretion by plasmacytoid dendritic cells (pDCs). The purpose of this study was to identify potential mechanisms by which IgE cross-linking mediates this inhibition of pDC antiviral responses.
METHODS: pDCs were purified from human blood samples using antibody-coated magnetic beads and cultured in the presence of human anti-IgE, rabbit IgG, or control media conditions. Gene expression patterns were determined by DNA microarray analysis (HumanHT-12 v4 Expression BeadChip) and expression of specific genes was confirmed by qPCR. In select experiments, pDCs were exposed to influenza A (FluA) and 2-deoxy-D-glucose (2-DG, a glycolysis inhibitor). IFNα was measured in supernatants by ELISA.
RESULTS: DNA microarray analysis demonstrated that genes encoding components of innate immune pathways such as IRF7 and IFNα were downregulated by IgE cross-linking. Surprisingly, IgE cross-linking also resulted in significant downregulation of multiple key genes in the glycolytic pathway including those encoding hexokinase 2 (HK2), phosphofructokinase (PFK) and lactate dehydrogenase A (LDHA). Results were confirmed by qPCR (p<0.001 vs control for all above). In separate experiments, exposure of pDCs to the glycolysis inhibitor 2-DG significantly inhibited FluA-induced pDC IFNα secretion (p<0.05).
CONCLUSIONS: IgE cross-linking, a surrogate for allergic stimulation, results in significant suppression of key glycolytic genes in pDCs. Our results also reveal that glycolysis is essential for FluA-induced pDC IFNα production. Taken together, these data suggest that alteration of metabolic pathways such as glycolysis represents a potential mechanism by which allergic stimulation impairs antiviral responses in human pDCs.
516 Increased Platelet Adherence to Leukocytes Results in Cysteinyl Leukotriene (cysLT) Overproduction in Aspirin Exacerbated Respiratory Disease (AERD)

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RATIONALE: Overproduction of cysLTs is a hallmark of AERD, but the mechanisms for their production are incompletely defined. Because leukocytes can provide the precursor leukotriene (LTA4) to adherent platelets for transcellular conversion to the parent cysLT, LTC4, we hypothesized that platelet-dependent leukotriene metabolism contributes to cysLT production in AERD.

METHODS: Blood, urine, and nasal polyp tissues were obtained from subjects with AERD and from aspirin-tolerant asthmatic and nonasthmatic controls. Circulating platelet-leukocyte aggregates were quantified cyto-fluorographically, as were the effects of adherent platelets on leukocyte expression of adhesion markers. Platelet contribution to granulocyte-derived leukotrienes in vitro was quantified by HPLC, by measuring release of 5-lipoxygenase products from freshly isolated or platelet-stripped granulocytes. Urinary LTC4 was quantified by mass spectrometry.

RESULTS: Percentages of circulating leukocytes with adherent platelets were markedly higher in subjects with AERD than in control groups, and platelet-adherent leukocytes were abundant in their nasal polyp tissues. Subsets of platelet-adherent monocytes and granulocytes expressed significantly higher levels of several adhesion markers than did platelet nonadherent subsets. Adherent platelets contributed more than half of the leukotriene C4 synthase (LTC4S) activity found in peripheral blood granulocytes from subjects with AERD and primed them for 5-lipoxygenase activity and conversion of endogenous LTA4 to LTC4. Urinary LTC4 levels correlated strongly with percentages of neutrophil-platelet (r=0.78) and eosinophil-platelet (r=0.73) aggregates.

CONCLUSIONS: The increased frequency of leukocyte-platelet aggregates in AERD permits both adherence to endothelial cells and augmented transcellular conversion of leukotrienes, and may be partly responsible for the tissue inflammation and systemic overproduction of cysLTs that characterize this disease.

517 MiR150 Transgenic Mice Exhibit Altered T Cell Response, Cytokine Profile and Lung Inflammation

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RATIONALE: Regulatory T (Treg) cells play a pivotal role in regulating T cell response and inflammation in asthmatic lungs; however the mechanism of Treg generation and function is poorly understood. A micro-RNA profile in Tregs from asthmatic mice showed a significant decrease in miR-150 expression. MiRNA150 may thus be useful as an anti-inflammatory treatment for asthma. Our goal was to determine the effect of miR-150 overexpression on T cell response and lung pathology in a mouse model of asthma.

METHODS: Total RNA was isolated from Treg and non-Treg cells and subjected to miRNA profiling. Transgenic mice overexpressing miR-150 were generated by pronuclear injection. T cell responses, inflammation and airway hyperreactivity were measured in asthmatic mice given miR-150 chitosan nanoparticles.

RESULTS: MiR-150 was significantly down-regulated during asthmatic inflammation in Treg and non-Treg CD4+ T cells and in Th1 and Th2 cells. Transgenic mice overexpressing miR-150 showed lower airway hyperreactivity, inflammatory cytokine production and NKT cells than WT. Overexpression of miR-150 delivered by chitosan nanoparticles inhibited lung inflammation and cytokines in asthmatic mice and did not cause adverse side effects. MiR-150 suppressed expression of Akt3, Cbl1 and Elk1 but up-regulated p53, inhibited cell proliferation and promoted apoptosis of Jurkat T cells.

CONCLUSIONS: MiR-150 decreases inflammation in asthma by inhibiting cytokine production, inducing apoptosis and repressing cell growth by regulating critical genes including Akt, Elk, Cbl1 and p53. Deregulation of miR-150 may be involved in the pathogenesis of asthma. Thus, overexpressing miR-150 may be useful as a safe anti-inflammatory therapeutic strategy for attenuating lung inflammation.

518 Meta-analysis of Transcriptome Data using Pathway-based Approach Reveals Biologically Relevant Asthma Genes

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RATIONALE: Asthma is a disease of chronic airway inflammation that affects over 300 million individuals worldwide including 20 million in the U.S. While the causes of asthma are not fully understood, genetic differences may be partly responsible for the variations in asthma susceptibility. Using microarray experiments, many independent groups have identified differentially expressed genes (DEGs) between healthy versus asthmatic patients. However, there is little overlap in the DEG lists identified among different experiments due to variation from random noise, biological and experimental differences, and differences in the extraction and handling of RNA samples. Therefore, a statistics-based meta-analysis is necessary to identify a set of genes that are consistently dysregulated among multiple independent microarray studies.

METHODS: A recent search of asthma-related expression data in the GEO dataset yielded 1379 experiments of which 1190 were human-specific (http://www.ncbi.nlm.nih.gov/, accessed on September 2, 2011). In this study, we focused on our meta-analysis and network/pathway analysis on the 5 microarray human data sets, which had individual experiment sample sizes of over 100.

RESULTS: Of the 5534 genes analyzed, 550 (12%) were up-regulated and 456 (8%) were down-regulated. Many key genes including SERPINB2, CLCA1 and P2RY14 were up-regulated and MUC5AC/MUC5B mucins and C6 were down-regulated. These genes were involved in inflammatory diseases, mucus over-production, airway obstructions, immunological diseases, and hypersensitivity responses.

