

Allergen Structures and Biologic Functions: The Cutting Edge of Allergy Research

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Studies of structure and function of allergens using state-of-the-art technologies have led to a better understanding of allergenicity, including aspects related to cross-reactivity, allergen nomenclature, and the identification of antigenic determinants. This information is being applied to the design and production of allergy vaccines, some of which already have proven efficacy and safety in clinical trials.

Introduction

A common structural feature that may explain why some proteins become allergens has not been found. Approximately 50 allergen structures are now available in the Protein Data Bank (<http://www.rcsb.org/pdb/>), revealing that structural variability among allergens is common (Table 1). Allergens are structurally heterogeneous, with only α -helices (Fel d 1, tropomyosins, group 5 dust mite allergens), only β -sheets (group 2 dust mite allergens), or, in most cases, a combination of both secondary structure elements. Structural variety is reflected in many biochemical functions, and little is known about function's relevance to allergenicity. A wide range of biologic functions have been attributed to allergens, including proteolytic and nonproteolytic enzymes; enzyme inhibitors; binding proteins; and structural, storage, and regulatory proteins. Most plant and animal food and pollen allergens recently have been reported to belong to a restricted number of protein superfamilies. Animal food allergens were classified into three main families (tropomyosins, EF-hand proteins, and caseins), most plant food allergens into just four (prolamin, cupin, profilin, and Bet v 1 superfamily) and the major pollen allergens into three (expansins,

profilins, and calcium-binding proteins) [1,2,3•]. An evolutionary analysis of the relationships between allergenic and nonallergenic proteins from plant foods also has been reported [4]. This information, combined with data on environmental availability of the proteins for human exposure, will provide a new perspective on why some proteins become allergens. This review covers published literature from 2004 to the present that is related to allergen structure and function.

New Three-Dimensional Allergen Structures

Recent improvement of biophysical methods such as mass spectrometry, x-ray crystallography, and nuclear magnetic resonance spectroscopy (NMR) has made detailed analysis of allergen structures attainable in less time [5•]. The three-dimensional structures of major allergens from the main indoor allergen sources have been solved since 2004: Bla g 2, Blo t 5, Der p 1 (pro- and mature allergen), Der f 2, and Fel d 1 (Table 1). Bla g 2 and Der p 1 are globular proteins with a combination of α -helices and β -sheets, whereas Der f 2 and Fel d 1 contain only β -sheets or α -helices, respectively (Fig. 1).

Bla g 2 has a bilobal structure typical of aspartic proteases and derived from gene duplication. The cleft between the two lobes, corresponding to the catalytic area in active enzymes, has important amino acid substitutions that render this allergen inactive in standard aspartic protease assays (Fig. 1) [6]. Five instead of the usual two or three disulfide bonds and an atom of zinc bound to the molecule may confer stability to the allergen and contribute to its persistence in the environment and, therefore, to asthma development [7••].

Der p 1 is a cysteine protease with two globular domains connected by a flexible outside loop formed by the central residues of the sequence. This allergen is secreted with an 80-amino acid N-terminal proregion that is cleaved autocatalytically under acidic conditions upon enzyme maturation. The proregion blocks not only the catalytic activity but also major conformational IgE antibody-binding epitopes [8]. A lack of serine protease activity, previously attributed to Der p 1,

