

**LATE-BREAKING ABSTRACTS  
PRESENTED AT SCIENTIFIC SESSIONS  
AAAAI ANNUAL MEETING  
FEBRUARY 23-27, 2007**

The following abstracts were accepted for presentation after the printing deadline for the abstract supplement that was mailed with the January 2007 issue of The JACI.

**LB1 Enhanced Allergic Responses And IL-13 Induced Airway Inflammation In Mice Carrying The Human IL-4 Receptor Alpha Chain Arginine 576 Polymorphism.**

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**RATIONALE:** Polymorphisms in the IL-4R alpha chain have been associated with both allergies and asthma. Of particular interest is the glutamine to arginine substitution at position 576 of IL-4R alpha (Q576R), which has been associated with atopy and with fatal or near fatal asthma. This polymorphism is particularly prevalent in the African-American population, which suffers disproportionately from complications of asthma.

**METHODS:** To determine whether or not the heightened risk of atopy and asthma are intrinsic to the Q576R polymorphism, we took advantage of the conservation of Q576 residue in the mouse IL-4R alpha to introduce the R576 residue into the murine IL-4R alpha by knockin mutagenesis. The mutant mice were analyzed for alterations in allergic responses.

**RESULTS:** The R576 mutant mice exhibited increased production of IL-4 in a Th2 polarized milieu and heightened total and allergen-induced IgE responses in vivo. Furthermore, the mutant mice exhibited augmented airway hyper-responsiveness, eosinophilia and goblet cell metaplasia following IL-13 inhalation. The R576 substitution was associated with enhanced activation of the extracellular signal-regulated kinases 1/2 (Erk1/2) while leaving unperturbed the activation of canonical IL-4R alpha-coupled pathways including Stat6 and PI3-kinase/Akt.

**CONCLUSIONS:** These results indicate a direct role for the human IL-4R alpha chain R576 substitution in promoting atopy and allergic airway inflammation, and suggest it directly contributes to asthma incidence and severity in carrier populations.

**Funding:** NIH

**LB2 Natural Killer (NK) Cells, Not T Cells, Produce the Cytokine IL-4 in Response to Primary Respiratory Syncytial Virus Infection**

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**RATIONALE:** IL-4 is an important contributor to respiratory syncytial virus (RSV)-induced lung inflammation in the mouse model. However, the cellular source of IL-4 in the setting of RSV infection has previously not been identified.

**METHODS:** Whole lung mononuclear cells were isolated from the lungs of RSV-infected mice four to seven days post-infection. Cells were stained for intracellular IL-4 and interferon-gamma (IFN- $\gamma$ ) and surface markers for NK, NKT, and T cells. Cellular analysis was performed by flow cytometry.

**RESULTS:** Primary RSV infection of wild-type BALB/c mice resulted in NK cell (CD3-CD49b<sup>+</sup>) expression of IL-4, which was not present in NKT or T cells. Further experiments in STAT1-deficient mice revealed a 2.5-fold increase in the number of NK cells expressing IL-4 in response to RSV infection, compared to BALB/c mice. We ruled out NKT cells as the IL-4 source using  $\alpha$ -GalCer-loaded CD1d tetramer analysis and NKT cell-deficient CD1d<sup>-/-</sup>STAT1<sup>-/-</sup> mice. Furthermore, IL-4 deficiency reduced lung IL-13 levels while increasing IFN- $\gamma$  as a result of RSV infection. IL-4 deficiency reduced RSV-induced lung consolidation.

**CONCLUSIONS:** We show for the first time that NK cells express IL-4 and that this is negatively regulated by STAT1 signaling. In addition, IL-4 regulates immune-mediated cytokine production and contributes to immunopathogenesis in RSV infection.