CONCLUSIONS: Meta-analytic dataset and network/pathway analysis of publicly available databases provide a starting point to identify biologically plausible potential candidate genes linked to asthma and to explore the mechanistic basis for the observed expression patterns in asthma.
519 Gene-Environment Interaction between CD14/TLR4 Polymorphisms and Use of Antibiotics in Infancy increases the Risk of Allergic Diseases

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RATIONALE: Antibiotics use by newborns alters infant microbiota profiles including long-term reduction in microbiota diversity. We investigated the interaction between CD14 -159C/T(rs2569190) and TLR4 +8595C/T(rs1927911) polymorphisms and use of antibiotics in infancy on the development of allergic diseases in Korean school children.

METHODS: A cross-sectional study (n=1803) aged 9-12 years was conducted with ISAAC questionnaire. Polymorphisms of CD14 and TLR4 were genotyped by TaqMan assay.

RESULTS: Antibiotic use during infancy was significantly associated with allergic rhinitis (AR) diagnosis and atopic dermatitis (AD) diagnosis, and current AR and current AD. When we analyzed interaction between use of antibiotics and genotype of the CD14 and TLR4, there was a significant association with development of allergic diseases. Children using antibiotics in infancy and TT+CC genotype of the CD14 had increased aOR of AR diagnosis (1.88) and any allergic disease diagnosis (2.06) compared with children having TT genotype of the CD14 and no antibiotics use. Children using antibiotics in infancy and CC genotype of the TLR4 had increased aOR of asthma diagnosis (2.85) and any allergic disease diagnosis (2.18), and children using antibiotics in infancy and CT+TT genotype of the TLR4 had significantly increased aOR of asthma diagnosis (4.00) and any allergic disease diagnosis (1.77) compared with children having CC of the TLR4 and no use of antibiotics.

CONCLUSIONS: The use of antibiotics in infancy is an important risk factor for the development of allergic diseases. The interaction of gene (CD14 -159C/T and TLR4 +8595C/T polymorphism) and use of antibiotics in infancy may influence the development of allergic diseases.

520 Exhaled Breath Condensate Formate Increases After Allergen Challenge in Human Atopic Asthmatics In Vivo

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RATIONALE: S-nitrosoglutathione (GSNO) belongs to a class of endogenous airway smooth muscle relaxants that are diminished in asthmatics due to increased activity of S-nitrosoglutathione reductase (GSNOR). Up-regulation of GSNOR results in both a loss of GSNO and an increased response (LAR).

METHODS: Ion chromatography analysis of formate was performed in EBC at baseline and IAC in 16 atopic asthmatics in vivo. Analysis of formate provides a new sensitive index of GSNOR activity in the asthmatic airways in vivo.

RESULTS: In asthmatics with isolated EAR, formate concentrations remained unchanged after IAC as compared to baseline. In contrast, in asthmatics with both EAR and LAR, formate concentrations were increased in all samples after IAC as compared to baseline, but reached statistical significance only at 1 h post challenge (p=0.019) (bas 2.76±2.5 (range 1.3-9.1), 1h 4.6±1.9 (2.2-8.1), 2h 3.1±1.5 (1.7-6), 4h 3.4±1.7 (1.3-7.2), 6h 3.2±1.6 (1.5-5.8), 8h 3.7±2.4 (1.25-7.5), mean±SD).

CONCLUSIONS: Increased EBC formate concentrations in patients with dual asthmatic response but not in asthmatics with isolated EAR suggests that upregulation of GSNOR may play pathomechanistic role in the development of allergen-provoked LAR in human atopic asthmatics in vivo. Analysis of formate provides a new sensitive index of GSNOR activity in the asthmatic airways in vivo.

521 Characterization of Genetic Epidemiology for GWAS-identified Asthma Susceptibility Loci in Chinese by Next Generation Sequencing

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RATIONALE: A recent large-scale genome-wide association study (GWAS) identified 10 asthma susceptibility loci in Europeans. However, the genetic epidemiology of these loci in Asians is poorly defined. This study characterized the minor allele frequencies (MAFs) of peak association signals and haplotype structures of these loci in Chinese.

METHODS: Genomic DNA from 24 healthy children recruited from local schools were subjected to next generation sequencing. The target 50 Mb-regions both upstream and downstream from peak association signals on these 10 asthma loci were characterized by pyrosequencing. These high-resolution sequences were then compared with reference Phase 3 HapMap and 1000 Genomes databases.

RESULTS: Presequencing check revealed excellent quality of DNA samples by Agilent 2100 Bioanalyzer. Compared with reference sequences, we observed significant differences up to 0.2 for MAF of single-nucleotide polymorphisms (SNPs) on 10 peak association signals between Chinese and Caucasians. Haplotype analyses by Haplovist revealed substantially discrepant haplotype structure with up to 5-block differences on 100 Mb-regions of the 10 asthma susceptibility loci between these populations. Tagging algorithm applied on these loci at MAF ≥ 0.05 and r² ≥ 0.8 yielded over 60 haplotype tagging (ht)-SNPs in our Chinese, which again represented striking differences up to 20 ht-SNPs when compared with CEU samples in the reference databases.

CONCLUSIONS: Genetic epidemiology of 10 GWAS-identified asthma loci is markedly different between Chinese and Caucasians. These sequence differences influence the selection of tagging SNPs for replicating these asthma susceptibility loci in the Chinese population.
Simultaneous Detection of TREC and KREC in Newborn DNA Isolated From Dried Blood Spots (Guthrie Cards)

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RATIONALE: NYSScreening for severe combined immunodeficiency (SCID) using TREC levels in dried blood spots successfully identified several SCID patients over 12 months. However, newborns with defective B-cell receptor rearrangement (BCRR) go undiagnosed. To develop a simultaneous screening method to detect SCID and BCRR defects at birth, we adapted published KREC-specific primers with a modified probe for a multiplex PCR compatible with current NYS approved TREC primers that detect SCID.

METHODS: The KREC probe (Sottini 2010) was modified to contain a 5' NED fluorescent label for multiplex PCR detection with a TREC probe (5' FAM) used by NYS. Blood-derived DNA from known XLA patients and age matched controls were obtained following informed consent. DNA from 25 blood spots from normal infants were also screened individually and simultaneously for KREC/TREC levels using an ABI 7900 HT, compared to Rnase-P (control gene).

RESULTS: Equivalent levels of KREC/TREC in blood were identified in controls (n=3), but not in XLA patients (n=3), who had control-like TREC levels, but 50-100 fold less KREC. Dried blood spot DNA from 25 control infants had equivalent levels of KREC/TRECs that were equal whether assayed alone or in multiplex, demonstrating primer/probe set compatibility.

CONCLUSIONS: Extending SCID newborn screening to include BCRR-specific primers that detect KREC's together with NYS approved TREC primer/probe sets is feasible. Dried blood spot DNA from XLA and other specific primers that detect KREC's together with NYS approved TREC primer/probe sets, is feasible. Dried blood spot DNA from 25 control infants had equivalent levels of KREC's/TRECs that were equal whether assayed alone or in multiplex, demonstrating primer/probe set compatibility.

Different Effects Of ADA And PNP Deficiency On Thymocytes Development

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RATIONALE: Defects in adenosine deaminase (ADA) and purine nucleoside phosphorylase (PNP), two consecutive enzymes important for purine metabolism, cause T cell deficiency. However, the immune abnormalities in ADA-deficient patients occur at earlier age and are more severe than those of PNP-deficient patients, suggesting different effects of the two enzymes on thymocytes.

