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Intrinsic properties of allergens and environmental exposure as determinants of allergenicity

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Introduction

Allergens are some of the best studied proteins associated with human disease, and yet, little is known about why these proteins induce production of specific IgE antibodies in susceptible individuals, and the mechanisms involved in causing sensitization. Most IgE responses are elicited by environmental exposure to inhaled allergens. Chronic exposure to low levels of indoor allergens (1-10 mg/year) leads to the development of IgE antibody responses and is strongly associated with the development of allergic asthma. Cockroach allergen exposure and sensitization, followed by dust mite and cat allergens, are strongly associated with asthma morbidity among inner-city children with asthma (1). In the past five years, several indoor allergens from cockroach, cat and dog were selected to study their molecular structure and function, by molecular cloning and/or molecular modeling based on the known three-dimensional structure of homologous proteins. Several hypotheses for allergenicity involving intrinsic properties of the allergen have been developed from these studies. These properties include integrity of the allergenic protein, resistance to degradation, proteolytic function and mimicry of the allergen with endogenous proteins. Key determinants for allergenicity involve dose and route of environmental exposure and genetic predisposition of the individual towards developing a Th2 response to specific allergens.

Results and Discussion

Structural and functional diversity of allergens

Given the importance of cockroach allergens in the development of allergic disease, the structure of several cockroach allergens was studied, including Bla g 1, the homologous American cockroach allergen Per a 1, and Bla g 2. The German cockroach (Blattella germanica) is the most common in the U.S.A., and the prevalence of IgE antibodies to Bla g 1 and Bla g 2 is high (30-50 % and 60 %, respectively). Bla g 2 is a very potent allergen, inducing IgE antibody responses at very low doses of exposure (0.33 µg/g) (2). Another cockroach allergen, Bla g 4, belongs to the same structural family of proteins, the lipocalins, as the dog allergens Can f 1 and Can f 2. Structural studies of Can f 1 and Can f 2 revealed common features with the cat cystatin Fel d 3, whose tertiary structure was modeled on cysteine proteinase inhibitors.

Relationship between integrity of the protein and allergenicity. The molecular cloning of the Group 1 cockroach allergens (Bla g 1 and Per a 1) revealed that these allergens are constituted by a novel structure containing several tandem 100 amino acid repeats (3-6). This structure was visualized as parallel lines to a diagonal in a dotplot matrix analysis (Figure 1).

![Figure 1. Repeated structure in Group 1 cockroach allergens is reflected at the amino acid (left) and nucleotide (right) level in a dotplot matrix analysis (Figure 1).](image_url)
Interestingly, several Bla g 1 molecular forms expressed in E. coli and P. pastoris contained different number of repeats and bound IgE as the natural allergen, indicating the existence of repeated IgE binding units in the molecule. Protein sequencing demonstrated that the natural allergen is also constituted by several fragments starting after trypsin cleavage sites, very similar to the molecular forms obtained in the recombinant allergen. This suggested that Bla g 1 could be trypsin cleaved in the cockroach digestive tract where the allergen is produced, in which case, once excreted to the environment, the different Bla g 1 molecular forms would be inhaled by atopic individuals during the process of sensitization. Therefore, in the case of the Group 1 cockroach allergens, digested fragments of the protein may be sufficient to produce sensitization.

The opposite argument can be applied to explain allergenicity of other proteins, especially food allergens. When the structure of the allergen is very resistant to degradation, conformational epitopes would persist in the body and stimulate the immune system for prolonged periods. For example, β-lactoglobulin is able to resist acidity and remain intact after passing through the stomach (7). Similarly, the peanut allergen Ara h 1 has a trimeric structure that may protect some epitopes from degradation in the stomach (8). Therefore, the relationship between integrity of a protein and allergenicity depends on the protein structure and the route of exposure to the protein.

The cockroach allergen Bla g 2 is an inactive aspartic proteinase. The potent cockroach allergen Bla g 2 was modeled using the X-ray structures of the homologous aspartic proteinases porcine pepsin and bovine chymosin (~26% identity with Bla g 2) (9, 10). The Bla g 2 model has a bilobal structure typical of aspartic proteinases (Figure 2A). Aspartic proteinases are widely distributed enzymes whose proteolytic mechanism involves the participation of two co-planar aspartic acid residues in the catalytic triads interacting with a water molecule (11). The catalytic site of these enzymes is located in the bottom of the binding pocket or cleft, and is formed by two loops, each containing the triad aspartate-threonine-glycine (DTG) (Figure 2).

