603 The Major Allergens of Birch Pollen and Cow Milk, Bet v 1 and Bos d 5, Are Structurally Related to Human Lipocalin 2, Enabling Them to Manipulate T-Helper Cells Depending on Their Load with Siderophore-Bound Iron

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RATIONALE: Human lipocalin 2, LCN2, is highly expressed in encounter sites of allergens, as lung and gut. It has immuno-regulatory properties when carrying iron via siderophores (holo) or not (apo). We investigated whether major allergens of birch pollen and milk, Bet v 1 and Bos d 5, might structurally and biologically interfere with LCN2.

METHODS: Structural comparison to LCN2 was performed using FATCATflex, CE algorithm and TM-Align methods. Ligand binding was analyzed with AutoDock Vina. Iron-binding was determined by Prussian blue staining. Activated human PBMCs were stimulated for 18h with apo- or holo-allergens, or controls. Subsequently, cells and supernatants were analysed by flow cytometry and for their cytokine-content.

RESULTS: Both tested allergens shared great structural homology to LCN2. Thus, besides Bos d 5, we could also classify Bet v 1 as a lipocalin-like protein. Both allergens were capable of binding iron via siderophores. When incubated with PBMCs, only the apo-forms of the allergens, but not the holo-forms, were able to promote CD4+ cells and the secretion of IL13.

CONCLUSIONS: We conclude that Bet v 1 and Bos d 5 not only structurally mimic human LCN2, but also functionally by their ability to bind iron via siderophores. The apo-forms promote Th2 cells, whereas the holo-forms appear to be immunosuppressive. These results provide for the first time a functional understanding on the principle of allergenicity of major allergens from entirely independent sources, like birch and milk.

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604 Analysis of GST Allergen Cross-Reactivity in a North American Population for Molecular Diagnosis

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RATIONALE: IgE reactivity to glutathione S-transferases (GST) has been reported in tropical and subtropical environments, where it is not clear if cross-reactivity or co-sensitization occurs. In the US, Bla g 5 is the most important GST allergen, and lack of co-exposure to GSTs from certain species allows a better assessment of cross-reactivity versus co-sensitization.

METHODS: The crystal structures of Bla g 5, Der p 8, Blo t 8, and *Ascaris suum* GST (GSTA) were determined and surface residues compared. Sera from North American cockroach and mite allergic patients were tested for IgE reactivity to these GSTs. A panel of six murine anti-Bla g 5 mAb was compared for cross-reactivity with the other three GSTs using antibody binding assays.

RESULTS: The allergen structures revealed few contiguous regions with similar exposed residues that would make cross-reactivity unlikely. Bla g 5 sensitive sera did not react with Der p 8, and vice versa. None of the sera reacted with Blo t 8 or GSTA. Only Der p 8 inhibited IgE binding to Der p 8. The anti-Bla g 5 mAb failed to interact strongly with the other three GSTs.

CONCLUSIONS: The lack of IgE cross-reactivity to Bla g 5, Der p 8, Blo t 8 and GSTA in allergic patients from temperate climates, is in agreement with the low shared amino acid surface identity. Previous results from tropical environments may be due to co-sensitization. This highlights the need for species-specific GSTs for accurate molecular diagnostics.

605 Structural and Stability Studies of Profilins Amb a 8 and Art v 4

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RATIONALE: Profilins form one of the largest family of allergens. Members of this family have highly conserved sequences and are responsible for many cross-reactions between different sources of pollen and food allergens. The goal of this analysis is to map IgE epitopes on allergenic profilins originating from pollens, and provide a detailed explanation at the molecular level of cross-reactivity of this allergens.

METHODS: X-ray diffraction analysis was used to investigate the molecular structures of Amb a 8 and Art v 4. Thermal shift assays were used to analyze the stability of both weed allergens and their homolog Bet v 2. Gel filtration, dynamic light scattering and electrophoresis were used to determine the oligomeric state of these allergens.

RESULTS: Structural data revealed that the both Amb a 8 and Art v 4 share the same overall fold and are structurally similar to Bet v 2 . Our studies revealed that pollen profilins may form oligomeric assemblies.

CONCLUSIONS: Determination of 3D models of Amb a 8 and Art v 4 allowed for their comparison with the already known structure of Bet v 2. This comparison confirmed a high degree of structure and sequence conservation among analyzed pollen profilins. In addition, we have found that the profilins we analyzed in solution may be present in different oligomeric states. However, detailed molecular structure of the oligomeric assemblies is yet to be determined. Sequence and structure conservation, as well as the propensity of profilins to oligomerize seem to be crucial for triggering IgE-dependent inflammatory response.