Table 1. Tertiary structures of inhaled allergens

Allergen	Function/structure	Species of origin	X-ray crystal structure	NMR structure
Inhaled (indoor)				
Bla g 2	Inactive aspartic protease	<i>Blattella germanica</i>	1YG9(m)	2JMH
Blo t 5	Unknown	<i>Blomia tropicalis</i>		
Bos d 2	Lipocalin	<i>Bos domesticus</i>	1BJ7	
Der f 1	Cysteine protease	<i>Dermatophagoides farinae</i>	3D6S	1AHK, 1AHM, 1WRF
Der f 2	Lipid-binding protein	<i>D. farinae</i>	1XWV, 2F08	
Der f 13	Fatty acid-binding protein	<i>D. farinae</i>		
Der p 1	Cysteine protease	<i>Dermatophagoides pteronyssinus</i>	1XKG, 2AS8(m)	1A9V(m)
Der p 2	Lipid-binding protein	<i>D. pteronyssinus</i>	1KTJ(m)	
Fel d 1	Uteroglobin	<i>Felis domesticus</i>	1PUO, 1ZKR, 2EJN	
Mus m 1	Lipocalin	<i>Mus musculus</i>	1MUP	2A0A
Rat n 1	Lipocalin	<i>Rattus norvegicus</i>	2A2G, 2A2U	
Inhaled (outdoor)				
Amb t 5	Unknown	<i>Ambrosia trifida</i>		1BBG, 2BBG, 3BBG
Ara t 8	Profilin	<i>Arabidopsis thaliana</i>	1A0K	1BTv, 1B6F
Bet v 1	Pathogenesis-related protein	<i>Betula verrucosa</i>	1BV1, 1FSK, 1QMR(m), 1FM4, 1LLT(m)	
Bet v 2	Profilin	<i>B. verrucosa</i>	1CQA	
Bet v 4	Calcium-binding protein	<i>B. verrucosa</i>		1H4B
Che a 3	Polcalcin	<i>Chenopodium album</i>	2OPO	1T0W, 1HEV
Chi t 1	Hemoglobin	<i>Chironomus thummi thummi</i>	1ECO	
Hev b 6	Hevein precursor	<i>Hevea brasiliensis</i>	1Q9B, 1WKX	
Hev b 8	Latex profilin	<i>H. brasiliensis</i>	1G5U	1SS3
Jun a 1	Pectate lyase	<i>Juniperus ashei</i>	1PXZ	
Ole e 6	Unknown	<i>Olea europaea</i>		
Phl p 1	Expansin	<i>Phleum pratense</i>	1N10	1BMW
Phl p 2	Grass group 2/3	<i>P. pratense</i>	1WHO, 1WHP	
Phl p 5	Unknown	<i>P. pratense</i>	1L3P	
Phl p 6	Unknown	<i>P. pratense</i>	1NLX	2HCZ
Phl p 7	Calcium-binding protein	<i>P. pratense</i>	1K9U	
Zea m 1	Beta-expansin	<i>Zea mays</i>		
NMR—nuclear magnetic resonance spectroscopy.				

NMR—nuclear magnetic resonance spectroscopy.

has been proven by the same Japanese group. The substrate-binding and catalytic residues (C34, Q28, H170, and N190) are in a cleft (smaller than the one in Bla g 2) that is formed by juxtaposition of both terminal domains. The crystal structures of a recombinant pro-Der p 1 mutant (C114A) and mature Der p 1 without the pro- part have been reported (Fig. 1D and Fig. 1E, respectively) [9••,10••].

Group 2 dust mite allergens have an immunoglobulin-like fold with two major β -sheets [11]. Two x-ray crystal structures and an NMR structure of Der f 2 were published in 2005 (Table 1). An internal cavity for ligand binding is present in the x-ray crystal structures but not in the NMR structures of Der p 2 and Der f 2, which could indicate the presence of an “open” and “closed” state of the molecules. The NMR structure of Der f 2 is more

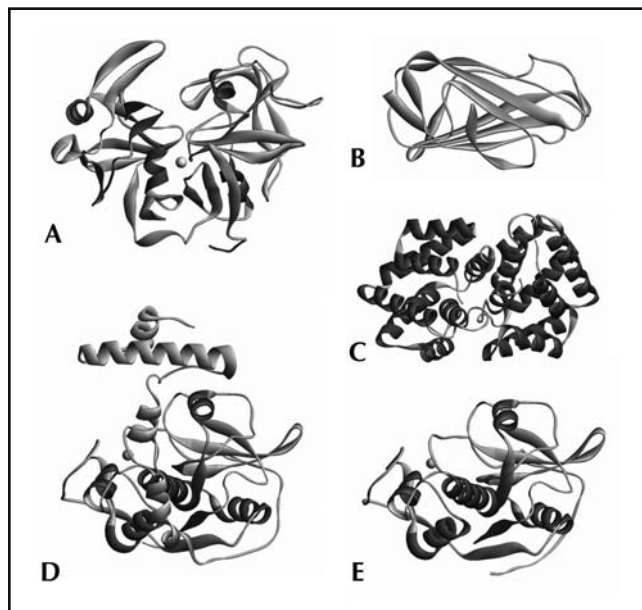


Figure 1. Three-dimensional structures of the indoor allergens Bla g 2 (A), Der f 2 (B), Fel d 1 (C), and pro- (D) and mature (E) Der p 1. The Protein Data Bank accession codes are 1YG9, 1XWV (chain A), 2EJN, 1XKG, and 2AS8, respectively. Spheres in Bla g 2 and Der p 1 are zinc and metal ions (magnesium for mature Der p 1) bound to the allergen, respectively.