**Funding:** NIH

**LB3 Thymic Stromal Lymphopoietin (TSLP) is Released by Human Epithelial Cells in Response to Microbes and Potently Activates Mast Cells**

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**RATIONALE:** There is accumulating evidence that atopic diseases may be initiated by epithelial cells via the production of thymic stromal lymphopoietin (TSLP) which promotes a dendritic cell-mediated Th2 response. The aim of this study was twofold: (1) to identify some clinically relevant stimuli inducing TSLP production by human airway epithelial cells and (2) to demonstrate that TSLP directly activates primary mast cells (MCs).

**METHODS:** Primary small airway epithelial cells (SAEC) were cultured with bacterial peptidoglycan (PGN), poly I:C or other TLR ligands. The presence of TSLP in culture supernatant was examined by bioassay and ELISA. The SAEC culture supernatants were tested in the presence or absence of TSLP neutralizing antibody for their ability to stimulate CD34<sup>+</sup> progenitor-derived MCs for degranulation, eicosanoids release and cytokines/chemokines production.

**RESULTS:** 1. SAEC released biologically active TSLP in response to PGN and poly I:C. 2. Epithelial cell-derived or recombinant TSLP stimulated MCs for the production of high levels of several Th2 cytokines and chemokines without inducing degranulation or synthesis of eicosanoids.

**CONCLUSIONS:** Direct epithelial cell-mediated and TSLP-dependent activation of MCs might play a central role in the aggravating role of viral infection in asthma and perhaps also in the induction of intrinsic forms of atopic diseases.

**LB4 An Essential Role For IL-21R In The Immune Response To Epicutaneous Sensitization In A Mouse Model Of Atopic Dermatitis**

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**RATIONALE:** Atopic dermatitis (AD) is a common allergic skin disease that affects 10-20% children in developed countries. Although the exact etiology of AD is not completely understood, it is T cell mediated. IL-21, a cytokine derived from activated CD4<sup>+</sup> T cells, modulates Th1 and Th2 immune response. The role of IL-21 in the development of AD and the associated lung inflammation has not been studied.

**METHODS:** We examined the skin, lung and systemic immune response to epicutaneous (EC) sensitization with ovalbumin (OVA) in wild-type and IL-21R KO mice.

**RESULTS:** IL-21R-deficient mice responded poorly to EC sensitization with OVA, resulting in significantly diminished spleen cell proliferation and production of Th1 and Th2 cytokines, reduced serum level of OVA-specific immunoglobulins, and decreased local inflammation in the skin and the lung. Dendritic cells (DCs) from IL-21R-deficient mice were

impaired in their ability to migrate from skin to the draining LN following skin painting with FITC.

**CONCLUSIONS:** Our study establishes a critical role for IL-21 in the migration of dermal DCs to draining LN and in the subsequent allergic response to EC introduced allergen.

**Funding:** NIH R01-AR-047417

## LB5 Understanding Immunological Pathways of Peanut-induced Anaphylaxis

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**RATIONALE:** The effector immune mechanisms underlying murine peanut-induced anaphylaxis (PIA) remain to be fully elucidated.

**OBJECTIVE:** To investigate the role of mast cell activity as a key effector mechanism in the generation of PIA.

**METHODS:** Wild-type C57BL/6, mast cell-deficient and FcγRIII-deficient mice were sensitized by gavage with peanut antigen plus cholera toxin, followed by an intraperitoneal challenge with crude peanut extract.

**RESULTS:** While littermate controls developed severe anaphylaxis (median clinical score, 3.8/5), mast cell-deficient (Kit W/Kit W-v) mice experienced no anaphylactic reactions. Clinical responses correlated with high levels of histamine and leukotrienes in plasma and peritoneal lavage (P.L.) respectively. These mediators were undetectable in Kit W/Kit W-v mice. Moreover, FcγRIII-deficient mice exhibited ~40% reduction in the degree of anaphylaxis compared to wild-type controls. Likewise, histamine levels were decreased by 5-fold in the P.L. of FcγRIII-deficient mice. We are currently investigating FcεRI-deficient mice to ascertain whether binding of IgE to the high-affinity receptor, FcεRI, represents the pre-eminent pathway involved in PIA. To evaluate the impact of leukotrienes, mice were treated with Zileuton, a 5-lipoxygenase (5-LO) inhibitor, orally prior to challenge. Both untreated and treated mice exhibited severe anaphylaxis.