The possibility that allergenicity of Bla g 2 was promoted by its putative proteolytic activity was investigated. Several features of the Bla g 2 binding pockets differ from the active site of catalytically active aspartic proteinases and suggested that Bla g 2 is an inactive aspartic proteinase. Firstly, Bla g 2 has amino acid substitutions in the catalytic triads which are absolutely conserved.
in all active aspartic proteinases: residues DTG 32-34 and DTG 215-217 (using pepsin numbering) are substituted by DST and DTS, respectively (Figure 2B). Secondly, the co-planar aspartates are inaccessible to water because the bulkier residue threonine 34 interferes with aspartate 215 (as shown in Figure 2B). Finally, there is a critical substitution in the "flap" region that comprises the residues 72-81 and forms a β-hairpin that partially covers the active site cleft (Figure 2A). Residue tyrosine 75, that is a highly conserved residue among active aspartic proteinases, is substituted by phenylalanine in Bla g 2. The lack of enzymatic activity was confirmed by milk clotting assay (9).

The specificity pocket of Bla g 2 was also analyzed by comparative studies using peptidomimetic inhibitors and showed that the binding cleft is able to accommodate a wide range of peptides, suggesting that Bla g 2 is a binding protein (9). Surprisingly, Bla g 2 is related to a family of mammalian glycoproteins called pregnancy associated glycoproteins (PAGs) that are thought to be binding proteins. PAGs are secreted by the outer layer (chorion) of the placentas of various ungulate species (12). They belong to the aspartic proteinases protein family, but most of them have critical amino acid substitutions similar to Bla g 2, that make them catalytically inactive (13).

Most known mammalian allergens are ligand binding proteins. The dog allergens Can f 1, Can f 2 and the recently cloned cat allergen Fel d 3 were studied by molecular modeling (Figures 3, 4) (14-15). Can f 1 and Can f 2 belong to a family of animal allergens called lipocalins (14, 16). This protein superfamily includes other major and minor allergens from a wide range of species: cockroach (Bla g 4), mouse (Mus m 1), rat (Rat n 1), cow (Bos d 2, Bos d 5 which is β-lactoglobulin) and horse (Equ c 1 and Equ c 2). Despite an amino acid homology as low as 20 %, the crystal structures of Mus m 1, Rat n 1, Bos d 2, β-lactoglobulin and Equ c 1 show the same characteristic lipocalin folding: an α-helix and a single eight-stranded (ten-stranded for β-lactoglobulin) continuously hydrogen-bonded antiparallel β-barrel enclosing an internal cavity (Figure 3) (17-21).

Interestingly, most of the known mammalian allergens are ligand-binding proteins (lipocalins or calycins) - except for albumins and the cat allergen Fel d 1- , and they are secreted by hair/dander and saliva. One of the major functions of the lipocalins is related to the ligand binding capacity of their hydrophobic pocket. These small extracellular proteins bind small hydrophobic ligands such as pheromones, retinoids, steroids and arachidonic acid (22). For example,
ß-lactoglobulin from cow’s milk, associated with food hypersensitivity, binds palmitate and retinol within its central cavity (20, 23), and the rodent urinary proteins Mus m 1 and Rat n 1 bind male pheromones (17).

Can f 1 is produced in the tongue epithelial tissue and shows high degree of homology (57 % identity) to the human von Ebner’s gland (VEG) salivary lipocalin which is a cysteine protease inhibitor (16, 24). Similarly, the cystatin cat allergen, Fel d 3, contains a cysteine protease inhibitor motif (Figure 4) (15). The Fel d 3 structure was modeled on the X-ray crystal structures of recombinant human cystatin A, human stefin A and human chain I of stefin B (15). The cat cystatin molecule includes a two and half turn α-helix and a five stranded antiparallel β-pleated sheets interconnected by a hairpin loop and an additional carboxy terminal strand (Figure 4). Both, Can f 1 and Fel d 3 could be functional cysteine protease inhibitors. These findings raise the possibility that these animal allergens could inhibit cysteine proteases such as Der p 1.