METHODS: Sequential T cell receptor gene rearrangements were measured in lymphocytes from ADA- and PNP-deficient patients. Expression of CD44, CD25 and intracellular TCRb were used to assess cell proliferation.

RESULTS: ADA- and PNP-deficient patients have defects in cell proliferation when compared to normal controls. ADA-deficient cells have significantly impaired cell proliferation than those of PNP-deficient patients, suggesting different effects of the two enzymes on thymocytes.

CONCLUSIONS: ADA deficiency causes severe defect in the maturation of very early thymocytes, while PNP deficiency is associated with a milder defect at a later maturation stage and at a later age.

Pioglitazone (pio), A Peroxisome Proliferator-activated Receptor (ppar) Agonist, Restores Efferocytosis Of Neutrophils In Chronic Granulomatous Disease (cgd) By Enhancing Oxidant Production And "Eat Me" Signaling

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RATIONALE: Clearance of apoptotic cells (efferocytosis) by macrophages (MF), a potentially anti-inflammatory process, is defective in CGD where absence of oxidase function causes: (i) classically activated, PPARglow MFs and (ii) impaired apoptosis and "eat me" signaling by neutrophils. Pio, a PPARg agonist, has been shown to normalize exaggerated sterile inflammation in CGD mice by enhancing MF programming and efferocytosis. We hypothesized that Pio treatment also reverses defective signaling by CGD neutrophils.

METHODS: Peritonitis was induced with zymosan in WT and CGD (gp91phox-/-) mice treated with vehicle or Pio. Inflammatory cells, mediators, and functions were determined.

RESULTS: As reported, Pio treatment, while not altering neutrophil recruitment, normalized resolution of neutrophilia and reduced accumulation of apoptotic neutrophils during CGD peritonitis. Pio (vs vehicle) treatment effects were assessed by isolating early peritonitis neutrophils and testing them for "palatability" to normal MF. Neutrophils from Pio-treated CGD mice were efferocytosed by MF significantly better, and equivalently to WT neutrophils. CGD neutrophils from Pio-treated mice were found to undergo timely apoptosis with enhanced display of "eat me" signals: phosphatidylserine (PS) and the modified species, lyso-PS. Given that these signals are oxidant-dependent, CGD neutrophils from Pio-treated mice were tested for oxidant production following ex vivo stimulation. Significant production of superoxide by an alternative flavochrome was demonstrated in neutrophils from Pio-treated CGD mice.

CONCLUSION: PPARg agonism restores proper resolution of sterile inflammation in CGD mice by enhancing efferocytosis, both by normalizing CGD MF programming and CGD neutrophil oxidant-mediated "eat me" signaling. Therapeutic intervention by PPARg agonism deserves further consideration in CGD.

Knockdown of Dock8 in Zebrafish Reveals a Role for Dock8 in Extra-Lymphoid Tissue Development

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RATIONALE: Since the function of dedicator of cytokinesis 8 (DOCK8), which is deficient in patients with an autosomal recessive form of hyper IgE syndrome, is currently unknown, we characterized its expression in zebrafish and studied the effects of gene knockout using morpholino technology.

METHODS: RNA extraction and PCR analysis was performed on morphants at 1 and 3 dpf to evaluate gene knockdown. Microinjection of zebrafish embryos (n=25) at the 1-2 cell stage was performed with a dock8/p53 morpholino to distinguish off target gene effects. Morphological changes were examined at 24hpf. Sense and antisense RNA probes for rag1 known to be expressed in zebrafish at 4dpf, were constructed and used for in situ hybridization to characterize rag1 expression in dock8 morphants (n=48).

RESULTS: PCR analysis revealed decreased dock8 gene expression in morphants. The dock8 and rescue dock8/p53 morphants both showed a dysmorphic phenotype as evidenced by smaller head size and developmental delay, with no difference in the deformity rates (p=0.32). Rag1 expression was detected in the thymus of dock8 morphants at 4dpf.

CONCLUSIONS: Morpholino treated embryos resulted in decreased dock8 expression. Dock8 deficiency did not impair early lymphoid development as evidenced by the normal expression of rag1. Knockdown of dock8 in zebrafish specifically results in a dysmorphic phenotype, reflective of a role for dock8 in brain development. These findings may be relevant to the association of the human DOCK8 locus with mental retardation and other brain anomalies.
526 Elucidating the Role of IgM Memory B Cells in Common Variable Immunodeficiency

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RATIONALE: There is a very limited data on CD27\(^+\) IgM\(^+\) B cells in CVID. Prior studies focused on their relation to bacterial pneumonia, but the precise role of these IgM memory B cells in CVID remains undefined, suggesting studies with broader objectives and a larger sample size. We therefore sought to elucidate the role of IgM memory B cells by characterizing immunological profiles and clinical parameters in a large cohort of CVID patients.

METHODS: Eighty-six CVID patients were divided into low vs normal IgM memory B cell groups using 9% as the cutoff. We analyzed immunological profiles and clinical parameters including history of recurrent pneumonia, anti-pneumococcal polysaccharide (anti-PnPS) IgG titers to 14 serotypes, lung CT scan findings, and associated non-infectious complications of CVID.

RESULTS: In the low IgM memory B cell group (N=50), 72% experienced recurrent pneumonia, whereas only 28% did in the normal IgM memory B cell group (N=36), \(P<0.0001\). Low IgM memory B cells were associated with lower number of switched memory B cells (\(<0.55\% \text{ of B cells})\, P=0.04. Mean serum IgM level (32.9 mg/dL vs 116.3 mg/dL) and the mean number of protective anti-PnPS IgG titers (1.57 vs 2.95) were lower in low IgM memory B cell group (\(P=0.24\) and \(P=0.18\), respectively). There was no statistically significant difference in the incidence of non-infectious complications.

CONCLUSIONS: Reduced IgM memory B cells are associated with higher incidence of pneumonia and lower numbers of switched memory B cells. Its measurement may help identify some CVID patients with greater risk of pneumonia.

527 Specific Induction Of CD203c Expression In Blood Basophils Discriminates Between CF Patients With Aspergillus Colonization And Those With CF-ABPA

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RATIONALE: A. fumigatus (Af) colonizes the airways of 25-50% of cystic fibrosis (CF) patients, with some further progressing towards allergic bronchopulmonary aspergillosis (ABPA). ABPA significantly impacts prognoses in CF, yet may be avoided by early diagnosis and treatment. However, the diagnosis of CF-ABPA is clinically challenging, due to the absence of an objective biological test. Since blood basophils play a major role in allergic responses, we hypothesized that changes on their surface activation pattern could discriminate CF patients with ABPA from those without.

METHODS: Blood basophils CD203c and CD63 levels, were measured by flow cytometry at baseline and upon in vitro activation with Af allergen, in 5 groups of subjects: (A) CF patients with Af colonization but without ABPA (N=11); (B) CF-ABPA patients (N=8); (C) CF-ABPA patients whose treatment includes omalizumab (N=2); (D) CF patients (N=5) and (E) healthy controls (N=11).