**CONCLUSIONS:** These findings demonstrate a critical role of mast cells in PIA. In addition, they suggest that an IgE triggering pathway of effector responses mediated through IgG Fc receptors (FcγRIII) could contribute to the anaphylactic response. Uncovering novel mast cell mediators of anaphylaxis may reveal new therapeutic targets that could improve clinical responses along with anti-IgE therapy.

**Funding:** Food Allergy Initiative, NY, USA and MedImmune Inc, MD, USA

## LB6 Effect of Human Alpha Defensin-5 (HAD-5) on Cytokine Expression by CaCo-2 Colonocytes: A Possible Mechanism of TH1 Predominance in Crohn's Disease

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**RATIONALE:** Previous Affymetrix Genechip analyses performed in Crohn's colitis patients identified HAD-5 as markedly upregulated in involved areas. IHC confirmed increased protein expression. The role of HAD-5 in CD remains unknown. We hypothesized HAD-5 may directly effect cytokine production by colonic epithelial cells.

**METHODS:** Caco-2 cells were incubated in RPMI-1640 5% FCS with and without 0.5-1 mcg/ml recombinant HAD-5 for 24-48 hours, and supernatants tested for G-CSF, HGF, IL-1β, IL-6, KGF, M-CSF, MIG, MIP-1α, MIP-1β, TGF-β1, TNF-α, VEGF, IL-1α, IP-10, MCP-1, RANTES, EGF, IL-11, LIF by Multiplex Cytokine Assay. Results were confirmed with ELISA (R&D), and also for eotaxin-1. RNA was extracted (Qiagen kits) and semiquantitative RT-PCR performed using Gene Amp EZ rTth RNA PCR kit (Perkin Elmer) followed by gel electrophoresis and ethidium bromide staining. Expected IP-10 product size was 192 bp; GAPDH 225 bp.

**RESULTS:** VEGF, IL-8, IP-10, and MCP-1 were produced by Caco-2 with and without HAD-5. HAD-5 did not induce any of the other cytokines tested. However, HAD-5 at 1 mcg/ml increased IP-10 expression by 24 hours with average control = 210 pg/ml; 0.5 mcg/ml = 210 pg/ml; 1.0 = 280 pg/ml; and doubled IP-10 expression by 48 hours with average control 270 pg/ml; 0.5 mcg/ml = 260 pg/ml; 1.0 mcg/ml = 500 pg/ml; (n=4 experiments). RT-PCR confirmed mRNA expression for IP-10 with no increase detected with HAD-5.

**CONCLUSIONS:** HAD-5 may play a pathogenic role in CD by increasing IP-10 expression, and favoring effector T cell recruitment and the TH1 skewing characteristic of CD. Antagonizing HAD-5 signaling may present a possible novel treatment strategy.

**Funding:** The McCutchen Foundation

## LB7 The First Placebo-Controlled Trial in Cryopyrin-Associated Periodic Syndromes (CAPS): IL-1 Trap (rilonacept) Markedly Reduces Clinical and Laboratory Abnormalities in Patients with Familial Cold Autoinflammatory Syndrome (FCAS) and Muckle-Wells Syndrome (MWS)

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**RATIONALE:** FCAS and MWS, rare autoinflammatory conditions caused by *CIAS1/NALP3* gene mutations, are mediated by IL-1 overproduction. This Phase III trial evaluated the efficacy and safety of IL-1 Trap (IL1T), an inhibitor of IL-1, in FCAS and MWS.