Figure 4. Cat cystatin modeled on the crystal structure of three human cystatins including human stefin B (cystatin B). Ribbon representation showing the short α-helix (red), five anti-parallel β sheets (blue) and the hairpin loops (grey). The cysteine protease inhibitor motif is indicated by yellow and orange spheres in the first hairpin loop with the orange spheres representing residues that are directly involved in forming complexes with the protease (also labeled with the amino acid residue numbers).

**Hypotheses for allergenicity**

*Intrinsic properties of the allergen: structure and biological function.* The broad spectrum of allergenic proteins, encompassing a wide variety of structures and functions, makes it unlikely that structure alone is responsible for IgE antibody responses (25). An increasing body of evidence suggests that having functional enzyme activity may explain why some allergens, notably the cysteine and serine protease allergens from house dust mite (Der p 1, Der p 3 and Der p 9) and phospholipase A from bee venom, are particularly potent (26-37). Der p 1 may directly promote IgE synthesis through cleavage of the low affinity IgE receptor (CD23) on B cells and, indirectly, through cleavage of or the α-subunit of the IL-2 receptor (CD25) on T cells (27-31). Mite protease allergens disrupt the bronchial epithelium and cause release of pro-
inflammatory cytokines from bronchial epithelial cells, mast cells and basophils (32-35). Der p 1 disrupts tight junctions and facilitates trans-epithelial allergen delivery and processing (36). Proteolytically active Der p 1 is reported to significantly enhance IgE production in mice compared with enzymatically inactive allergen (37). These lines of evidence suggest that having enzyme activity enhances allergenicity: the "enzyme hypothesis" (30). This hypothesis was originally developed to explain why mite allergens are so strongly associated to the development of allergic responses. The finding that most mite allergens were enzymes made this a logical avenue to pursue and several potentially interesting mechanisms whereby mite protease allergens could enhance IgE responses have been described (26-37). However, our data on both molecular and functional studies show that Bla g 2 is an inactive aspartic proteinase, yet is a potent allergen, inducing IgE responses at exposure levels that are often 1-2 orders of magnitude lower than for Der p 1 (2).

Several other major allergens provide exceptions to the "enzyme hypothesis". For example, the other cloned cockroach allergens (Bla g 4, Bla g 5, Bla g 6) have diverse biological functions and none of them are proteolytic enzymes. Mammalian allergens (Can f 1, Can f 2, Rat n 1, Mus m 1, Bos d 2, Equ c 1) are lipocalins and function as pheromone binding proteins or transporter proteins (14). Cat cystatin and Can f 1 may be cysteine protease inhibitors. Der p 2 has an immunoglobulin fold structure, with primary sequence homology to moth molting protein and human epididymal protein, but no known enzyme function (38, 39). Der p 2 causes sensitization in > 90% of mite allergic patients, at exposure levels that are usually 2-10 fold lower than for Der p 1. This evidence confirms that enzymatic activity of the allergen is not a pre-requisite for allergenicity. Recent structural studies have shown that allergens have diverse biological functions – they may be enzymes, structural proteins, binding proteins and also enzyme inhibitors (14). The fact that several mite allergens are enzymes appears to represent a special case.

Although enzyme function per se is not required for proteins to elicit IgE responses, the results do not rule out the possibility that enzymes inhaled into the respiratory tract could contribute to inflammation. For example, some enzymes including Der p 1 may contribute to inflammation by inactivating the $\alpha_1$-proteinase inhibitor which is the major natural inhibitor of neutrophil enzymes (40). This could occur if particles that are inhaled into the lung carry both allergens and enzymes simultaneously, and clearly both characteristics may co-exist in certain molecules, such as Der p 1.

Other hypotheses for allergenicity have been proposed suggesting that intrinsic properties of allergens modulate the immune response and stimulate IgE production. As mentioned earlier, resistance to degradation may be important for sensitization to some food allergens such as $\beta$-lactoglobulin and Ara h 1 (7, 8). In these sense, food allergens tend to be heat stable and pH resistant proteins.

