24 Common and Rare Variation in the T Helper 2 Gene Pathway Predicts Allergic Asthma Phenotypes

Rebecca Slager, Wake Forest University
Wendy Moore, Wake Forest University
Huashi Li, Wake Forest University
William Busse, University of Wisconsin
Maria Castro, Washington University
Serpi Erzurum, Lerner Research Institute
Anne M Fitzpatrick, Emory University
Sally Wenzel, University of Pittsburgh
Deborah Meyers, Wake Forest University
Eugene R. Bleecker, Wake Forest University

Journal Title: World Allergy Organization Journal
Volume: Volume 5
Publisher: BioMed Central | 2012-02, Pages S8-S9
Type of Work: Article | Final Publisher PDF
Publisher DOI: 10.1097/01.WOX.0000411769.14940.76
Permanent URL: https://pid.emory.edu/ark:/25593/s5nnq

Final published version: http://dx.doi.org/10.1097/01.WOX.0000411769.14940.76

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Results: EXPB1, an Arabidopsis protein (belonging to the beta expansin multi gene family), showed significant sequence and structural similarity to Cyn d 1. This protein was expressed in E. coli and the recombinant protein did not react with serum IgE from grass pollen allergic patients, suggesting that EXPB1 represented a non-allergenic homologue of grass group 1 allergens. It is proposed that differences in the amino acid sequence are responsible for the difference in the allergenicity profile of the Arabidopsis and grass pollen proteins.

Conclusions: Our study provides valuable data for further investigations of the molecular basis of allergenicity and cross-reactivity of grass group 1 allergens.

22 Protein-Protein Interactions Determine IgE Reactivity to Polygalacturonase From Cupressus sempervirens Pollen

Youcef Shahali, Jean-Pierre Sutra, PhD, Sylvie Chollet-Martin, Iman Haddad, Joëlle Vinh, PhD, Adriano Mari, MD, Denis Charpin, Hélène Sénéchal, PhD, and Pascal Poncet, PhD.

Background: In a recent proteomic study, we identified in Italian cypress (Cupressus sempervirens, Cups) pollen grains, 2 proteins at 43 and 60 kDa, homologous to already known Cupressaceae polygalacturonase (PG) proteins. The 60-kDa PG is suspected to be a multi-protein complex including the 43-kDa PG and one or more proteins with lectin-like properties.

Objective: In the present study, cypress pollen PGs were further characterized and the molecular basis of their allergenicity including the presence of specific IgE directed against cross-reactive carbohydrate determinants (CCDs) were investigated.

Methods: Cups pollen PBS extracts were characterized using 2- and double one-dimensional electrophoresis followed by IgE immunoblotting. The IgE reactivity to carbohydrate- versus peptide-specific determinants was investigated using both bromelain inhibition and Con A-binding assays. Pollen proteins were also prefractionated in their native forms using size exclusion chromatography. The presence of multi-protein complexes were investigated by using 2-D blue native (BN)-PAGE/SDS-PAGE electrophoresis.

Results: Upon bromelain inhibition assay, we revealed that 70% of tested patients displayed CCD-specific IgE to the 43-kDa PG while its isoenzyme of 60 kDa appeared to be exclusively recognized for its peptide-specific determinants. The specific binding of the Con A lectin to the 43-kDa PG, and not to the 60-kDa isoenzyme, demonstrated the presence of exposed mannose-containing oligosaccharides only on the 43-kDa protein. This fact reflects fundamental differences between specific IgE-binding epitopes involved in the recognition of the 43-kDa and 60-kDa proteins making these 2 cypress pollen PGs immunologically distinguishable. The present results suggest that in the 60-kDa protein complex, the CCDs of the 43-kDa PG are not exposed due to the binding of a lectin-like protein exhibiting peptidic IgE reactive epitopes recognized by 25% of tested patients.

Conclusion: The current study demonstrates that the sensitization to the Cups pollen PG is mainly due to CCD bromelain-type epitopes and directly associated with an increased prevalence of IgE reactivity to cypress pollen extracts due to CCD interference. However, the Cups pollen PG and its carbohydrate-specific determinants seem to play a key role in the dynamics of protein-protein interaction in cypress pollen and may confer to protein complexes a higher allergenicity.

23 Grafting of BET V 1 Epitopes onto its Homologue API G 1 Reveals Patient-Specific IgE Recognition Profiles

Barbara Gepp, Nina Balazs, Wolfgang Hemmer, Christian Radauer, PhD, and Heimo Breitenleier, PhD. Department of Pathophysiology and Allergy Research, Medical University of Vienna, Vienna, Austria; 2 Floridsdorfer Allergiezentrum, Vienna, Austria.

Background: Up to 70% of birch pollen-allergic individuals show adverse reactions to certain plant foods. This cross-reactivity is caused by sensitisation to the major birch pollen allergen Bet v 1 and binding of Bet v 1-specific IgE antibodies to homologous plant food allergens. We aimed to assess the importance of selected conformational epitopes for IgE binding to Bet v 1.