**METHODS:** Randomized, double-blind placebo-controlled studies (A and B) were conducted sequentially in a FCAS/MWS population of 47 subjects. Subjects received weekly IL1T 160 mg or matching placebo (PBO) subcutaneously. A entailed a 6-week treatment comparison. After single blind IL1T treatment, B comprised a 9-week randomized withdrawal comparison. Primary efficacy endpoints were change in disease activity (rash, fever/chills, joint pain, eye redness/pain, and fatigue) scores.

**RESULTS:** In A, subjects treated with IL1T experienced substantial reductions in signs and symptoms. Mean changes at A endpoint were: primary efficacy: PBO, -0.3 (-13%) vs. IL1T, -2.6 (-85%) (P < 0.0001 vs. PBO); serum amyloid A (SAA) in mg/L: PBO, -0.08 (-0%) vs. IL1T, -54.5 (-93%) (P = 0.006 vs. PBO). In B, subjects treated with PBO experienced gradual return of disease activity. Mean changes at B endpoint were: primary efficacy: PBO, 0.94 vs. IL1T, 0.09 (P < 0.0001 vs. PBO); SAA: PBO, 67.4 vs. IL1T, -0.3 (P = 0.01 vs. PBO). There was a greater incidence of injection site reaction (mostly mild) and upper respiratory tract infection (mild to moderate) with IL1T. There were no drug-related serious adverse events.

**CONCLUSIONS:** IL1T produced rapid, profound, and lasting reductions of signs and symptoms. IL1T normalized SAA from levels associated with risk for amyloidosis. A favorable safety and tolerability profile was observed.

**Funding:** Regeneron Pharmaceuticals

## LB8 Effect of Polymorphisms in the Beta<sub>2</sub>-Adrenergic Receptor Gene (*ADRB2*) on Response to Long-Acting Beta<sub>2</sub>-Agonist (LABA) Therapy

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**RATIONALE:** Because of recent interest concerning LABA safety and reports of interaction between regular short-acting  $\beta$ -agonists and *ADRB2* gene polymorphisms, we assessed whether *ADRB2* polymorphisms at amino acid 16, or other single nucleotide polymorphisms (SNPs) in that gene, affect the efficacy and safety of combined inhaled corticosteroid (ICS)/LABA therapy in asthma.

**METHODS:** We analyzed data from a large 6-month double-blind trial (SD-039-0735) from 2250 patients who consented to provide DNA for genetic studies. Patients  $\geq 12$  years old with reversible asthma and symptomatic on ICS received budesonide/formoterol dry powder inhaler (DPI) 160/4.5  $\mu\text{g}$  bid plus additional inhalations as needed, budesonide/formoterol DPI 320/9  $\mu\text{g}$  bid plus as-needed terbutaline, or salmeterol/fluticasone pressurized metered-dose inhaler 50/250  $\mu\text{g}$  bid plus as-needed terbutaline. Effects of SNPs on severe asthma exacerbations, symptoms, lung function, and safety were analyzed.

**RESULTS:** 833 patients were homozygous Gly/Gly, 1029 heterozygous Gly/Arg, and 363 homozygous Arg/Arg. Time to first severe exacerbation was similar among the 3 genotypes for the Gly16Arg polymorphism ( $P=0.312$ ). Across all treatment groups, Gly16Arg genotype had no negative effect on the percentage of patients experiencing severe exacerbations (12% Gly/Gly, 11% Gly/Arg, 9% Arg/Arg). Other clinical outcomes, including symptoms and pulmonary function, did not differ between Gly16Arg genotypes, and no relationship was observed between *ADRB2* haplotypes and study endpoints.

**CONCLUSIONS:** This pharmacogenetic study on the largest *ADRB2* polymorphism dataset studied so far suggests that polymorphisms in this gene do not determine efficacy or safety in reversible asthma patients receiving combined ICS/LABA therapy. For all study endpoints outcomes were similar in Arg/Arg and Gly/Gly genotypes.