Molecular mimicry of the allergens with endogenous proteins. Another hypothesis for allergenicity based on molecular mimicry between allergens and endogenous proteins, and involving mechanisms of self-tolerance, has been proposed for lipocalins (41, 42). For example, endogenous lipocalins, such as retinol binding protein (RBP), apolipoprotein D (APD) and von Ebner’s gland protein (VEG), are homologous to exogenous lipocalin allergens. Experimental support for this hypothesis comes from mapping studies of T cell epitopes that revealed that one of the main epitopes of Bos d 2 overlapped with the conserved regions of lipocalins (43).
However, it is noticeable that at the level of IgE epitopes, several studied allergens differ from homologous endogenous proteins. This is the case for the lipocalin allergen Equ c 1, albumins and tropomyosins (14, 21). Albumins represent the most conserved family of cross-reactive mammalian allergens. Cat and dog albumins are cross-reacting allergens that share a high degree of homology. The existence of human endogenous albumin (which shows 82.6% homology with dog albumin), and lack of reactivity to self-albumin may indicate that the allergenic epitopes of the molecule are in non homologous areas of the molecules (44). This argument can also be applied to the invertebrate tropomyosin allergens that are structural proteins from shrimp, crab, lobster, snails, mite and cockroach allergens. The presence of IgE epitopes in the parts of the allergen that do not share homology with human and meat tropomyosins could explain the lack of sensitization to human endogenous and meat tropomyosins.

**Effect of allergen dose and route of environmental exposure on allergenicity**

The key elements that affect production of IgE antibodies appear to be route of exposure, allergen dose and host immune response genes, all of which preferentially stimulate Th2 responses. The common feature of all the inhaled allergens is that they are secreted into the environment. Some of them are especially ubiquitous, like Fel d 1 which can induce sensitization even in the absence of cats at home. Under natural conditions, repeated exposure to low doses of allergen (1-10 mg/year) without adjuvant on airborne particles of 1-40 µm diameter is required for sensitization in genetically predisposed individuals. These conditions have been reproduced by administration of subclinical doses of allergen to atopic subjects, showing a shift to allergen-specific Th2 response in vitro (45). An opposite response, with IgE antibody reduction is achieved during immunotherapy by administration of increasing doses of allergen, in order to desensitize the allergic individual (46). The critical effect of allergen dose is emphasized by recent studies which show that the prevalence of allergen specific sensitization in atopic children is associated with the highest domestic concentration of Bla g 2 (median levels of 0.33 µg/g; range > 0.08-15 µg/g) and Group 1 mite allergens (38 µg/g; range 24-150 µg/g), but not with Fel d 1 (2). Low dose exposure to Fel d 1 (0.5-5 µg/g) posed the strongest risk for sensitization. High dose exposure to Fel d 1 (> 20 µg/g up to 3840 µg/g) results in reduced prevalence of IgE antibody responses to Fel d 1, and is associated with increased specific IgG and IgG4 antibody levels (2, 47). Bla g 2 and mite allergen exposure routinely falls in a window that represents the highest risk for sensitization (2). These results have recently been confirmed in a study of 2,502 adults, indicating that the prevalence of sensitization to cat is decreased in the lowest and highest cat allergen exposure groups (48). Exposure to low levels of Bla g 1 and Bla g 2 has also been associated with wheezing among infants in the first three months of life and with increased proliferative T cell responses (49). Genetic factors are also involved in allergenicity, and recognition of low doses of allergens may be associated with certain HLA DR genes. For example, association of the HLA-DRB1*0101 allele in the Hutterite population and the HLA-DRB1*0102 allele in African Americans with sensitization to cockroach allergens has been reported (50).
Future directions

Once the tertiary structure of an allergen such as Bla g 2 is known, site-directed mutagenesis studies will allow the determination of crucial amino acids for IgE antibody binding on the surface of the molecule, as well as substitution of key cysteine residues for the correct folding of the molecule. The administration of modified allergens or peptides with low IgE binding capacity, could potentially avoid the side-effects of conventional immunotherapy (IT) by inducing specific IgG4 instead of IgE production (46).

Another approach to be considered for IT in the future is the use of recombinant allergens and adjuvants(s) to down-regulate Th2 responses. The adjuvants that are now under study are the immunostimulatory sequences CpG nucleotides, which induce a proinflammatory Th1 response to the mammalian immune system (51). CpG sequences are added to allergen DNA and administered during IT in animal models and clinical trials. Other approaches to IT are the use of other adjuvants including allergens coupled to IL-12, IL-18, or lectins, and peptide-based therapy (52, 53).