similar to the crystal structure of the distant mammalian homologue NPC2 (a molecule that binds cholesterol and is responsible for Niemann-Pick type C2 disease) than to that of Der p 2 in terms of the separation and angle between the two major β -sheets (Fig. 1). Another allergen with an immunoglobulin-like fold is Zea m 1, a β -expansin from maize with a role of extension and stress relaxation of grass cell walls by binding to xylans and other polysaccharides [12].

Fel d 1 is a 35-kD tetrameric glycoprotein formed by two heterodimers that elicits IgE antibody responses in 95% of cat-allergic patients. The crystal structure of a construct of chain 2 plus chain 1 (Fel d 1 [2 + 1]) revealed a striking structural similarity to that of uteroglobin, a steroid-inducible, cytokine-like molecule with anti-inflammatory and immunomodulatory properties [13]. Recently, the crystal structure of the Fel d 1 (1 + 2) tetramer at 1.6-Å resolution has been reported, revealing two different calcium-binding sites. The authors speculated that Fel d 1 could provoke an allergic response through the modulation of phospholipase A2 by sequestering Ca^{++} ions in a similar manner as previously suggested for uteroglobin (Fig. 1) [14].

Additional new structures of outdoor allergens have been solved since 2004 from outdoor allergens (Che a 3, Hev b 6, Ole e 6), foods (Api g 1, Ara h 6, Gal d 3, Bos d 4, Bos d 5, Pru av 2, Pru p 3), injected allergens (Api m 2, Ves v 2), and allergens that sensitize through skin (Mala s 1, 6, and 13) (Table 2). Interesting structures have been described: a triple-helical bundle fold of Blo t 5; two anti-parallel α -helices and a long, unstructured C-terminal tail

of Ole e 6; a typical fold of a fatty acid-binding protein of Der f 13; a β -propeller fold of Mala s 1 that binds lipids; and an eightfold β/α architecture (TIM-barrel motif) typical for glycoside hydrolases of the banana fruit allergen. Table 1 and Table 2 contain the Protein Data Bank accession codes to access the structural information at <http://www.rcsb.org/pdb/>.

How Does Structure Define Function?

The shape of the allergenic molecule, as defined by its three-dimensional structure, determines in part the biochemical function. For example, globular molecules with a pocket provide the structural environment for a catalytic site to proteolytic enzymes such as Der p 1 [9••,10••], whereas the linear shape of tropomyosins contributes to this protein's structural function during muscle contraction. As mentioned, some allergens have an internal cavity that is associated with the transport of a ligand (eg, rodent lipocalins bind pheromones; cat Fel d 1 or group 2 mite allergens bind a ligand of unknown function).

Flexibility is another determinant of function. Calcium-binding proteins such as the cockroach allergen Bla g 6, a troponin C, have a regulatory function due to their capacity to modify the structure upon calcium binding [15].

Finally, allergen function is also defined by specific amino acids. The cysteine protease Der p 1, as well as serine and aspartic proteases, has few amino acids involved in the enzyme's catalytic mechanism. Specific motifs are also involved in enzymatic inhibition (such as the cysteine protease inhibitor motifs in Fel d 3 and Can f 1). Ion binding, as described for zinc bound to Bla g 2 or a metal bound to Der p 1, may confer stability to allergens [7••,9••,10••].

