Background: Airway eosinophilia and Th2 lymphocytes-recruitment to the lung are one of the main pathological features of asthma. It is clear now that the axis chemokine/chemokine receptors have a role in controlling leukocyte recruitment and development of the inflammatory process observed in asthma. Although it has been reported that CCR9 receptor is expressed in asthmatic patients, it is not known whether CCR9 may have a regulatory role of the development of this disease. Our aim was to analyze the expression of CCR9 in a murine model of allergic airway inflammation (WT) and compared to CCR9-deficient (KO) mice.

Methods: Four groups of 6 to 8 weeks female CCR9-deficient mice were sensitized by intraperitoneal injections of 10 micrograms of ovalbumin (OVA) in alun (ALO3) diluted in PBS, on days 1 and 8 of the established sensitization protocol. Aerosolised OVA was administered (1% in PBS) on days 15, 20 and 34. 24 hours after last OVA exposure, mice were sacrificed and bronchoalveolar lavage (BAL) fluid and cells were obtained. Total and differential cell numbers were obtained and characterized cell subpopulations by FACS analysis. Cytokine/chemokine levels were quantified by ELISA and qRT-PCR respectively.

Results: Total cell numbers in BAL were no significantly different between WT and KO mice. Interestingly, reduction in the numbers of eosinophils was observed in CCR9 KO mice compared to WT mice. Histological analysis of lung tissue demonstrated a reduction in the granulocytic population (eosinophils) in CCR9 KO mice. Analysis of cell subpopulations by FACS demonstrated that CD4+ lymphocytes were significantly reduced but CD8+ and CD19+ lymphocytes numbers were not different between WT and CCR9-deficient mice. A population of CCR9+ Gr1+ was altered in KO mice and it correlated with cytological analysis. Furthermore, histological analysis demonstrated alteration in mucus production in allergic airway in CCR9 deficient mice, accompanied with a non-significant reduction of OVA-specific anti-IgE antibodies in serum at the time of analysis.

Conclusions: Altogether, these results suggest that CCR9 may be involved in recruitment of granulocytic cell subpopulation into the allergic airways and have an impact in the regulation of the chronic inflammatory process.

MECHANISMS OF ASThma AND ALLERGIC INFLAMMATION

123 Serum IL6 and Soluble IL6R Are Correlated With Lung Function in Non-Hispanic Whites with Asthma

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Background: Interleukin 6 (IL6) belongs to a family of cytokines with both pro- and anti-inflammatory properties. The functional relationship between IL6 signaling and airway disease has not be well characterized; however, IL6 expression is increased during lung inflammation and injury. In this study, serum IL6 and soluble IL6R levels were assessed in non-Hispanic whites with asthma from the Severe Asthma Research Program. Correlations between serum IL6 and IL6R levels, lung function, phenotypic asthma clusters, and asthma severity were evaluated.

Methods: Serum IL6 and soluble IL6R was measured in 149 subjects with mild to severe asthma. Serum sIL6R levels were measured using the sIL-6R DuoSet (R&D Systems, Minneapolis, MN) ELISA kit and reported as ng/ml. Serum IL6 measurements were determined using the IL-6 ELISA kit (R&D Systems, Minneapolis, MN) and reported as pg/ml. Serum IL6 and sIL6R measurements were transformed to normalize distribution. The continuous variables analyzed included: % predicted FEV1 [ppFEV1], % predicted FVC [ppFVC], and FEV1/FVC. Serum samples were collected at Wake Forest. Phenotypic asthma clusters were derived as previously described (Am J Respir Crit Care Med. 2010;181:315–323).

Results: Elevated serum IL6 was associated with lower ppFEV1 (P = 0.02) and lower ppFVC (P = 0.003), while elevated serum soluble IL6R was associated with lower ppFEV1 (P = 0.02) and lower ppFVC (P = 0.008). Increasing trends in serum IL6 were observed in atopic asthma Clusters 2 and 4 and the later onset fixed airways obstruction Cluster 5. The highest IL6 serum levels were observed in Cluster 3 characterized has having late onset asthma and elevated BMI. Serum IL6 levels were elevated in subjects with severe asthma (log IL6 = 0.33; N = 25) compared to subjects with mild/ moderate asthma (log IL6 = 0.16; N = 69).

