Methods: Extracts from North American short ragweed (Ambrosia artemisiifolia) pollen were investigated by mass spectrometry (MS), 2D-PAGE and immunoblotting. Furthermore, Amb a 1 isoallergens were purified and IgE reactivity determined by immunoblotting and IgE inhibition.

Results: 2D-PAGE and MS of ragweed extract proved the presence of all 5 known Amb a 1 isoallergens, of which Amb a 1.01 represents the dominant form. Additionally all other ragweed allergens known by sequence (Amb a 3, Amb a 4, Amb a 5, Amb a 6, Amb a 8, Amb a 9, Amb a 10) were identified. The highest IgE reactivity by immunoblotting was observed for Amb a 1.01 followed by Amb a 1.03; other Amb a 1 isoallergens as well as other detected ragweed allergens showed only weak IgE reactivity. All isoallergens with the exception of Amb a 1.04, which is only of low abundance in ragweed extract, were purified. Similar to the immunoblot analysis with crude extract, the purified isoallergens Amb a 1.02 and Amb a 1.05 showed weak IgE binding, whereas Amb a 1.01 and Amb a 1.03 had high IgE reactivity. First IgE inhibition experiments suggest that Amb a 1.01 contains all relevant IgE epitopes.

Conclusions: Amb a 1.01 is the most abundant Amb a 1 isoallergen, and presumably the most important ragweed allergen. However, a larger panel of ragweed-allergic subjects has to be analyzed with regard to IgE and T cell reactivities, to be able to choose a candidate for a recombinant vaccine for specific immunotherapy of ragweed allergy.
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 group 1 allergens.

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

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Background: In a recent proteomic study, we identified in Italian cypress (Cuppressus 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 peptide 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 confers 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

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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: Chimeras 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 chimera. 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.

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ALLERGIC MODELS OF INFLAMMATION

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

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Background: The T helper 2 (Th2) inflammatory pathway, including the Th2-activating cytokine interleukin 33 and its receptor interleukin 1 receptor-like 1 have been strongly implicated in asthma susceptibility (Moffatt MF, et al NEJM 2010). However, the role of Th2 pathway genetic variation in asthma progression and severity is not well understood. Our research group recently developed a clustering algorithm based on comprehensive phenotype information to assign subjects with asthma in the Severe Asthma Research Program (SARP) to 5 primary clusters; of which represent increasing severe