RESULTS: In the CF-ABPA group, basophil CD203c levels and histamine levels increased significantly upon Af allergen stimulation (CD203c levels: P<0.001, compared to the four other groups). This increase was Af allergen-specific, since stimulation with a non-offending allergen did not increase surface CD203c or plasma histamine from subjects in any of the groups (CD203c levels: P>0.1). Basophil CD203c levels upon Af allergen stimulation provided excellent predictive value for discriminating CF-ABPA from CF patients with Af colonization but without ABPA (ROC curve = 0.92; P<0.01). Basophil CD203c levels increased also upon Asp f 8 stimulation.

CONCLUSIONS: These results support the notion that basophils can be used to diagnose and monitor CF-ABPA clinically.

528 Inflammatory Gene Expression Differentiates With Airway Neutrophil Response To Ozone

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RATIONALE: Exposure to ozone is known to cause neutrophilic airways inflammation in humans. Despite its adverse health effects, the genes and associated pathways that underlie ozone-induced inflammation and disease remain largely unknown.

METHODS: We sought to determine whether genes associated with inflammatory pathways differ according to the magnitude of the neutrophil (PMN) response in the airways of human volunteers following ozone exposure. Sputum samples were collected from 27 individuals (13 healthy, 4 allergic non asthmatics, 10 allergic asthmatics) pre and 6hr post a 2hr exposure to 0.4 ppm ozone. RNA was extracted from sputum samples, hybridized to gene arrays and analyzed for genes that differentiate between minimal and robust PMN response to ozone.

RESULTS: We found a significant difference between the genomic response of those with a minimal PMN response over baseline (ave. = 1.0%, N=9) vs those with a robust PMN response (ave. = 35%, N=18). A total of 140 genes were modified by ozone between the two levels of PMN responsiveness. Robust PMN responders showed a muted response at the gene expression level (20 genes) while minimal PMN responders showed changes in 127 genes. Few genes overlapped between the two groups, but both groups showed gene expression changes in the Nuclear Factor kappa beta (NF-kb) pathway.

CONCLUSIONS: Gene expression signatures differ according to the magnitude of the airway neutrophil response following ozone exposure. The significance of this effect and the role it plays of how ozone impacts individuals with pre-existing allergic airways inflammation (atopic asthma) needs to be examined.

529 Functional Interaction of Cockroach Allergens and Mannose Receptor on Human Circulating Fibrocytes

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RATIONALE: Fibrocytes have been suggested to play a role in the regulation of allergic asthma with cockroach allergens (CR) being one of the important triggers. The innate pattern recognition C-type-lectin receptors (CLRs), including mannose receptor (MR, CD206), have been suggested to functionally interact with allergens. We aimed to test the hypothesis that cockroach allergens are the natural ligands for CLRs and functionally influence fibrocyte’s response.

METHODS: Expression of CLRs, including CD206, on human circulating and cultured fibrocytes was examined by Flow cytometry, and solid-phase binding assays were used to determine the binding activity of CR or Bga g2, a major CR allergen, to MR. The levels of cytokine response in fibrocytes were measured by ELISA.

RESULTS: Flow cytometric analyses of human circulating and cultured fibrocytes (CD45\(^+\) and collagen-1\(^+\)) showed selective expression of CD206 on the cell surface and in the cytosol. In binding assays, significant and saturable bindings of CR and Bga g2 to CD206 were observed, which was calcium-dependent and could be inhibited by mannann and anti-CD206 Abs. Functionally, Bga g2 induced, in both time- and dose-dependent manners, significant levels of cytokine secretion for IL-6, IL-10 and TNF-alpha in cultured fibrocytes. Further, this effect was abrogated by the addition of a calcium chelator, EDTA, mannann or blocking Abs for CD206, and in fibrocytes with CD206 knockdown.

CONCLUSIONS: Taken together, these results provide evidence supporting the existence of a functional CR-CD206 axis in human fibrocytes, suggesting its likely importance in regulating airway remodeling in asthma.
531 Transient Receptor Potential Melastatin 8 Mediates Airway Inflammation Of Toluene-diisocyanate Induced Asthma
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RATIONALE: Neurogenic inflammation is one of the pathogenic mechanisms of toluene diisocyanate induced asthma (TDI-OA) where transient receptor potential melastatin family member 8 (TRPM8) receptor is a well-established cold- and menthol-sensing caution channel. Our previous GWAS study suggested this gene as a potential candidate in the pathogenic mechanism of TDI-OA. This study was aimed to investigate the functional effect of TRPM8 receptor in the pathogenesis of TDI-OA.

METHODS: Human airway epithelial cell line, BEAS-2B, was treated with TDI-HSA conjugate. The mRNA and protein expressions of TRPM8 receptor were determined by real time PCR and western blotting. The change of surface expression of TRPM8 receptor was examined by flow cytometry. IL-8 was measured by ELISA method.

RESULTS: TRPM8 receptor was expressed primarily in human airway epithelial cells, which increased significantly with IL8 production after TDI exposure in dose- and time- dependent manners. Flow cytometry identified a significant increased surface expression of TRPM8 receptor after TDI exposure.

CONCLUSIONS: TDI exposure could activate TRPM8 receptor expression and production from airway epithelial cells, leading to airway inflammation in patients with TDI-OA.

532 Comparison of Sublingual Immunotherapy (SLIT) versus Oral Immunotherapy (OIT) in the Treatment of Peanut Allergy
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RATIONALE: Both SLIT and OIT have been used to induce clinical desensitization in peanut-allergic subjects. To our knowledge, there has been no direct comparison of the two modes of treatment for peanut allergy.

METHODS: Subjects were enrolled in double-blind, placebo-controlled studies using either peanut SLIT or peanut OIT with maintenance doses of 2mg and 4000mg respectively. Immunologic parameters were measured longitudinally. Double-blind, placebo-controlled food challenges (DBPCFC) were performed after 12 months of treatment.

RESULTS: 26 subjects on SLIT and 23 subjects on OIT (median age 8.8 and 8.6 years respectively) were evaluated after 2 years of treatment. Baseline and 24 month peanut-IgE levels were similar between the two groups but were higher at 12 months in the OIT group (median 204kU/L vs 57kU/L, p = 0.03). Compared to SLIT, OIT resulted in higher peanut-IgG4 levels at 12 and 24 months (median 20mg/L vs 3.5mg/L, p < 0.001, and 20mg/L vs 9.7mg/L, p = 0.001). The absolute decrease in SPT was greater following 12 and 24 months of OIT versus SLIT (median -7.5mm vs -3.8mm, p = 0.02, and -8.5mm vs -3.5mm, p = 0.02). After 12 months of treatment, 16/18 subjects on OIT and 7/26 subjects on SLIT passed a DBPCFC (p=0.001; RR = 0.3, 95% CI = 0.13 to 0.52). The 23 subjects who passed the DBPCFC had greater absolute change in peanut-IgG4 (median 9.9mg/L vs 3.07mg/L, p = 0.05).

CONCLUSIONS: During this limited observation period at the doses used, OIT induced more extensive immunologic changes associated with desensitization. These findings are reflected in the more variable degree of desensitization demonstrated by SLIT versus OIT subjects during DBPCFCs.
534 Real-time Imaging Assessment of Blood Flow, Temperature, and Skin Color on Patients with Cold-induced Urticaria: Correlation with Histamine and Tryptase Release

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RATIONALE: Acquired cold-induced urticaria has been characterized primarily by visual observation and measurement of mediators released by mast cells during challenge testing. We sought to characterize cold-induced urticaria with real-time optical imaging.