Elucidating Allergen Function

The biochemical function of some allergens was predicted from sequence homology to proteins of known function. A sequence homology as low as 25% to 30% may reflect the same overall protein folding, and some homologous allergens with the same three-dimensional structure can have a similar function. For example, lipocalins are ligand-binding proteins that act as transport proteins. Co-crystallization of Mus m 1 and Rat n 1 with their respective ligands revealed that these rodent allergens act as pheromone transporters [16]. Other allergens are also ligand-binding proteins, and the protein's molecular structure has provided clues to elucidate the biochemical function. Fel d 1 has an internal cavity similar to that of uteroglobin. The cavity in Fel d 1 is asymmetric, and the residues lining this cavity suggest that such ligand must be amphipathic [13]. The nonspecific lipid transfer proteins are a group of allergens of plant origin that bind and transport lipids [17]. The major allergen from birch tree pollen, Bet v 1, showed affinity for a broad spectrum of physiologic ligands. It recently has been reported

Table 2. Tertiary structures of allergens that sensitize by ingestion, injection, or through skin

Allergen	Function/structure	Species of origin	X-ray crystal structure	NMR structure
Ingested (food)				
Api g 1	Pathogenesis-related protein	<i>Apium graveolens</i>	2BK0	
Ara h 6	Conglutin	<i>Arachis hypogaea</i>		1W2Q
Bos d 4	Alpha-lactalbumin	<i>Bos domesticus</i>	1F6R, 1F6S, 1HFZ, 2G4N	
Bos d 5	Beta-lactoglobulin	<i>B. domesticus</i>	1GX8, 1GX9, 2AKQ, 1BSO, 1UZ2(m)	
Gal d 2	Ovalbumin	<i>Gallus domesticus</i>	1JTI, 1OVA, 1UHG	
Gal d 3	Ovotransferrin	<i>G. domesticus</i>	1RYX, 2D3I, 1OVT, 1AIV, 1TFA, 1NNT	1IEJ
Gal d 4	Lysozyme	<i>G. domesticus</i>	1FLQ(m), 1H6M(m)	1GXV, 1GXX
Mus a x	Banana glucanase	<i>Musa acuminata</i>	2CYG	
Pru av 2	Thaumatin-like protein	<i>Prunus avium</i>	2AHN	
Pru p 3	Nonspecific lipid transfer protein	<i>Prunus persica</i>	2ALG, 2B5S	
Ric c 3	Albumin storage protein	<i>Ricinus communis</i>		1PSY
Injected				
Api m 1	Phospholipase A2	<i>Apis mellifera</i>	1POC	
Api m 2	Hyaluronidase	<i>A. mellifera</i>	1FCQ, 1FCU, 1FCV, 2J88	
Api m 4	Mellitin	<i>A. mellifera</i>	1BH1(m), 2MLT	
Ves v 2	Hyaluronidase	<i>Vespula vulgaris</i>	2ATM	
Ves v 5	Antigen 5	<i>V. vulgaris</i>	1QNX	
Through skin				
Mala s 1	Beta-propeller fold	<i>Malassezia sympodialis</i>	2P9W	
Mala s 6	Cyclophilin	<i>M. sympodialis</i>	2CFE	
Mala s 13	Thioredoxin	<i>M. sympodialis</i>	2J23	

NMR—nuclear magnetic resonance spectroscopy.

to bind to and permeabilize membranes. Binding is accompanied by major structural rearrangements involving an increase in α -helical structure and a decrease in β -structure [18]. The group 2 mite allergens share structural similarity with the lipopolysaccharide (LPS)-binding protein myeloid differentiation protein 2, which has an immunoglobulin-like, β -rich fold. However, Der p 2 binds LPS only weakly, probably due to the lack of clusters of basic residues that are very important for LPS binding and signaling in myeloid differentiation protein 2 [19]. In the x-ray crystal structure of Der p 2, the β -sheets create an internal cavity that is occupied by an unidentified hydrophobic ligand [11].

Despite overall amino acid sequence and structural homology to a protein of known function, substitutions of specific amino acids in some allergens may lead to a different function, which can be investigated by enzymatic assays. Bla g 2, a German cockroach allergen, shares homology with aspartic proteases but, as mentioned previously, has important substitutions at the level of the catalytic site that impairs proteolytic activity [6,7••].

Tissue localization of the allergen is another way to elucidate its biologic function. Bla g 4 involvement in a

male reproductive function has been shown by in situ hybridization studies that localized this cockroach allergen in the utricles and conglobate gland of the adult male reproductive system. Bla g 4 is transferred to the female within the spermatophore during copulation [20]. The olive pollen allergen Ole e 10 was immunolocalized in mature and germinating pollen by transmission electron microscopy and confocal laser scanning microscopy. The allergen was co-localized with callose (1,3- β -glucan) in the growing pollen tube, suggesting a role for Ole e 10 in the metabolism of carbohydrates and pollen tube wall reformation during germination [21].

