**508** Transcriptional analysis of B cells from patients with alpha-gal allergy

Shailesh Choudhary, PhD; 1Univ. of North Carolina at Chapel Hill.

**RATIONALE:** Patients who develop alpha-gal allergy have tolerated mammalian meat and products for years. Understanding the shift in transcriptional programming of alpha-gal IgE-producing B cells is critical to elucidating the switch from immune tolerance to allergen reactivity.

**METHODS:** B cells were enriched from alpha-gal allergic and control subjects by negative selection and sorted for CD22hi/CD38hi/IgE+aGal+CD138+ plasmablasts, one cell/well into a 96-well BD precise plate. Target genes were amplified, sequenced and data were analyzed using BD genomic data view software. In conjunction, additional enriched B cell preparations from control and alpha-gal-allergic subjects were analyzed for targeted gene expression using digital barcoded platform.

**RESULTS:** We detected AG IgE plasmablast in the blood of recent tick bitten subject with median percentage of 0.054 (25% Percentile 0.013, 75th percentile 0.105, N=13). Further a positive correlation was observed between alpha-gal slgE and alpha-gal IgE plasmablast. Projection of data with tSNE plot suggested that genes from subjects with high slgE annotated together. An increase in gene expression of transcription factors and pseudogenes involved in transcriptional regulation were observed in subjects with high slgE. Upregulation of TNF gene expression as well as other inflammation-related products was found in alpha-gal allergic subjects without influence of alpha-gal slgE titer.

**CONCLUSIONS:** Subjects with alpha-gal allergy appear to have a strikingly higher percentage of circulating plasmablasts than control subjects. Moreover, these plasmablasts express a distinct transcriptional repertoire consistent with a robust inflammatory stimulus which likely explains the shift from immune tolerance of red meat to clinical food allergy.

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**509** Expression of T-helper Cytokines by γδ T Cell Subsets in Respiratory Allergic Diseases

Nunu Mitskevich¹, Tamar Tsertsvadze², Natela Melikidze¹, Mariam Sukhiashvili¹, Ketevan Machavariani³, ¹Ivane Javakhishvili Tbilisi State University, ²Ivane Javakhishvili Tbilisi State University, ³Tbilisi State Medical University.

**RATIONALE:** Respiratory allergic diseases like bronchial asthma (BA) and atopic rhinitis (AR) are prevalent and on the rise. T cells play a fundamental role in allergic diseases through the recognition of antigen and secretion of T-helper cytokines. Recent studies have suggested that the less common γδ TCR bearing cells may play important roles in allergic inflammation as effector cells. The controversial information is available about phenotype of cytokine producing CD8+ and γδT cells in humans. This study focused on the characteristics of γδ T cells and their cytokines expression in the patients with BA and AR.

**METHODS:** PBMC were isolated from patients with BA (n=30) and AR (n=28) and healthy controls (n=18). The percentage of T cells producing the cytokines IFN-γ, IL-4, IL-13, IL-17 respectively, of CD4⁺, CD8⁺, γδ T cells was analyzed by Flow Cytometry.

**RESULTS:** The percentages of IL-4⁺ and IL-17⁺ CD4⁺ T cells were increased in patients with BA than in patients with AR, whereas IFN-γ⁺ CD4⁺ T cells decreased in both group of patients (with BA and AR) compared with healthy controls. Higher frequency of IL-4⁺-producing and lower frequency of IFN-γ⁺-producing CD8⁺ γδ T cells were found in patients with BA and AR compared with control group. The ratio IFN-γ⁺/IL-17⁺ among CD4⁺, CD8⁺, γδ T cells was significantly decreased in patients with BA and AR compared with healthy controls.

**CONCLUSIONS:** CD8⁺ and γδ T cells are involved in the pathogenesis of bronchial asthma and atopic rhinitis through the expression of Th1, Th2 and Th17 type cytokines.

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**510** P2Y2 Is an Epithelial Brush Cell Receptor For ATP-Elicited Cysteinyl Leukotrienes Generation

Saltanat Ualiyeva¹, nils hallen², Wolfgang Junger³, Carola Lederose³, Nora Barrett, MD FAAAI¹, Lora Bankova, MD¹, ²Brigham and Women’s Hospital, ³Department of Surgery, Beth Israel Deaconess Medical Center.