METHODS: Laser speckle contrast (LSCI), infrared (IR), and polarized light colorimetry (PLC) imaging collected real-time blood flow, temperature, and skin color data during cold-hand immersion challenge testing in patients with cold-induced urticaria and controls, in concert with measurement of serum mediators. Some patients repeated this procedure on cetirizine (10mg).

RESULTS: LSCI, IR and PLC detected significant differences in cold challenge responses in patients compared to controls. LSCI displayed significantly higher maxima, delayed onset and slower recovery of mean blood flow (p=0.0006) than challenged controls. IR detected rapid rewarming and temperature increase above baseline (p=0.0017) and a delay in time to reach maximal color index as determined by PLC. The mean post challenge histamine peak of 45.5 nM was achieved at 5 minutes. No peak was found in tryptase. Evidence for mast cell degranulation and localized release of tryptase was seen in post-challenge biopsies. Significant correlations were found between histamine release, and blood flow and temperature. Using LSCI, treatment of three patients with cetirizine revealed variable effectiveness.

CONCLUSIONS: Real-time imaging quantifies skin tissue hemodynamic responses to cold challenge in patients with cold urticaria, which correlated with evidence of mast cell activation as revealed through the release of histamine. This approach offers a unique opportunity to follow the effectiveness of treatment intervention.

535 Vitamin D Insufficiency is Strongly Associated with Challenge-proven Infantile Food Allergy in the Healthnits Population-based Study

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RATIONALE: Recent reports have shown a latitude gradient in food allergy prevalence with those furthest from the equator recording the highest admissions to hospital for food allergy related events. We aimed to investigate the role of Vitamin D in the development of infantile food allergy in Melbourne, Australia, a city with a high population prevalence of both Vitamin D insufficiency and food allergy and no Vitamin D food-chain fortification.

METHODS: The population-based HealthNuts study screened 5,302 infants using skin prick tests. Serum was obtained from 358 with challenge-proven food allergy (peanut, egg and/or sesame), 242 with sensitisation only and 108 negative controls and was analysed for serum 25(OH)D using liquid chromatography tandem mass spectrometry. The association between serum 25(OH)D and food allergy was examined using multiple logistic regression.

RESULTS: Low vitamin D (<=50nM/L) at 1 year of age was associated with having a blood draw in winter or spring (OR 4.5, 95% CI 3.0-6.8), breastfeeding > 10 months (OR 1.5, 95% CI 1.3-1.7), never consuming formula (OR 3.3, 95% CI 2.2-5.0) and never consuming semi-cooked eggs (OR 1.6, 95% CI 1.1-2.3). Infants with vitamin D insufficiency (26-50nM/L) and deficiency (<26nM/L) were more likely to be food allergic even after adjusting for these factors (adjusted OR 1.7, 95% CI 1.0, 2.7, p=0.028 and aOR 3.7, 95% CI 1.4, 10.1, p=0.009 respectively).

CONCLUSIONS: These results provide a rationale for investigation of fetal or early postnatal life factors and Vitamin D status and its impact on development of infantile food allergy.

536 Genetic Variants In Cep68 In Patients With Hypersensitivity Reactions To Nsaids

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RATIONALE: NSAIDs are the compounds more frequently involved in hypersensitivity drug reactions (HDRs) which include selective responses (IgE- or T cells-mediated) (SR) and cross-intolerance (non immunological immunemecme) (CI), being the latter quantitatively the most important. Genetic association studies have been carried out only in patients with CI and consist mainly in the analysis of polymorphisms related with the arachidonic acid pathway. However, other possibilities should be consid- ered. Recently CEP68 has been suggested as a susceptible gen in asthmatic patients that showed CI to NSAIDs. In this study, we analyzed the potential association of six tSNPs in a large group of patients with SR or CI to NSAIDs.

METHODS: Samples were obtained from several Allergy Services integrated into the Spanish network for allergic diseases RIRAAF. Patients who developed several episodes with the same NSAID and good tolerance to a strong COX inhibitor were considered to have a SR, whereas patients with episodes with two or more different NSAIDs were included in the CI group. We studied 6 tSNPs in CEP68 by using TaqMan® probes.

RESULTS: We included 812 subjects with HDRs to NSAIDs, 636 with CI and 176 with SR, and 350 age, sex-matched controls. Both groups showed statistically significant differences compared with the control group in rs3732098 (p<0.001 for both), and in rs7572857 (p=0.002 and p=0.025, respectively).

CONCLUSIONS: Our results suggest the importance of genetic variants in CEP68 in HDRs to NSAIDs. Further studies are required to analyze their functional role and the involvement of other potential genes nearby CEP68.
537 A Speech Recognition (SR) Reminder System Improves Adherence to ICS Among Pediatric Asthma Patients

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RATIONALE: Prior efforts to improve adherence to asthma controller medications have been characterized by minimal effectiveness and limited reach. We hypothesized that a speech recognition (SR) reminder system would improve adherence to an ICS in a large unselected population of pediatric asthma patients.

METHODS: 1393 children, ages 3-12 years, with persistent asthma were randomized to the SR intervention or control groups and followed for up to 1 year. Leveraging the EHR system, the intervention group received up to 3 tailored SR reminder calls when they were due to refill their ICS. The calls provided information about asthma, facilitated a rapid ICS refill, and offered an opportunity to receive a call back from an asthma nurse specialist.

RESULTS: There were no statistically significant differences between the intervention and control groups in age, gender, co-morbidities, and length of ICS enrollment. Time to first ICS refill was significantly shorter for the SR intervention group (Median 52 days) than the control group (Median 78 days), HR= 1.26 (95% CI 1.12, 1.42). Proportion of days with medication on hand was greater in the SR intervention than the control group (38% versus 28%, p<0.0001). Two-thirds of intervention parents reported the SR calls were helpful and that the program improved the care of their child’s asthma.

CONCLUSIONS: These results indicated that an SR reminder system, supported by asthma nurses and a convenient pharmacy refilling process, can be implemented in a large HMO to positively influence medication adherence.

538 Short Message Service (SMS) For Asthma Management: A Pilot Study Utilizing Text Messaging To Promote Asthma Self-management

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RATIONALE: Medication containing inhaled corticosteroids (ICS) is the preferred therapy for persistent asthma. However, adherence to daily ICS is low compared to other medication regimens for chronic disease. Poor adherence in asthmatics is associated with a higher risk for morbidity and mortality. Simple, effective strategies to improve medication adherence are needed.

METHODS: 43 adolescents with asthma were enrolled in a randomized, prospective, controlled trial. All subjects utilized their own personal cellular phones and pre-existing SMS plans for the study. Participants undergoing changes to their medication regimen at the time of enrollment were excluded. Subjects completed an interactive voice response survey on their phones at the beginning and conclusion of the study. Survey responses included the Asthma Control Test, as well as self-reported adherence and satisfaction with the texting program graded on a scale of 1-5. Subjects in the intervention group received randomly generated text messages pertaining to asthma education at variable frequencies (once every other day to twice per day) for 30 days.