Why Do Proteins Become Allergens?

Information on the primary structure of allergens derived from molecular cloning in combination with structural data has helped us to formulate some factors that contribute to a protein becoming an allergen and define parts of the molecule involved in allergenicity. The classification of animal and plant food and pollen allergens in few protein families led to the conclusion that certain protein

structures are more allergenic than others [1,2]. This conclusion contrasts with the idea that any antigen may be allergenic [22]. A complete analysis of the presence of the allergens in the environment and the accessibility of the antigens to the human immune system will help us to understand if the ideas are two sides of the same coin.

A recent hypothesis for allergenicity postulates that allergens are eukaryotic proteins lacking bacterial homologues. From 30 allergens randomly selected from the 391 protein sequences reported as allergens in the Universal Protein Resource database, 25 were found not to have bacterial homologues, whereas control, nonallergenic proteins from the same species as the respective allergens showed homology to many bacterial proteins. This hypothesis is in agreement with the fact that early exposure to bacterial proteins could induce tolerance against homologous proteins inhaled later in life [23].

At the level of the three-dimensional structure, IgE antibody-binding epitopes generally are expected to be in areas of the allergen surface that differ from the structurally equivalent areas of endogenous proteins. Analysis of the animal food allergens, classified into three main families (tropomyosins, EF-hand proteins, and caseins), showed that in general, proteins with a sequence identity to a human homologue above approximately 62% were rarely allergenic [1].

Resistance to heat and proteolytic digestion in the gastrointestinal tract is another factor that may contribute to the allergenic potential of food proteins by allowing sufficiently intact (or a large fragment of) protein to be taken up by the gut and sensitized through the mucosal immune system [24,25]. There is a structural basis for this resistance to digestion. For example, all the 2S albumin allergens characterized to date contain an intrachain disulfide bond that holds the protein core together, even after extensive proteolysis, contributing to stability of a fragment that contains IgE antibody-binding epitopes [25,26]. A study investigated the stability of natural and recombinant cherry allergens Pru av 3 (nonspecific lipid transfer proteins), Pru av 1 (Bet v 1 homologue), and Pru av 4 (profilin) to pepsin digestion and thermal processing. In contrast to birch pollen-related allergens, nonspecific lipid transfer proteins were highly stable to pepsin treatment and thermal processing and showed higher allergenic potency [27].

The first mite allergen to be cloned was a cysteine protease, Der p 1. Other proteolytic enzymes were subsequently identified: the serine proteases Der p 3, Der p 6, and Der p 9. Several studies associated proteolytic activity with allergenicity based on increased bronchial permeability via disruption of epithelial tight junctions; the promotion of IgE antibody synthesis via CD23 cleavage in activated B cells and CD25 in T cells; and the induction of release of proinflammatory cytokines from bronchial epithelial cells, mast cells, and basophils [28]. New evidence has been reported supporting this "enzyme hypothesis" that postulates that

proteolytic activity of allergens contributes to allergenicity. A murine model was used to show that immunization with E-64-treated or heat-denatured rDer p 1 elicited much less production of serum total IgE and rDer p 1-specific IgE and IgGs compared with immunization with active rDer p 1 [29]. Most allergens are not proteolytic enzymes, and biologic function does not distinguish allergens from nonallergenic proteins.

Prediction of Allergenicity and Cross-Reactivity

New Internet-accessible allergen databases are available, and bioinformatic tools to classify and compare proteins have been improved. This has led to the development of computational methods for predicting allergenicity and cross-reactivity based on the allergens' linear sequence [30]. These tools are especially useful for predicting linear epitopes that are abundant in food allergens due to the proteolytic digestion of the allergen through the digestive tract. With the increasing knowledge of allergens' three-dimensional structures, these predictions also will soon be based on the homology among surface-exposed residues [31]. Along these lines, the three-dimensional structures of rChe a 3, rPhl p 7, and rBet v 4 were compared. Conserved amino acids with high surface exposure explained the extensive cross-reactivity of allergic patients' IgE antibodies with two EF-hand allergens from unrelated plants [32].

