SUNDAY

## 527 A New Immunotherapy Approach: Mucosal Immunization With An Il-13 Vaccine Suppresses Murine Airway Allergic Responses

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**RATIONALE:** Previously we have shown that subcutaneous administration of an IL-13 peptide-based vaccine, developed in our laboratory, significantly suppresses murine airway allergic inflammation. Now, we sought to assess whether mucosal immunization with the IL-13 vaccine is better than subcutaneous immunization and its underlying mechanisms.

**METHODS:** An IL-13 vaccine employing Hepatitis B core antigen as a carrier with an insert of the selected IL-13 peptide was constructed using gene engineering method and expressed as a fusion protein. BALB/c mice (8/group) were immunized intranasally three times with the vaccine, vaccine carrier, or phosphate-buffered-saline. Another group of mice was immunized subcutaneously with the vaccine. Following immunization, mice were sensitized twice intraperitoneally and challenged once intranasally with ovalbumin. Methacholine-induced airway hyperresponsiveness was measured 2 days and bronchoalveolar lavage fluids (BALF) were collected 5 days after intranasal challenge. IL-13 specific-IgA in BALF and -IgG in serum were assayed using ELISA. Differential cell counts of inflammatory cells in BALF were performed.

**RESULTS:** Mice immunized intranasally, not subcutaneously, with vaccine produced significantly higher levels of IL-13 specific-IgA in BALF. Intranasal immunization also produced stronger serum IL-13 specific-IgG responses than subcutaneous immunization. BALF eosinophil percentages and airway hyperresponsiveness were also significantly reduced in mice receiving intranasal administration of vaccine, when compared to mice receiving carrier intranasally, or receiving vaccine subcutaneously as well as receiving saline.

**CONCLUSIONS:** Intranasal administration of IL-13 vaccine is more effective in suppressing airway allergic responses than subcutaneous immunization, which may provide a potential therapeutic approach in asthma.

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## 528 Role of the RNA-binding Protein Tristetraprolin (TTP) in Glucocorticoid (GC)-mediated Gene Regulation

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**RATIONALE:** The TTP protein family includes three RNA-binding factors, TTP, Butyrate-response Factor (BRF)-1 and BRF-2, that mediate acceleration of mRNA decay of early-response and inflammatory genes. We investigated whether GC treatment would influence their expression in order to exert posttranscriptional gene regulation, and whether lack of TTP would affect GC response.

**METHODS:** Expression of TTP, BRF-1 and BRF-2 was evaluated by real-time PCR and Western blot in the human airway epithelial cell line BEAS-2B cultured in the presence of the topical GC budesonide ($10^{-7}$ M) or diluent for different time points. Mouse embryonic fibroblasts (MEFs) from $TTP^{-/-}$ mice and their wild-type (WT) littermates were stimulated with medium or TNFalpha in the presence of budesonide or diluent, and total RNA was extracted to examine gene expression using Illumina arrays.

**RESULTS:** Budesonide time-dependently upregulated TTP and BRF-1 mRNA in BEAS-2B (mean ± SEM fold over control, 5.1 ± 0.8 [n=3] and 2.3 [n=2], respectively). TTP protein was also upregulated by budesonide in BEAS-2B and WT MEFs (3.0 ± 0.7 [n=4] and 2.3 [n=1]). Preliminary analysis of the array-based results revealed major differences between WT and $TTP^{-/-}$ MEFs in gene expression driven by either GC or TNFalpha, as well as for genes whose regulation was shared by TNFalpha and GC. Inhibition by GC of TNFalpha-induced chemokine and inflammatory genes (CCL5, CX3CLl1, CXCL5, CXCL10 and IL-6) was highly significant in WT (*p* values between $10^{-5}$ and $10^{-3}$), but not in in $TTP^{-/-}$ MEFs.

**CONCLUSIONS:** TTP is a GC-inducible gene and a potentially critical mediator of GC anti-inflammatory action.

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## 529 Comparison of Expression and Cellular Provenance of Thymic Stromal lymphopoietin and Chemokines in Patients with Severe Asthma, COPD and Controls

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**RATIONALE:** Asthma and chronic obstructive pulmonary disease (COPD) are suggested to result from Th2-type and Th1-type airway inflammation respectively. Thymic stromal lymphopoietin (TSLP) favours Th2 type inflammation. We hypothesised that airways expression of TSLP and Th2-attracting chemokines is increased in asthma, but not COPD, where Th1-attracting chemokines predominate.

**METHODS:** We used *in situ* hybridization and immunohistochemistry to examine the expression and cellular provenance of TSLP, Th2-attracting (TARC/CCL17, MDC/CCL22, and I-309/CCL1) and Th1-attracting (IP-10/CXCL10 and TAC/CXCL11) chemokines in bronchial biopsies from 13 patients with severe asthma, 15 with COPD (ex- and smokers) and 30 normal controls (non-smoker, ex-smoker and smoker, 10 for each group).

**RESULTS:** The numbers of cells within the bronchial epithelium and submucosa expressing mRNA for TSLP, TARC/CCL17, MDC/CCL22 and IP-10/CXCL10, but not I-TAC/CXCL11 and I-309/CCL1, were significantly increased in severe asthma and COPD as compared with non-smoker controls. Expression of these molecules was also increased in ex- and smokers as compared with normal non-smokers. TSLP and TARC/CCL17 expression correlated inversely with airways obstruction in asthma and COPD. Sequential IHC/ISH showed that epithelial cells, endothelial cells, neutrophils, macrophages and mast cells were the sources of TSLP and Th1 and Th2 attracting chemokines. The cellular provenance of these mediators was strikingly similar in severe asthma and COPD.

**CONCLUSIONS:** Our data implicate TSLP and both Th1- and Th2-attracting chemokines in the pathogenesis of asthma and COPD, and provide evidence for uniformity in the origins and expression of these mediators, particularly in the epithelium, in obstructive airways disease.

## 530 The Role Of Il-25 In Airway Allergic Response

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**RATIONALE:** IL-25 is a novel cytokine belonging to the IL-17 family. Systemic overexpression of IL-25 in mice resulted in Th2 type pathologies, including eosinophilia, increases in Th2 cytokines and serum IgE, suggesting that IL-25 may be important in pathogenesis of allergic diseases such as asthma.

**METHODS & RESULTS:** To test this idea and to understand the function of IL-25, we first characterized the distribution of IL-25 receptor (IL-17RB). IL-17RB mRNA was found expressed by lung epithelial cells, T cells, and B cells. Treatment of a lung epithelial cell line by IL-25 induced the upregulation of type II chemokines and mucin gene, implicating a role of IL-25 in regulating innate allergic response. We further examine the function of IL-25 in asthmatic disease by generating lung-specific IL-25 transgenic mice. Pathological analysis revealed mucus hyperplasia, eosinophilia, CD4+ T cell and dendritic cell infiltration, supporting a role of IL-25 in initiating allergic responses in the airway. Treatment with an IL-25 neutralizing antibody in an allergen-induced experimental asthma model resulted in the reduction of lung eosinophil and CD4+ T cell infiltration and Th2 cytokine production in BAL fluid.

**CONCLUSIONS**: Our results indicated an important role of IL-25 in initiating allergic responses. IL-25 is thus a potential target in treatment of allergic diseases.

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