Conclusions: Serum IL6 and sIL6R levels are elevated in non-Hispanic white asthma subjects with lower lung function. Serum IL6 and sIL6R are potentially important biomarkers that may distinguish between non-severe and severe asthma and between atopic asthma Clusters.

124 Heterogeneity of Allergen Epitope-specific CD4+ T Cells Responses: Steps Toward Optimal Composition for Peptide-based Immunotherapy

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Background: Peptide-based allergen immunotherapy is a promising alternative to conventional allergy vaccine. However, the optimal composition of such vaccines, in terms of the choice of the appropriate peptides, has remained unclear. Knowledge of the epitope-specific T cell responses to allergens can give important information on the pathogenesis and regulation of allergic inflammation. In this study we sought to identify candidate allergen-epitopes that can be used to improve peptide-based allergen immunotherapy.

Methods: Tetramer Guided Epitope Mapping was first used to identify CD4+ T cell epitopes for group 1 and group 5 timothy grass pollen allergens. MHC class II tetramer technology was then used in an ex vivo approach to assess the grass pollen-specific CD4+ T cell responses in allergic and non-allergic individuals. The frequency, surface marker phenotype and cytokine profile of these cells were directly analysed by flow cytometry.

Results: CD4+ T cell responses to Timothy grass allergens are directed to a broad range of epitopes characterized by defined immunodominance hierarchy patterns. We observed heterogeneity of phenotype within the allergen-specific CD4+ T cells that depends on the epitope for which the cells are specific. T cell epitopes associated with production of IL-10 or IFN-γ are recognized at low frequencies in both allergic and healthy individuals. In contrast, allergy-associated epitopes are only recognized in allergic individuals by high frequency, terminally differentiated allergen-specific CD4+ T cells, which are susceptible to deletion by repeated stimulation with high doses of antigen. Allergen-specific immunotherapy caused significant changes in the epitopes hierarchy of the grass pollen allergen-specific memory CD4 T cell pool.

Conclusions: The ability to evaluate epitope-specific T cell responses to allergens can give important information on the pathogenesis and regulation of allergic inflammation and could be of great use in designing peptide-based allergy vaccination strategies. Some epitopes may play a prominent role in
driving a protective response, while others may directly impair the pathogenic response.

125
The Association between Mast Cells and Remodelling of the Small Airways in Chronic Asthma

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Background: Repeated airway challenges with House Dust Mite (HDM) allergen results in marked remodelling and mast cell hyperplasia in the small airways of allergic sheep. We now examine mast cell activation and its association with small airway function and remodelling in these sheep using a novel segmental allergen challenge approach.

Methods: Eight allergic sheep received weekly intra-lung challenges of HDM to the left caudal lung for 24 weeks. Eight separate sheep were used as controls. Baseline lung function was assessed in the left caudal segments of all sheep throughout the challenge regime using a wedged-bronchoscope technique. Airway tissue was collected from challenged segments from all sheep, 7 days following the final intra-lung challenge. The airway tissues were immunohistochemically labelled for chymase-mast cells and eosinophils. Collagen and airway smooth muscle content were assessed on Masson’s Trichrome stained sections.

Results: Resting lung function in the left caudal segment is elevated in 4 out of 8 sheep at the end of the repeated allergen challenge regime. Chymase mast cell density was significantly increased in the small bronchial walls of the HDM-challenged group compared to the control group (52 ± 8 vs 8 ± 4, P < 0.01). There were significant increases in bronchial collagen deposition in HDM-exposed segments compared to control segments (0.17 ± 0.02 vs 0.11 ± 0.02 mm²/BM, P < 0.05). A correlation analysis of individual sheep data showed that there was a trend for a direct association between the increases in bronchial collagen deposition and the density of chymase-labelled mast cells (rs = 0.71, P = 0.088). Eosinophil density in the small bronchial walls of HDM-challenged segments was also significantly increased compared to controls (65 ± 19 vs 11 ± 3 cells/mm², P < 0.001), but not associated with collagen content. The bronchial smooth muscle content was not different between HDM-challenged and unexposed control segments.