The biologic activity of cross-reactive carbohydrate determinant-specific IgE is being reconsidered under the light of new evidence. Foetisch et al. [33] proved that the cross-reactive carbohydrate determinant-specific IgE of approximately one third of patients with tomato allergy was biologically active and mediated histamine releases of up to 82%. Similarly, a carbohydrate determinant for IgE antibody binding was proven in the major latex allergen Hev b 13, a lipolytic esterase from *Hevea brasiliensis* [34].

Structural "Relatives" Among Different Allergenic Groups

Cross-reactivity occurs not only among homologue allergens from different sources but also among homologues from the same species. The World Health Organization/International Union of Immunological Societies Allergen Nomenclature arbitrarily decided that if two allergens shared 67% or greater amino acid sequence identity, they would be considered isoallergens. Under this definition, structurally related allergens have been assigned to different allergen groups. In some cases, new allergen identification can challenge the established allergen nomenclature or provide new information about closely related allergens. Groups 2 and 3 of grass pollen allergens are a good example of this situation. Specifically, Phl p 2 and Phl p 3 show sequence identities of 57% and share sequence homology (41% and 29%, respectively) with the C-terminal half of Phl p 1 [35]. The sequence homology of

the three allergen groups suggests a common origin: Phl p 2/3 may have been generated by partial gene duplication of the C-terminal part of the group 1 molecule, and Phl p 2 and 3 would have segregated later, as they have higher structural homology. Cross-reactivity of IgE antibodies was detected between Phl p 2 and 3 but not between these two allergens and Phl p 1 [35]. Similarly, the olive pollen allergen Ole e 10 showed homology with the C-terminal domain of Ole e 9 (1,2- β -glucanase, 53% identity) [21]. Finally, groups 5 and 21 from dust mites, sharing approximately 30% to 40% sequence identity, also belong to different groups of nomenclature. Although most (> 75%) sensitized individuals were cosensitized to Blo t 5 and Blo t 21, there was a low to moderate degree of cross-reactivity between both allergens [36].

Allergen Epitope Mapping

Most strategies reported for epitope mapping are not based on the proteins' tertiary structure but involve approaches that exclude conformational epitopes, such as the use of libraries of overlapping synthetic peptides (eg, for food allergens), the expression of allergen peptides as recombinant proteins, and the creation of epitope expression cDNA libraries. The most precise way to identify antibody-binding epitopes on an allergen is by cocrystallization of the allergen in complex with antibody fragments and the resolution of the three-dimensional structure of the complex by x-ray crystallography. Only four allergen-antibody complex structures have been solved to date. Three are complexes of Fab or Fab' fragments of IgG antibodies with the birch pollen allergen Bet v 1 [37], the bee venom allergen Api m 2 (hyaluronidase) [38], and the German cockroach allergen Bla g 2 [39]. The complex of a β -lactoglobulin with Fab fragments of a recombinant IgE monoclonal antibody generated from a phage display library was recently reported. Two IgE/Fab molecules bind dimeric β -lactoglobulin. The epitope consists of six different short fragments of the polypeptide chain located especially in the β -strands and covering a flat area on the allergen surface [40••].

Other strategies for epitope mapping, also based on tertiary structure, involve the use of NMR. A study reported NMR differential exchange broadening experiments for complexes of intact monoclonal antibodies and antigens to identify conformational epitopes for two monoclonal antibodies as two separate surface patches on Der f 2 [41]. Transverse relaxation-optimized spectroscopy/NMR experiments studied the interaction of Blo t 5 with the Fab' of a specific monoclonal antibody. The authors mapped a discontinuous epitope that overlaps with a human IgE antibody-binding epitope in Blo t 5 [42].

Finally, phage display technology has been used to select mimotopes that resemble the epitope of the allergen where the antibody used to screen the library binds [43]. Mimotopes from a few allergens (Bet v 1, Bla g 2, Der p 1,

Der p 2, Lol p 1, Pru p 3, and Phl p 5a) have been identified using this technology.

Space Distribution of IgE Antibody-Binding Epitopes: Clustering and Oligomerization Influence IgE Cross-Linking

The way in which B-cell epitopes are distributed in the space on the allergen's molecular surface in monomeric or oligomeric form determines the chances of cross-linking IgE antibodies. A monomeric allergen with two spatially accessible epitopes on the same side of the molecule will cross-link antibodies more easily than a monomeric allergen with two epitopes located on opposite sides. Flicker et al. [44] isolated human allergen-specific IgE antibody fragments (Fab) from lymphocytes of an individual with grass pollen allergy from a combinatorial library technology. Using rPhl p 1-derived allergen fragments, several IgE-binding sites of these Fab were located in one cluster on the C-terminal domain of Phl p 1 in a sterically oriented manner such that they could induce cross-linking of effector-bound IgE antibody [44].