RESULTS: 93% of the subjects felt receiving text messages helped them take better care of their asthma. 86% liked receiving text messages about asthma. 7% thought too many messages were sent. 71% were interested in continuing to receive text messages about their asthma. Following the study, subjects receiving texts reported significantly higher adherence (p = 0.045) and confidence in taking care of their asthma (p = 0.01) compared to the control group.

CONCLUSIONS: Adolescents with asthma are accepting of educational text messaging and find texting beneficial for their asthma care.
541 Associations Between Self-Reported Adherence to Asthma Anti-Inflammatory Therapy and Child/Parent Attitudes and Behaviors Regarding Disease Management

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BACKGROUND: Assessment of patient adherence status and reasons for non-adherence can assist physicians in helping patients to overcome these barriers and ultimately improve asthma outcomes.

METHODS: 361 parents of children (59.6% male; 64.1% Caucasian; mean age 8.07 years) with intermittent and persistent asthma completed the AsthmaPACT from (8/2009 to 6/2011). This survey, hosted by the Asthma and Allergy Foundation of America website, serves to identify barriers to adherence to asthma treatment.

RESULTS: 259 parents (72%) reported giving their child anti-inflammatory medications (AI) prescribed by their physician. Of these, 69 (27%) were classified as non-adherent. Non-adherence was operationalized as parental report of giving at least one AI “less than prescribed by their physician.” During the 4 weeks prior to completing the survey, 43% of those receiving AI reported having daily symptoms. In this cross-sectional data set, items intended to relate risk factors to non-adherence were examined using chi square analysis. Individuals classified as non-adherent were more likely to report: 1) Ineffective asthma management behaviors by the child such as medication forgetfulness (p = 0.001); poor trigger avoidance, (p = 0.013); lack of perception of worsening asthma, (p = 0.008); and delaying treatment (p = 0.001) and 2) Negative attitudes about medication by the parent: medication does not work, (p = 0.002); child is taking too much medicine; (p = 0.001); refusal to administer oral steroids (p = 0.002) as well as inhaled corticosteroids, (p < 0.001).

CONCLUSIONS: By providing both patient self-report of adherence and identifying risk factors for non-adherence, the AsthmaPACT can help physicians to have meaningful conversations with patients to overcome these barriers.

542 Efficacy and Safety of a Novel Ragweed Allergy Immunotherapy Tablet (AIT) During Peak Season in North America

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RATIONALE: Ragweed-allergic patients suffer most during peak season when traditional pharmacotherapy may not control symptoms adequately. The efficacy of ragweed AIT has not been studied before. We report peak-season efficacy and safety from a trial investigating two strengths of ragweed AIT (Ambrosia artemisiifolia) in allergic rhinoconjunctivitis (ARC) subjects with or without asthma.

METHODS: 565 adults with ragweed-pollen induced ARC were randomized 1:1:1 to daily ragweed AIT 1.5, 6, or 12 Amb a 1-U or placebo for approximately 4 months before, throughout, and following ragweed pollen season for a total of approximately 52 weeks. Symptoms and rescue medications were recorded daily in e-diaries. Efficacy endpoints included total combined daily symptom/medication score (TCS), daily symptom score (DSS), and daily medication score (DMS) during peak season. Safety was monitored through adverse event (AE) reporting and by an external data/safety monitoring committee.

RESULTS: The majority of subjects were multisensitized; 17% had asthma. 12, 6, and 1.5 Amb a 1-U showed mean TCS improvement versus placebo of 24% (-2.0; p = 0.0015), 19% (-1.6; p = 0.0113), and 9% (-0.8; p = 0.2192), respectively. DSS for 12 Amb a 1-U improved by 18% (-0.9; p = 0.0118) versus placebo; 6 and 1.5 Amb a 1-U showed numerical improvement (9% and 5%). DMS improvement of 36% and 35% for 12 and 6 Amb a 1-U was significant (-1.10; p = 0.0058 and -1.08; p = 0.0053). Treatment effects were similar between continents. The majority of treatment-related AEs were mild, local, application-site reactions with no observed difference between 12 and 6 Amb a 1-U or systemic allergic reactions.

CONCLUSIONS: Ragweed 12 Amb a 1-U AIT showed greatest efficacy, with 1.5 Amb a 1-U being ineffective. 12 Amb a 1-U AIT may present a novel treatment option for ragweed allergic rhinoconjunctivitis.
All abstracts are strictly embargoed until the date of presentation at the 2012 Annual Meeting

**SUNDAY**

**544** Timothy Grass Allergy Immunotherapy Tablets Reduce Nasal and Ocular Symptoms Associated With Allergic Rhinoconjunctivitis During Grass Pollen Season in North American Children and Adults: 2 Randomized, Placebo-Controlled Trials

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**RATIONALE:** The nasal and ocular symptoms of allergic rhinoconjunctivitis (ARC) are bothersome with a substantial negative impact on quality of life. The effect of Timothy grass allergy immunotherapy tablet (grass AIT) treatment on individual ARC symptoms was assessed in 2 randomized, double-blind trials in North American adults and children with grass pollen-induced ARC.

**METHODS:** The pediatric trial randomized 345 children (5-17 years); the adult trial randomized 439 adults. Subjects received once-daily 2800 BAU grass AIT or placebo for approximately 16 weeks before and during the pollen season. ARC symptoms (runny nose, blocked nose, sneezing, itchy nose, gritty feeling/red/itchy eyes, and watery eyes) were recorded daily and measured on a scale of 0 (no symptoms) to 3 (severe).

**RESULTS:** In children, total nasal symptoms, runny nose, sneezing, itching, and congestion scores were reduced by 23%, 23%, 21%, 34%, and 16%*, respectively, in those receiving grass AIT versus placebo. The differences between treatment were ~0.81 points for total nasal symptoms and ranged -0.14 to -0.26 for its components, P<0.05; *P=0.06. In adults, nasal symptoms scores were reduced 15%, 17%, 15%, 16%, and 12%* for grass AIT versus placebo. The differences between treatment were -0.48 points (0.003) and ranged from ~0.09 to ~0.13 for its components, P<0.05; *P=0.13. Total ocular symptoms were reduced by 28% (-0.39points, p=0.0003) and 26% (-0.38points, p<0.001) in children and adults, respectively.

**CONCLUSIONS:** These data indicate that grass AIT effectively reduced nasal and ocular symptoms in North American children and adults with grass pollen ARC.

**545** Post-treatment, Long-term Clinical Efficacy Of A 300 IR Sublingual Tablet of 5-grass Pollen Allergen Extract In Adults With Grass Pollen-induced Allergic Rhinoconjunctivitis

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**RATIONALE:** The sustained efficacy (i.e., after 3 treatment years) of discontinuous treatment with 300IR sublingual tablet of 5-grass pollen allergen extract, initiated 4 or 2 months before each grass pollen season and continued for its duration, has been demonstrated in an ongoing 5-year study. Here we report the persistence of efficacy during the first post-treatment pollen season.

**METHODS:** 633 adults were randomized to placebo or 300IR pre- and co-seasonally for three grass pollen seasons starting 4 months[4M] or 2 months[2M] prior to the season each year. Patients were followed during the subsequent, treatment-free, season. Primary endpoint for post treatment efficacy was the Average Adjusted Symptom Score (AASS). Secondary efficacy criteria included overall Rhinoconjunctivitis Quality of Life Questionnaire (RQLQ) score. AASS and overall RQLQ score were analyzed using an ANCOVA model.