Conclusions: The results show that repeated exposure to allergen results in significant increases in density of chymase-labelled mast cells, together with increased levels of collagen content in the small airways. The segmental challenge protocol allows for a novel approach to characterise the progressive remodelling events occurring in the small airways in chronic asthma.

126
Creation of a Humanized Model for Respiratory Allergy Using a Human Mugwort-specific T-Cell Receptor and HLA-DR1

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Background: Currently, T cell receptor (TCR) transgenic (tg) mice with a murine TCR specific for chicken ovalbumin in the context of a murine restriction element (I-A<sup>4</sup>) are frequently used in allergy research to investigate T helper cell differentiation and allergy treatment in vivo.

Methods: We here aimed to generate double tg mice expressing a human TCR specific for the immuno-dominant epitope of the major mugwort (Artemisia vulgaris) pollen allergen Art v 1 in the context of the human restriction element HLA-DR1 to provide a valid model for studying allergy development and treatment in vivo. To obtain high expression levels the allergen-specific human TCR variable sequences were chimerized with murine TCR constant sequences. Resulting transgenes were cloned into the pT<sub>max</sub> vector system and thus put under the transcriptional control of the natural TCR alpha and beta promoter/enhancer elements. Allergen-specific TCR tg founder mice were cross-bred with HLA-DR1<sup>+</sup>B10.M-DR1<sup>+</sup>Aa mice.

Results: Immunophenotyping of double tg TCR/HLA-DR1 mice revealed clear-cut expression of the Art v 1-specific TRBV18 chain on peripheral blood CD3<sup>+</sup> T lymphocytes and HLA-DR1 expression on CD14<sup>+</sup> monocytes and B220<sup>+</sup> B lymphocytes. In vitro, splenocytes from TCR/HLA-DR1 double tg mice but not of HLA-DR1 single tg mice or wt mice specifically proliferated upon incubation with the human-relevant immuno-dominant Art v 1<sub>15 to 36</sub> Peptide or whole Art v 1 protein. No proliferation was observed upon incubation with control peptides or proteins. Allergen-specific cellular proliferation is accompanied by the production of a balanced cytokine milieu including IFN-gamma, IL-2, IL-4, IL-6, IL-13 and IL-17 (>50 pg/mL per 2 x 10<sup>5</sup> splenocytes). No cytokine secretion was evident upon incubation of splenocytes with a control peptide or medium alone. Importantly, double tg mice are proficient to mount both IgG2a and IgG1, IgE responses when i.p. immunized with antigen plus alum.

Conclusions: A fully humanized allergy model, in which all components of the allergen-specific synapse are well-defined enables to analyze the relevant T-cell dependent (and independent) pathways by which allergic diseases can be influenced in vivo and will provide important insights into the pathophysiologic of allergic diseases and their possible cure in the future.

127
Eosinophils Enhance Airway Smooth Muscle Cell Proliferation Via the Release of Cysteinyl Leukotrienes

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Background: Asthma is a chronic inflammatory disorder of the lung airways that is associated with airway remodeling and hyperresponsiveness. Its is well documented that the smooth muscle mass in asthmatic airways is increased due to hypertrophy and hyperplasia of the ASM cells. Moreover, eosinophils have been proposed in different studies to play a major role in airway remodeling. Here, we hypothesized that eosinophils modulate the airways through enhancing ASM cell proliferation. The aim of this study is to examine the effect of eosinophils on ASM cell proliferation using eosinophils isolated from asthmatic and normal control subjects.

Methods: Eosinophils were isolated from peripheral blood of 6 mild asthmatics and 6 normal control subjects. ASM cells were incubated with eosinophils or eosinophil membranes and ASM proliferation was estimated using thymidine incorporation. The mRNA expression of extracellular matrix (ECM) in ASM cells was measured using quantitative real-time PCR. The effect of eosinophil-derived proliferative cytokines on ASM cells was determined using neutralizing antibodies. The role of eosinophil derived Cysteinyl Leukotrienes in enhancing ASM was also investigated.

Results: Co-culture with eosinophils significantly increased ASM cell proliferation. However, there was no significant difference in ASM proliferation following incubation with eosinophils from asthmatic versus normal control subjects. Co-culture with eosinophil membranes had no effect on ASM proliferation. Moreover, there was no significant change in the mRNA