Concluding remarks

A combination of structural and functional studies shows that allergens have diverse biological functions and they include enzymes, structural proteins, binding proteins and enzyme inhibitors. Some intrinsic allergen characteristics, like resistance to degradation, may be important to elicit IgE antibody responses, specially in the case of food allergens. Regarding allergen function, the strong capacity of Bla g 2 to stimulate IgE production following low dose environmental exposure is unrelated to aspartic proteinase activity. Although proteolytic function may modulate allergenicity in some cases, including mite Der p 1 or bee venom phospholipase A2, allergen enzymatic activity is not a pre-requisite for allergenicity. Other factors such as dose and route of allergen exposure and genetic predisposition are important determinants for allergenicity. Knowledge of the molecular structure of allergens such as Bla g 2 will provide a better understanding of the immune response to the allergen and should allow rational immuno-therapeutic strategies for treatment of allergy to be developed.

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References


Genetically engineered allergen derivatives with reduced allergenic activity for specific immunotherapy of type I allergy

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1. Introduction

Type I allergy represents a major health problem in industrialized countries where more than 20% of the population suffers from type I allergic reactions (allergic rhinitis, conjunctivitis, allergic asthma and anaphylactic shock) (1). Specific immunotherapy, the only causative treatment of Type I allergy, is based on the administration of increasing doses of allergens to allergic patients. Although specific immunotherapy was introduced already 90 years ago (2) and is recognized as clinically effective treatment, the immunological mechanisms are not completely understood (3, 4). Major disadvantages of immunotherapy are that the treatment is performed with allergen extracts, which consist of mixtures of allergenic and non-allergenic components that are difficult to standardize and cannot be applied according to the patient's reactivity profile. Moreover, due to the administration of active allergens, severe anaphylactic side effects can occur. During the last years, many recombinant allergens have been produced by recombinant DNA technology, which equal the natural allergens regarding biochemical, biological, immunological, and structural properties (5, 6). Recombinant allergens can be used for component-resolved diagnosis, which may represent the basis for patient-tailored immunotherapy (7).

Recently several research groups have started to produce hypoallergenic allergen derivatives by genetic engineering as well as by synthetic peptide chemistry (8). Hypoallergenic allergen derivatives can be used for patient-tailored immunotherapy and are expected to induce a lower rate of anaphylactic side effects, when used for immunotherapy.

The present article describes the strategies which we have applied for the production and evaluation of hypoallergenic allergen derivatives and the experience obtained in first clinical studies. Perspectives, how hypoallergenic allergen derivatives may improve specific immunotherapy, are finally discussed.

2. Results and discussion

A strategy for the production and evaluation of hypoallergenic allergen derivatives

The key step for the production of recombinant allergens and hypoallergenic derivatives is the selection of the relevant allergen sources and of those allergens which contain most of the important B cell and T cell epitopes present in these extracts (9, 10) (Figure 1). cDNAs coding for the most relevant allergens are isolated from the selected allergen source to produce recombinant allergens equaling the immunological and biological properties of the natural allergens. Based on the knowledge gained through molecular, immunological and structural characterization of recombinant allergens, hypoallergenic allergen derivatives can be produced by recombinant DNA technology and synthetic peptide chemistry (11, 12) (Figure 1). In order to identify the best-suited hypoallergenic derivatives for immunotherapy, we have established an evaluation scheme (Figure 1).

In vitro assays for the evaluation of hypoallergenic allergens comprise the comparison of the derivatives with the corresponding wild-type allergens regarding IgE binding capacity, induction of basophil histamine release, T cell proliferation and cytokine release (11, 12, 13) (Figure 1). Those derivatives with preserved T cell epitopes and reduced histamine release activity are further evaluated in vivo. In vivo evaluation of hypoallergenic allergen derivatives comprises animal studies and provocation testing in patients (13 – 17) (Figure 1). Immunization of
animals should demonstrate whether the derivatives induce blocking antibodies, which recognize the wildtype allergen and inhibit the binding of patient’s IgE to the wildtype allergen (13, 14). To select safe allergen derivatives for immunotherapy, provocation testing (skin prick testing, intradermal testing) in patients has to be performed with derivatives which have been shown to induce blocking antibodies in animal studies (15 – 17) (Figure 1). We have applied the strategy described in Figure 1 for the production and evaluation of hypoallergenic derivatives of the major birch pollen allergen, Bet v 1.