**RESULTS:** Statistically significant differences compared to Placebo in mean AASS during the Year 4 pollen period were observed for both 300IR[4M] and 300IR[2M]. 300IR[4M]: difference in LS Means -1.14 (95% CI: [-2.03,-0.26], p = 0.0114), relative LS Mean difference from Placebo -22.9%. 300IR [2M]: difference in LS Means -1.43 (95% CI: [-2.32,-0.53]), p = 0.0019, relative LS Mean difference from Placebo -28.5%. Active treatment groups also showed statistically significant LS Mean differences in changes from baseline in overall RQLQ score (pg<0.001). There were no serious drug-related post-treatment adverse events.

**CONCLUSIONS:** The long-term efficacy of 300IR sublingual tablets of grass pollen allergen extract was demonstrated during the first post-treatment pollen season. This persistent improvement was clinically meaningful to patients.

**546** Persistent Treatment Effect Achieved at One Year After 4 Doses of Fel d 1-Derived Peptide Immunotherapy in an Environmental Exposure Chamber (EEC) Model of Cat Allergy

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**RATIONALE:** Previously, we identified a series of T-cell epitopes (ToleroMune Cat) from the major cat allergen Fel d 1 and showed that these were safe and well-tolerated when administered to cat allergic individuals (Worm et al., JACI 2011). In the current study, we hypothesized that after treatment with four doses of ToleroMune Cat, a persistent treatment effect (tolerance) could be demonstrated one year after dosing start.

**METHODS:** Subjects attended a Baseline Challenge in the EEC on 4 consecutive days, 3-hour allergen exposures (Fel d1 50.19±3.70ng/m³). Total Rhinoconjunctivitis Symptom Score (TRSS) was scored every 30 minutes on a scale of 0 - 24. 202 subjects were randomised to placebo, 8.3nmol ToleroMune Cat 2-weeks apart or 4x6nmol ToleroMune Cat 4-weeks apart. Subjects attended a Post-Treatment Challenge of 4 consecutive days, 3-hours in the EEC 18-22weeks after the start of treatment. 94 subjects were enrolled into a follow-on study without further dosing and had a further 4 consecutive days, 3-hour visits to the EEC 50-54weeks after treatment start.

**RESULTS:** Treatment with 4x6nmol ToleroMune Cat showed a mean change in the TRSS score at the one year EEC visit of -6.78±5.71 versus a change of -2.79±5.28 on placebo (p = 0.01). Secondary endpoints showed statistically significant treatment effects on both nasal and ocular components of the TRSS.

**CONCLUSIONS:** Treatment with four injections of ToleroMune Cat showed a substantial reduction in patients’ cat allergy symptom scores in the EEC model that persists one year after the start of treatment.
547 Eosinophil Derived LTC4 Acts Via CysLT2R to Promote Skin Thickening and Collagen Deposition in a Mouse Model of Allergic Skin Inflammation

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RATIONALE: Eosinophils and cysteinyl leukotrienes (Cys-LTs) have been implicated in tissue remodeling. Atopic dermatitis (AD) is associated with skin thickening and increased collagen deposition in the skin. We studied the role of eosinophils in skin thickening and collagen deposition in a murine model of AD.

METHODS: ddblGATA, the Cys-LT-generating enzyme LTC4 synthase (LTCS S-), CysLT2R-/-, CysLT2R+/- mice and WT controls on BALB/c background were epicutaneously (EC) sensitized with ovalbumin (OVA) over 7 weeks using three one-week sensitization cycles separated by two-week rest intervals. Skin histology was assessed by H&E staining and immunohistochemistry. Collagen content was measured by Sircol collagen dye-binding assay. Cytokine mRNA expression was examined by quantitative RT-PCR.

RESULTS: EC sensitization of WT mice, but not ddblGATA mice, which lack eosinophils, resulted in skin thickening and increased collagen deposition. mRNA expression for LTCS S was increased in WT but not ddblGATA mice. Skin thickening and collagen deposition, but not infiltration with CD4+ cells and eosinophils, were significantly reduced in OVA sensitized skin of ddblGATA mice. Adoptive transfer of bone marrow-derived eosinophils from WT, but not ddblGATA mice, restored skin thickening and collagen deposition in EC sensitized skin of ddblGATA recipients. CysLT2R-/-, but not CysLT2R+/- mice, exhibited reduced skin thickening and collagen deposition following EC sensitization. LTC4 stimulation increased collagen synthesis by human skin fibroblast.

CONCLUSIONS: These results suggest that eosinophil-derived CysLTs signal via CysLT2R to promote skin thickening and collagen deposition in allergic skin inflammation. Strategies that block eosinophil infiltration, CysLT production or CysLT2R might be useful in the treatment of AD.

549 Reduced Regulatory T Cells Due To Streptomycin Treatment Increases Mortality To Respiratory Viral Infection

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RATIONALE: Alterations in the composition of the gastrointestinal microbiota alter the immune system in the intestinal tract and at other mucosal sites. We examined whether antibiotic-induced alteration of the gastrointestinal microbiota affected the respiratory mucosal immune response to Sendai virus (SeV).

METHODS: To alter the intestinal microbiota, mice were given water with or without the non-absorbable antibiotic streptomycin for 2 weeks. They were then intranasally inoculated with a non-lethal dose of SeV (2 x 10^3 pfu). To deplete regulatory T cells (Treg), Foxp3-GFP-DTR mice were given diphtheria toxin intraperitoneally. Flow cytometry was used to quantify T cell subsets.

RESULTS: Addition of streptomycin to the water led to a significantly increased mortality from SeV infection (81% versus 0%, n=23-26; p<0.0001). Surprisingly, the Treg number was decreased in the lung by 47% in mice given the antibiotic (streptomycin, 1.05+/-0.13 x10^7; n=4; versus no streptomycin, 2.0+/-0.18 x10^7; n=3; p=0.01). Similarly, Treg cells in the distal small intestine were decreased by 92% with streptomycin (1.57+/-1.28 x10^5; n=3; versus no streptomycin, 1.97+/-0.44 x10^6; n=3; p=0.03). To determine if reduced Tregs lead to increased mortality, Tregs were depleted in Foxp3-GFP-DTR mice, and we observed 100% mortality to SeV even in absence of streptomycin (n=4; p=0.01 versus PBS treatment).

CONCLUSIONS: Administration of streptomycin markedly increased mortality to SeV and was associated with decreased Treg cells in the gastrointestinal and respiratory tracts. Even in the absence of an antibiotic, depleting Tregs led to high mortality to SeV. Tregs may link gastrointestinal and respiratory mucosal immune systems, especially in response to respiratory viral infections.

548 Exposure to IL-1-family Cytokines Regulates Differential Development of Antigen-Specific Tfh Cells and T Follicular Helper (Tfh) Cells in the Airway

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RATIONALE: IL-1-family cytokines, such as IL-1β and IL-33, are produced by innate immune cells and are implicated in development of CD4+ T cells. To better understand the immunologic mechanisms of allergic diseases, we investigated the roles for IL-1β and IL-33 in development of adaptive immune responses in the airway in vivo.