**RATIONALE:** Brush cells (BrCs) are IL-25 producing cells in the airways best known as chemosensors for bacterial-derived metabolites. We have previously reported a high functional capacity of BrCs to generate cysteinyl leukotrienes (CysLT). However, specific BrC activating receptors that induce CysLT generation and their importance for type 2 immunity have not been defined.

**METHODS:** Nasal BrCs were sorted from WT, ChAT-eGFP, P2ry2⁻/⁻ (lacking the P2Y2 ATP receptor) and Li-4s⁺ mice (that cannot generate CysLT) for ex vivo stimulation with the fungal aeroallergen Alternaria or with the nucleotide ATP, which is released by Alternaria-stimulated BrCs. CysLT generation was measured by ELISA. The specificity of purinergic receptors was assessed using pharmacologic inhibitors and the P2ry2⁻/⁻ strain. Nasal and bronchoalveolar lavages (BAL) were obtained 1 or 24 hours after inhalation of Alternaria or ATP in WT and BrC-deficient Pou2f3⁻/⁻ mice.

**RESULTS:** Stimulation of BrCs with ATP, UTP or Alternaria induced CysLT production in a dose-dependent manner. Pre-treatment of BrCs with a P2Y2 receptor inhibitor significantly reduced nucleotide and Alternaria-elicited CysLTs. P2ry2⁻/⁻ BrCs did not generate CysLTs in response to ATP. Finally, intranasal ATP or Alternaria triggered CysLT generation, which was abolished in Pou2f3⁻/⁻ mice. Furthermore, Alternaria-elicited BAL eosinophilia was reduced in Pou2f3⁻/⁻ mice.

**CONCLUSIONS:** CysLT generation is an effector function of airway BrCs, triggered by P2Y2 recognition of endogenously generated alarmins in response to aeroallergen. These results identify a novel epithelial cell activation pathway of innate epithelial generation of CysLTs leading to type 2 inflammation.

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**511** Dual blockade of IL-4 and IL-13 with dupilumab, an IL-4Rα antibody, is required to broadly inhibit type 2 inflammation

Audrey Le Floch-ramondu¹, Kirsten Nagashima¹, George Scot¹, Dylan Birchard¹, Seblewongel Asrat, PhD², Yu Bai, PhD³, Wei Keat Lim¹, Andrew Murphy, PhD², Matthew Sleeman, PhD¹, Jamie Orenge, PhD³, ¹Regeneron Pharmaceuticals, ²Regeneron, ³Regeneron Pharmaceuticals Inc.

**RATIONALE:** Dupilumab, a fully human monoclonal antibody that binds IL-4Rα and inhibits signaling of both IL-4 and IL-13, has shown efficacy across multiple diseases with underlying type 2 signatures and is approved for treatment of asthma, atopic dermatitis and chronic sinusitis with nasal polyposis. We sought to provide a comprehensive analysis of the redundant and distinct roles of IL-4 and IL-13 in type 2 inflammation and report the mechanism of action dupilumab.

**METHODS:** Using primary cell assays and a mouse model of house dust mite (HDM) induced asthma, we directly compared IL-4 vs IL-13 vs IL-4Rα blockers.

**RESULTS:** Intranasal administration of either IL-4 or IL-13 in mice causes lung inflammation by increasing immune cell infiltration, including eosinophils, increasing lung cytokine and chemokine expression as well as GCM, thus demonstrating redundant functions of these cytokines. We further teased out their contributions using human in vitro culture system. Then, in a mouse asthma model by comparing in head to head studies, either IL-4 or IL-13 inhibition to dual IL-4/IL-13 inhibition, we demonstrate that blockade of both IL-4 and IL-13 is required to broadly block type 2 inflammation, which translates to protection from allergen-induced lung function impairment. Notably, only dual IL-4/IL-13 blockade prevented eosinophil infiltration into lung tissue without affecting circulating eosinophils, demonstrating that tissue, but not circulating eosinophils contribute to disease pathology.

**CONCLUSIONS:** Overall, these data support IL-4 and IL-13 as key drivers of type 2 inflammation and help provide insight into the mechanism of action of dupilumab, a dual IL-4/IL-13 blocker.