**Production of hypoallergenic allergen derivatives**

For improved immunotherapy, several strategies may be applied to modify allergens into hypoallergenic variants, based on the knowledge obtained through the molecular, immunological and structural characterization of allergens. In principle, recombinant DNA technology and synthetic peptide chemistry can be used for the production of hypoallergenic allergen derivatives. One possibility is to produce T cell epitope containing peptides lacking IgE-binding capacity and allergenic activity for tolerance induction at the T cell level (18 - 20). Another strategy is the generation of hypoallergenic mutants with reduced IgE binding capacity by *in vitro* mutagenesis. Point mutations outside the IgE binding sites may change the allergen conformation and, thereby, destroy discontinuous IgE epitopes (21, 22) and mutations within IgE epitopes may affect single amino acids that are directly involved in the interaction with IgE antibodies (23, 24) or may delete several amino acids in a row to remove portions of the IgE epitopes (25). Strategies which we have applied comprise the production of recombinant allergen fragments which contain the entire sequence information of the allergens but possess reduced allergenic activity due to a loss of their three-dimensional fold (11, 26, 27). Furthermore we have constructed allergen oligomers with reduced allergenic activity but preserved B cell and T cell epitopes (12).

The use of such hypoallergenic derivatives should allow to inject much higher doses of the derivatives with reduced risk of anaphylactic side effects. Higher doses may allow a reduction of the number of injections given and will likely induce preferentially an allergen-specific Th1 immuneresponse (28, 29), accompanied by the production of blocking IgG antibodies (14).

**In vitro and in vivo evaluation of hypoallergenic derivatives**

In order to identify those hypoallergenic allergen derivatives which represent the best-suited candidate molecules for immunotherapy, the derivatives must be thoroughly evaluated. To deliver the proof of principle, we selected Bet v 1, the major birch pollen allergen, as model system to construct hypoallergenic derivatives. Bet v 1 represents one of the most common allergens which is recognized by more than 90% of tree pollen allergic individuals and almost 60% of them are sensitized exclusively to Bet v 1 (30). In addition Bet v 1 possesses conformational IgE epitopes and shares epitopes with homologous proteins present in the pollen of trees of the order *Fagales* and in plant-derived food (9, 10).

By genetic engineering, we produced two Bet v 1 fragments, comprising aa 1-74 and aa 75-160, and Bet v 1 oligomers as recombinant proteins in *E. coli* (11, 12). The Bet v 1 derivatives induced strong lymphoproliferative responses in peripheral blood mononuclear cells (PBMC) of birch pollen allergic patients and contained the relevant Bet v 1-specific T-cell epitopes (11 - 13). The Bet v 1 fragments lost their IgE binding capacity due to a loss of their native-like structure. The other modification, a Bet v 1 trimer, retained its IgE-binding capacity when compared with the recombinant Bet v 1 wildtype but, unexpectedly, exhibited a markedly reduced capacity to induce histamine release of basophils from birch pollen allergic patients.
Figure 2 shows a histamine release assay, performed with Bet v 1 monomer and two Bet v 1 oligomers (dimer, trimer) which shows that the trimer has an approximately 100-fold reduced capacity to release histamine from patients' basophils.