METHODS: Naïve IL-4 reporter mice were exposed intranasally to endotoxin-free ovalbumin (OVA) with or without IL-33 or IL-1β. Development of adaptive immune responses to OVA was analyzed by collecting draining lymph nodes and by challenging animals with OVA.

RESULTS: Airway exposure of naïve mice to IL-33 plus OVA produced OVA-specific IgE and IgG1. Airway challenge of these mice with OVA induced airway eosinophilia and robust IL-4, IL-5, and IL-13. Exposure to IL-1β plus OVA induced anti-OVA IgE/IgG1 and IL-4, but less pronounced eosinophilia. IL-5 or IL-3, IL-4-producing CD4+ T cells induced by OVA plus IL-33 consisted of ST2+ and ST2- populations. IL-4/ST2+ cells expressed GATA3 and produced large quantities of IL-5 and IL-13. IL-4/ST2- cells expressed Thf markers, including CXCR5, PD-1, ICOS and Bcl-6, and produced less IL-5 and IL-13 as compared to IL-4/ST2+ cells. Mice exposed to OVA plus IL-1β developed distinct IL-4/ST2-CD4+ T cells, but few IL-4/ST2+CD4+ T cells.

CONCLUSIONS: IL-1-family cytokines regulate development of antigen-specific Th2 cells and Thf cells in the airway; these CD4+ T cell populations may be involved in IL-5/IL-13-mediated allergic inflammation and IL-4-mediated IgE/IgG1 antibody response, respectively. Th2-type airway inflammation and antibody response may be regulated by two distinct CD4+ T cell subsets.
Selective Deficiency Of Prostaglandin E2 Uncovers A Dominant Effector Role For Thromboxane A2 In Allergen-Induced Pulmonary Inflammation And Vascular Remodeling

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RATIONALE: Prostaglandin E2 (PGE2) has prominent anti-inflammatory and anti-fibrotic actions in the lung. PGE2-deficient mice are deficient in PGE2 and developed exaggerated pulmonary eosinophilia and hyperplasia of the pulmonary arteriolar smooth muscle compared with PGE2-sufficient controls when challenged intranasally with low doses of a house dust mite extract (Df). We hypothesized that both pulmonary eosinophilia and remodeling observed in the setting of selective PGE2 deficiency depend on thromboxane A2 (TXA2).

METHODS: Double knockout mice lacking both ptges and T prostanoid (TP) receptor (ptges/tpr) mice were generated. After six challenges with Df, bronchial alveolar lavage fluid was collected and cell differential was counted; parabronchial lymph node was harvested and restimulated for cytokine generation analysis; and pulmonary pathological data was collected. E prostanoid (EP) receptor-dependent blockade of intracellular adhesion molecule (ICAM)-1 induction by TP receptor stimulation was studied in vitro using human umbilical vein endothelial cells and antibody blocking of ICAM-1 was performed in vivo.

RESULTS: The deletion of the TP receptor completely eliminated the increments in inflammation, vascular remodeling, effector cytokine generation, and ICAM-1 induction observed in the ptges mice. Dysregulation of TP-dependent ICAM-1 induction was essential for the effects of PGE2 deficiency in vivo. In vitro studies demonstrated that TP receptor signaling was controlled hierarchically by EP1 and EP2 receptor-dependent pathways involving separate protein kinases.

CONCLUSIONS: PGE2-mediated bronchoprotection involves prominent control of TP-dependent effector functions through a novel heterologous mechanism. TP receptor antagonism could be useful therapeutically in asthma associated with low levels of PGE2 production.

Vitamin D Inhibits Monocyte/Macrophage Pro-Inflammatory Cytokine Production By Targeting Mitogen-Activated Protein Kinase Phosphatase-1

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RATIONALE: Vitamin D deficiency has been linked to severity of various allergic diseases. However, the mechanisms of vitamin D-mediated inhibition of inflammation remain poorly understood. In this study, we investigated the inhibitory effects of physiologic levels of vitamin D on lipopolysaccharide (LPS)-stimulated immune responses.

METHODS: Peripheral blood mononuclear cells (PBMC) from normal and asthmatic subjects, and murine bone marrow-derived macrophages (BMM) were pre-incubated with 1,25(OH)2D3 or 25(OH)D3, followed by LPS stimulation. p38 phosphorylation was detected by flow cytometry and Western Blotting. mRNA expression was analyzed by real-time PCR. Vitamin D receptor (VDR) binding and histone acetylation at vitamin D response element in the murine mitogen-activated protein kinase phosphatase-1 (MKP-1) promoter were detected by Chromatin Immunoprecipitation.

RESULTS: Physiologic concentrations of 1,25(OH)2D3, and 25(OH)D3 inhibited LPS induced p38 phosphorylation and IL-6 production in normal subjects by 78% (p<0.01, n=4) and 77% (p<0.01, n=4) respectively. 1,25(OH)2D3 inhibited LPS induced p38 phosphorylation in asthmatic subjects by 57% (p<0.01, n=5). No inhibition of LPS induced p38 phosphorylation was found at 15ng/ml of 25(OH)D3. 1,25(OH)2D3 significantly up-regulated the expression of MKP-1 mRNA in adherent PBMC which consisted mainly of monocytes (2.2±0.2 fold, p<0.01, n=5) and BMM (2.6 ± 0.2 fold, p<0.01, n=3), increased binding of VDR (3.7±0.4 fold, p<0.05, n=3) and histone H4 acetylation (6.26±0.04 fold, p<0.05, n=3) at MKP-1 promoter. In BMM from MKP1-/- mice, the inhibition of LPS-induced p38 phosphorylation by vitamin D was completely abolished.

CONCLUSIONS: This study demonstrates that up-regulation of MKP-1 by vitamin D inhibits LPS-induced p38 activation and cytokine production in monocytes/macrophages.

Oral Food Challenge Tolerance Rates are Higher in Patients with Asthma

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RATIONALE: Patients with food allergy often have asthma and the risk of reaction to food reintroduction is thought to be higher in these individuals. Oral food challenges (OFC) are used to evaluate suspected food allergy or to establish tolerance. We compared reaction rates of patients with and without asthma undergoing OFC.

METHODS: Retrospective chart review was performed for 105 patients (0.75-74 years, 57% male) with clinical food allergy (defined as history of symptoms on exposure and positive skin prick test (SPT) and/or elevated serum specific IgE (sIgE)) or history of sensitization defined by positive SPT and/or elevated sIgE who underwent consecutive OFC between January and December 2011. An OFC failure was defined as immediate or delayed symptoms during or following an OFC. The failure rate of patients with physician diagnosed asthma (n=32) was compared to those without asthma (n=73).

RESULTS: 12.5% (4/32) of patient with asthma failed OFC with reactions including irritability (1), pruritus (1), urticaria (1), emesis (1), cough and wheeze (1). Those without history of asthma failed OFC at a rate of 20.5% (15/73) and had similar reactions including food refusal (2), erythema (3), pruritus (2), emesis (1), urticaria (3), cough and wheeze (3). Treatment of symptoms was similar between groups.

CONCLUSIONS: Patients with asthma had a lower failure rate than those without asthma. A history of asthma was not associated with severity of OFC reaction or resulting intervention.