**Funding:** AstraZeneca

## **LB9** *Chlamydomydia pneumoniae* (Cpn) Mediated IgE Production by Peripheral Blood Mononuclear Cells (PBMCs) of Allergic Asthmatics is Suppressed by Doxycycline

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**RATIONALE:** Infection with Cpn is associated with exacerbation of asthma. We have previously demonstrated increased Th2 and decreased Th1 cytokine responses to *ex vivo* Cpn infection of PBMC from allergic asthmatics indicating an allergic response to the bacterium. The cell wall of Cpn lacks the peptidoglycan component, which can suppress IgE production. Tetracyclines strongly suppress the *ex vivo* IgE response of allergic asthmatics. This study investigated the effect of *ex vivo* Cpn infection on IgE production in PBMCs of allergic asthmatics.

**METHODS:** PBMC from allergic asthmatics (serum IgE+) and healthy nonatopic controls (IgE-) were mock-infected or infected with Cpn TW-183 at a MOI=1 for 1h. PBMCs were then cultured  $\pm$  Doxycycline (1  $\mu\text{g}/\text{ml}$ ) and supernatants collected on days 2 and 10 post infection. Th1 (IFN- $\gamma$ , IL-12) and Th2 (IL-4) cytokines and levels of total IgE were assayed in supernatants by ELISA.

**RESULTS:** IgE production by PBMC from allergic asthmatics dramatically increased in the presence of Cpn on day 10 (35%,  $p<0.05$ ). IgE increases were accompanied by a Th1 to Th2 switch on day 2. In controls IgE production did not change. In contrast, Cpn mediated IgE production in PBMCs from asthmatic patients was suppressed ( $p<0.05$ ) when doxycycline was added to cultures.

**CONCLUSIONS:** Taken together the results support the hypothesis that Cpn upregulates IgE in allergic asthmatics by causing a Th1 to Th2 switch. Further, tetracyclines show potential as anti-inflammatory drugs in allergic asthmatics.

**Funding:** SUNY Downstate Medical Center

## **LB10** Interleukin-10 In Nasal Mucosa Protects Against Allergic Symptoms

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**RATIONALE:** In allergic diseases, an immune reaction is initiated in response to a harmless antigen. A better understanding of factors crucial in initiating this response could contribute to the development of new therapeutic strategies to cure allergy. Since *in vitro* experiments and mouse models have demonstrated the regulatory capacities of the cytokine Interleukin-10 (IL-10), we investigated the expression of IL-10 in human nasal mucosa.

**METHODS:** We evaluated the expression levels of IL-10 in nasal biopsies from allergic rhinitis patients before and after provocation. All patients had a positive house dust mite (HDM) skin-prick test or radioallergen sorbent test (RAST). IL-10 expression levels were correlated with symptoms (nasal obstruction, rhinorrhoea, sneezing) before and after provocation.

**RESULTS:** IL-10 is expressed in the nasal mucosa of HDM allergic patients. This expression is both located in the epithelium and in the endothelial cells in the blood vessels. Furthermore, it reveals a large variation between individuals. This variation was significantly correlated with allergic symptoms. Patients with high epithelial IL-10 levels at baseline have little symptoms and vice versa ( $r=-0.704$ ). After provocation, patients with high endothelial IL-10 levels have little symptoms and vice versa ( $r=-0.908$ ). A rise in IL-10 level was observed after allergen provocation ( $p=0.032$ ), which normalized to baseline levels 2-4 weeks after provocation ( $p=0.059$ ).

**CONCLUSIONS:** The main immunosuppressive cytokine, IL-10, plays a role in the pathogenesis of allergic rhinitis and could be targeted therapeutically in the treatment of allergic rhinitis.