Methods: Chimera of Bet v 1.0101 and its homologue Api g 1.0101 were constructed. In each of the 4 chimeras, roughly one fourth of the surface residues of Api g 1.0101 were replaced by corresponding residues of Bet v 1.0101. The proteins were expressed in Escherichia coli and purified by chromatographic methods. Secondary structures were checked by CD-spectroscopy. IgE ELISA with Bet v 1.0101, Api g 1.0101 and the chimeras were performed with sera of 63 Bet v 1-sensitized birch pollen allergic patients. For inhibition ELISAs, chimeras were coated and inhibition was performed with the chimeras or Api g 1.0101.

Results: IgE binding to Api g 1.0101, Api-Bet-1, -2, -3 and -4 was observed for 22, 81, 79, 70 and 38% of the sera, respectively. To assess the relevance of the grafted regions for IgE binding to Bet v 1, the amounts of IgE binding to the chimeras were compared with those to Api g 1.0101. Most of the sera recognised either 3 chimeras (39%) or all 4 chimeras (21%) better than Api g 1.0101. Only a minority of the sera showed increased binding to a single chimesa. Inhibition ELISAs confirmed the presence of IgE specific for the grafted regions.

Conclusions: Our study indicates that the epitope recognition profile of Bet v 1-specific IgE is highly patient specific. Due to the different IgE binding patterns to Bet v 1, determined by binding of IgE to different chimeras, the existence of a single major IgE epitope on Bet v 1 can be excluded. Moreover, the Bet v 1-specific IgE repertoire is polyclonal and the IgE epitopes are distributed over the whole surface of Bet v 1.

This study was supported by grants P22559-B11 (CR) and SFB-F01802 (HB) from the Austrian Science Fund.
allergic asthma (Moore WC, et al AJRCCM, 2010). We hypothesized that common and potentially deleterious rare variation in this pathway would be associated with severe asthma based on SARP cluster designation.

**Methods:** To evaluate common variants (minor allele frequency or MAF >5%), 419 SARP non-Hispanic white participants with a cluster assignment were genotyped for 182 single nucleotide polymorphisms (SNPs) in Th2 pathway genes using whole-genome SNP data. Individual SNPs and a cumulative model of significant SNPs were evaluated using contingency tables with a chi-square test for trend and ordinal regression models adjusted for age, sex, and principal components. Rare (MAF <5%) amino acid changes and splice site alterations in this pathway were tested for association with asthma severity outcomes in 20 SARP subjects with whole exome sequence data.

**Results:** Individual Th2 pathway variants were associated with overall SARP cluster assignment, and allergic clusters of increasing severity (1, 2, and 4), including GATA3 polymorphism rs1244186 ($P = 0.005$). In an 18-SNP additive model, an increasing number of Th2 pathway risk genotypes were highly associated with severe allergic asthma ($P = 3.9 \times 10^{-6}$). For example, in cluster 4, the percentage of subjects with at least 9 risk genotypes was 83% compared to 35% in cluster 1. Additionally, there was evidence that subjects with rare variants in this pathway were more likely to report allergy symptoms ($P = 0.006$), especially in the fall ($P = 0.003$), compared to subjects with no rare variants.

**Conclusions:** Common Th2 pathway variants predict an increased likelihood of severe allergic asthma and rare variants were associated with increased seasonal allergy symptoms.

### 25 Role of Myeloid Derived Suppressor Cells in Asthma

Allison Nelson, Jim Parkerson, DO, Richard F. Lockey, MD, Subhra Mohapatra, PhD, Shyam Mohapatra, PhD, and Srinivas Nagaraj, PhD. 
1Nanomedicine Research Center and Divisions of Translational Medicine and Allergy and Immunology, Department of Internal Medicine, University of South Florida, Tampa, FL; 2Division of Allergy & Immunology, University of South Florida and James A. Haley Veterans’ Hospital, Tampa, FL; 3Nanomedicine Research Center and Division of Translational Medicine, Departments of Molecular Medicine and Internal Medicine, University of South Florida and James A. Haley Veterans’ Hospital, Tampa, FL; 4USF Nanomedicine Research Center, University of South Florida College of Medicine & VA Hospital, Tampa, FL.

**Background:** We know that a heterogeneous group of myeloid cells termed myeloid derived suppressor cells (MDSC) accumulate in almost all pathological conditions, which elicit an inflammatory signal. The exact role played by these cells in asthma is not known. In this study we investigated the function and role of these cells in asthma.

**Methods:** Accumulation of MDSC and other subsets of myeloid cells were analyzed from peripheral blood mononuclear cells from patients with non-severe asthma (FEV$_1$ >60) and severe asthma (FEV$_1$ <60) by multicolor-flow cytometry and compared to healthy controls. Allergic mouse models were used to determine the role of microRNA-142 (miR-142) in regulation and expansion of MDSC.