Similarly, a dimer or oligomeric form of an allergen may favor cross-linking versus the monomer. Recently, dimeric β -lactoglobulin was reported in the crystal structure of the allergen in complex with Fab from IgE antibodies of a combinatorial scFv phage display library. The Fab fragments were located in positions such that the dimeric β -lactoglobulin would be able to cross-link two IgE antibodies [40••]. Oligomerization of allergens in their natural form is very common and has been observed in crystallized allergens (eg, Fel d 1, Bla g 2-Fab' complex, β -lactoglobulin) and in allergens purified by size exclusion chromatography (Ara h 1) [14,39,40••,45]. For example, purification of the native peanut allergen Ara h 1 by size exclusion chromatography led to an oligomeric rather than the reported trimeric structure. The monomers in Ara h 1 oligomers had a more compact tertiary structure compared with the monomers in Ara h 1 trimers, which may imply a different allergenicity for Ara h 1 than previously described [45].

Hypoallergens for Immunotherapy

The production of modified allergens with reduced IgE antibody-binding capacity but retained T-cell reactivity has been proposed to improve safety and efficacy of allergen extract-based immunotherapy. These vaccines' design is based on changing the allergen structure to reduce IgE antibody binding. Along these lines, several strategies have been attempted that involve production of allergen peptides or recombinant allergen fragments, creation of unfolded mutants by disruption of disulfide bonds or substitution of certain residues for proline, hypoallergens with point mutations, restructuring the molecules by swapping the N by the C-terminus, directed

molecular evolution by DNA shuffling, or the creation of hybrid molecules or dimers [46–48]. Since 2004, several hypoallergens have been engineered from Bet v 1, Phl p 1, Phl p 2, Phl p 5, Phl p 6, Phl p 7, Phl p 12, group 2 mite allergens, Fel d 1, fish parvalbumin, Pen a 1, and Dau c 1. It is not possible to cover all these studies in this review, but two examples are described.

Directed molecular evolution by DNA shuffling followed by allergen expression and screening for low IgE antibody binding was performed to identify and express three hypoallergenic forms of group 2 dust mite allergens (two isoforms of Lep d 2 and Gly d 2). The shuffled allergens showed 80-fold reduced to completely abolished IgE antibody binding compared with the parental allergens and comparable capacity to stimulate allergen-specific T-cell proliferation and cytokine production [47]. In another study, a dimer composed of mutants of Dau c 1.0104 and Dau c 1.0201 was a promising candidate vaccine for treating carrot allergy because of its high immunogenicity and drastically reduced allergenicity (by 81% on average). This study's results proved that destruction of native conformation rather than oligomerization is the appropriate strategy to reduce the allergenicity of Bet v 1-homologous food allergens [48].

Molecular and structural studies are beginning to produce potential new forms of immunotherapy. A recombinant vaccine of a mixture of five wild-type, recombinant grass pollen extracts has been successful in clinical trials with patients with allergies [49••]. Vaccination with genetically engineered hypoallergenic rBet v 1 derivatives (fragments or trimer) also has shown potential to reduce allergic responses [50].

Conclusions

In the past decade, allergen function and structure have been studied in depth using modern technologies (eg, x-ray crystallography, NMR, mass spectrometry, and immunohistochemistry). The acquired knowledge is being applied to help us better understand allergenicity and to identify antigenic epitopes relevant to the allergic disease. Comparison of the surface-accessible residues on the three-dimensional structures of homologue allergens provides information about amino acids involved in cross-reactivity. As clinical sensitivities tend to reflect allergenic cross-reactivity, diagnosis and therapy can be simplified, taking into account allergenic cross-reactivity. The application of recombinant technology to the design and production of hypoallergenic forms for immunotherapy is beginning to yield positive results in clinical trials.

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Disclosure

Dr. Pomés is a full-time employee of Indoor Biotechnologies, Inc.

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- Of importance
- Of major importance

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