**Funding:** University of Amsterdam

## **LB11** Distinct Association Between Nonsynonymous Snps Of Candidate Genes And Bronchodilatory Response To Short-acting Inhaled $\beta_2$ Agonist According To Atopic Status

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**RATIONALE:** Inhaled short-acting  $\beta_2$ -agonist is the most effective reliever for exacerbated asthma symptoms, but response to this drug is known to have genetic components. The cellular mechanism that  $\beta_2$ -agonist induce smooth muscle relaxation may interact with lots of signal pathway induced by other molecules. Our previous study revealed that atopic subjects may have less bronchodilatory response to this drug when compared with nonatopic subjects. Under the hypothesis that bronchodilatory response (BDR) to inhaled short-acting  $\beta_2$  agonist is associated with genetic polymorphisms of candidate genes that may influence signaling pathway of  $\beta_2$  adrenoceptor, association study was done in general population.

**METHODS:** BDR at 5 minutes after inhalation of 2 puffs of albuterol inhaler were done in 471 subjects with a reduction of FEV1 more than 20% after methacholine inhalation when compared with baseline FEV1. After screening of informative nonsynonymous coding single nucleotide polymorphism (SNP) with restriction fragment mass polymorphism for candidate genes, SNP scoring was determined by high throughput technique. The statistical significance was evaluated by multiple logistic regression analysis.

**RESULTS:** BDR was associated with nonsynonymous SNPs of GM-CSF2 (I179T,  $p=0.04$ ), IL12 receptor beta1 (M365T,  $p=0.04$ ) in atopic subjects. In contrast, BDR was associated with nonsynonymous SNPs of Fce receptor 1, chain (E237G,  $p=0.03$ ), FGF receptor 4 (I10V,  $p=0.03$ ; R388G,  $p=0.04$ ), IL3 (P27S,  $p=0.04$ ), IL4 receptor (Q576R,  $p=0.02$ ), and IL12 (S226N,  $p=0.04$ ) in non-atopic subjects.

**CONCLUSIONS:** BDR to short-acting inhaled  $\beta_2$  agonist was significantly associated with nonsynonymous SNPs of candidate genes that influence signaling pathway of  $\beta_2$  adrenoceptor, and the association significance was different according to atopic status.

## **LB12** Transcriptome Seasonal Regulation in Ragweed Allergy

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**RATIONALE:** To examine transcriptional regulation in a subset of Immune Tolerance Network ragweed allergy clinical trial participants treated with Omalizumab plus Rush Immunotherapy (RIT), Omalizumab or RIT alone, and Placebo.

**METHODS:** A subgroup of 35 ITN019AD participants as per protocol was randomly chosen and balanced between the 4 treatment groups from one clinical site. 201 peripheral blood samples were collected at study

weeks -9 (baseline, 34 samples), 0 (administration of RIT, 34 samples), 1 (one week post RIT, 33 samples), 5 (early ragweed season, 31 samples), 9 (late ragweed season, 35 samples) and 13 (1 week post ragweed season, 34 samples). Study weeks -9, 0, and 1 are pre ragweed season. Affymetrix U133 2.0 Plus microarrays were hybridized. Clinical average daily allergy severity scores were binned into a high, medium and low categorical scale for correlation to transcriptional regulation.

**RESULTS:** Gene expression differences greater than 1.5 fold were observed between study week 1 to 5 and study week 9 to 13 in all treatment groups ( $p=0.05$ ). These observed transcripts corresponded to immune regulation pathways for IL-10, IL-2, IL-4, and IL-6 and was most pronounced in the Placebo group. An analysis of high and low severity score participants, independent of treatment, showed immune function related genes differentially expressed greater than 1.5 fold at study week 5, early ragweed season ( $p=0.05$ ).

**CONCLUSIONS:** Transcriptional regulation in ragweed allergy is predominantly seasonal, with activation of immune regulation pathways mainly in the Placebo group. Clinical symptoms mirrored gene expression signatures.

**Funding:** ITN, NIAID, NIH, Genentech