Our in vivo evaluation scheme for hypoallergenic allergen derivatives suggests immunization of animals and provocation testing of patients as next steps. Immunization of animals should indicate whether the hypoallergenic derivatives can induce antibodies which recognize the wild-type allergen and cross-react with homologous allergens. To evaluate whether the hypoallergenic derivatives induce blocking antibodies the inhibition of the binding of patient's IgE to the wild-type allergen has to be tested. Table 1 shows that rabbit IgG antibodies induced with rBet v 1 fragments inhibited substantially (mean 40%) the binding of patient's IgE to natural birch pollen extract. Skin prick- and intradermal testing with hypoallergenic Bet v 1 fragments and Bet v 1 oligomers was performed in a Swedish and a French study with 60 birch pollen allergic patients (15, 16). The recombinant Bet v 1 derivatives exhibited a more than 100-fold reduced allergenic activity compared to Bet v 1 wildtype and no late phase reactions were observed. Figure 2 illustrates the lack of allergenic activity of the recombinant Bet v 1 fragments in a birch pollen allergic patient. The ability of the rBet v 1 derivatives (trimer, fragments) to induce late phase reactions and eosinophil activation was studied in a skin blister model in birch pollen allergic patients. The hypoallergenic rBet v 1 derivatives induced less activation of eosinophils and lower release of ECP and GM-CSF compared to Bet v 1 wildtype (17).

3. Plans for future research

Hypoallergenic allergen derivatives for specific immunotherapy
According to our in vitro and animal studies, we suggest to use adjuvant-bound hypoallergenic allergen derivatives for injection immunotherapy. Immunotherapy with hypoallergenic derivatives will allow patient-tailored treatment with defined molecules, so that therapy-induced de novo sensitization to previously not recognized allergens cannot occur (31). Due to the reduced allergenic activity of the derivatives, it will be possible to inject higher doses with reduced risk of anaphylactic side effects. It is expected that immunization with high doses will induce a preferential Th1-like immune response which will be accompanied by the production of blocking IgG antibodies (28, 29). Blocking antibodies may suppress allergen-induced activation of effector cells and thus block mediator release (32). Furthermore, they may inhibit IgE-mediated presentation of allergens to T cells as well as suppress T cell activation (33). Due to the fact that antibodies induced with rBet v 1 derivatives, cross-reacted with Bet v 1-homologous allergens present in tree pollen and plant-derived food, these birch pollen-related allergies may also be treated with hypoallergenic Bet v 1 derivatives. Our ongoing immunotherapy studies with hypoallergenic Bet v 1 derivatives will provide information, whether they represent effective tools for immunotherapy and allow to analyse the mechanisms underlying this treatment on a molecular level. Should clinical studies with Bet v 1 derivatives deliver the proof of principle, our concept can be applied for other important allergen sources (e.g. grasses, mites and animals).
4. Concluding remarks

The introduction of molecular biological techniques into allergy research has allowed the production of several active recombinant allergens for component-resolved allergy diagnosis (CRD). Based on sequence, epitope and structural analysis of the recombinant allergens, it is now possible to develop and evaluate synthetic and genetically engineered hypoallergenic derivatives as candidate molecules for safe and patient-tailored forms of immunotherapy (CRIT). Clinical immunotherapy trials will evaluate the safety and efficacy of hypoallergenic allergen derivatives and provide detailed knowledge regarding the mechanisms of specific immunotherapy.

5. References:


Figure legends:

Figure 1: Production and evaluation of hypoallergenic Bet v 1 derivatives.

Identification of birch pollen as the most relevant allergen source for tree pollen and associated food allergy (9, 10)

Genetic engineering of hypoallergenic Bet v 1 derivatives (fragments and oligomers) (11, 12)

In vitro evaluation of hypoallergenic Bet v 1 derivatives
- IgE binding capacity (11, 12)
- Induction of histamine release (11, 12)
- T-cell proliferation (11 -13)

In vivo evaluation of hypoallergenic Bet v 1 derivatives
- Immunogenicity in animal models (13, 14)
- Induction of blocking antibodies (13, 14)
- Provocation testing in patients (15 - 17)

Figure 2: Reduced allergenic activity of recombinant Bet v 1 oligomers demonstrated by histamine release. Granulocytes from a birch pollen allergic patient were incubated with different doses of purified recombinant Bet v 1, rBet v 1 dimer and rBet v 1 trimer. Histamine released in the cell-free supernatant was measured by RIA and is expressed as percentage of total histamine release.
Table 1: Rabbit antibodies induced by immunization with rBet v 1 fragments inhibit allergic patient’s IgE binding to natural birch pollen extract (*Betula verrucosa*).
The percentage inhibition of serum IgE binding of 13 patients to ELISA plate-bound natural birch pollen extract after preincubation with rabbit anti rBet v 1 aa 1-74 and rabbit anti rBet v 1 aa 75-160 is displayed.

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