**Results:** There is a significant increase in the proportion of MDSC in severe versus non-severe asthmatics and controls, corresponding to a decrease in myeloid dendritic cells. Allergic mice had significant increased levels of MDSC expansion which were associated with increased levels of IL-6 and downregulation of miR-142. miR-142 overexpression induced MDSC differentiation.

**Conclusions:** An accumulation of MDSC is associated with severe asthma in humans and mice. In an allergic mouse model, IL-6 levels increase. miR-142 may play an important role in regulation and differentiation of MDSC, leading to altered immunity.

### 26 MIR-150 Suppresses Lung Inflammation in a Mouse Model of Experimental Asthma

Jia-Wang Wang, PhD, Kunyu Li, BS, Gary Hellermann, PhD, Richard F. Lockey, MD, Subhra Mohapatra, PhD, and Shyam Mohapatra, PhD.
1Internal Medicine, University of South Florida, Tampa, FL; 2Division of Allergy & Immunology, University of South Florida and James A. Haley Veterans’ Hospital, Tampa, FL; 3Nanomedicine Research Center and Division of Translational Medicine, Departments of Molecular Medicine and Internal Medicine, University of South Florida and James A. Haley Veterans’ Hospital, Tampa, FL; 4USF Nanomedicine Research Center, University of South Florida College of Medicine & VA Hospital, Tampa, FL.

**Background:** Asthma is a complex disorder of the immune system caused by a combination of genetic predisposition with environmental exposures. The environmental factors play a predominant role in the etiology of asthma. It is hypothesized that epigenetic changes in miRNAs play a critical role in pathogenesis of asthma as an interface between genetic makeup and environmental exposures. (Wang, Jia-wang; Li, Kunyu; Hellermann, Gary; Lockey, Richard F.; Mohapatra, Subhra; and Mohapatra, Shyam. Regulating the Regulators: microRNA and Asthma. World Allergy Organization Journal. June 2011, Volume 4, Issue 6).

**Methods:** In the present study, we used miRNA array profiling in a mouse model of ovalbumin-induced asthma to identify differentially regulated miRNAs and characterized miR-150 in terms of cellular and humoral involvement and analysis of lung inflammation markers.

**Results:** We found that miR-150 was downregulated in CD4 T lymphocytes during asthmatic inflammation and Th1 and Th2 induction. Over-expression of miR-150 delivered by chitosan nanoparticles inhibited lung inflammation and decreased Th1 and Th2 cytokine levels. miR-150 suppressed Akt3, Cbl1 and Elk1 oncoproteins, which are involved in inflammation and cytokine production. Transgenic mice overexpressing miR-150 are resistant to asthma induction, demonstrated by reduced AHR and cytokine inflammation production.

**Conclusions:** These results suggest that deregulation of miRNAs may be involved in the pathogenesis of asthma and miR-150 may suppress inflammation in asthma by inhibiting cytokine production by downregulating critical genes such as Akt, Elk1 and Cbl1. miR-150 may be an attractive candidate for asthma gene therapy.

### 27 Serine Protease Inhibitor Attenuates Ova Induced Inflammation in Mouse Model of Allergic Airway Disease

Sanjay Sawc, MSc, Sagar Kallec, MSc, Bhanu Pratap Singh, PhD, and Naveen Arora, PhD. Allergy and Immunology, Institute of Genomics and Integrative Biology, Delhi, India.

**Background:** Serine proteases promote inflammation and tissue remodeling by activating proteinase-activated receptors, urokinase, metalloproteinases and angiotensin. In the present study, AEBSF (4-(2-Aminooethyl) benzene-sulfonyl fluoride) a serine protease inhibitor, was evaluated for prophylactic and therapeutic treatment in mouse model of airway allergy.

**Methods:** BALB/c mice were sensitized by i.p route on 0 and 14 day and challenged with OVA (25, 26 and 27 day) by i.n. route. Mice were treated i.n. with AEBSF, 1 hour before/after challenge and sacrificed on day 29 to collect BALF, blood and lungs. OVA specific immunoglobulins were measured in serum. Proteolytic activity, total cell/eosinophil count, eosinophil peroxidase activity (EPO), IL-4, IL-5, IL-10, cysteinyl leukotrienes and 8-isoprostane (oxidative stress marker) were determined in BALF. Haematoxylin and eosin stained lung sections were examined for cellular infiltration and airway inflammation.

**Results:** Mice exposed to OVA and treated with PBS showed significantly high levels of IgE, IgG1 and IgG2a as compared to sham mice. Both prophylactic and symptomatic AEBSF treatment reduced serum IgE and IgG1 significantly ($P \leq 0.05$) than control, however there was little increment