

Immunoglobulin E-Binding Epitopes of Mite Allergens: From Characterization to Immunotherapy

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Abstract House dust mites and storage mites produce a number of allergens that can induce hypersensitivity reactions in humans and result in allergic diseases like asthma, rhinitis, and dermatitis. Recent advances in identifying and characterizing these allergens—and, in particular, their immunoglobulin E (IgE)-binding epitopes—have produced a wealth of knowledge. Here, methods for identifying IgE-binding epitopes, from immunoassays to in silico approaches, are summarized and placed in context with the identification of epitopes of mite allergens, particularly from the *Dermatophagoides* spp. major allergen groups 1 and 2. Finally, the transfer of this information to the clinical development and application of new diagnostic and immunotherapeutic approaches is discussed. While progress in recent years has built on the specific immunotherapies established decades ago, much work remains to be done to mitigate mite allergic disease. Future studies should seek to identify epitopes for mite species beyond *Dermatophagoides* and for minor allergens. Efforts in translational medicine should use the current epitope data to develop modified allergens for immunotherapy.

Keywords Immunoglobulin E · IgE · Epitopes · Mite allergens · Specific immunotherapy

Introduction

Most allergic disease is caused by allergens produced by plants and animals that, when ingested, inhaled, or in contact

with skin, trigger an immune response in a sensitized individual. Exposure to an allergen can lead to a complex hypersensitivity reaction [1]. Importantly, allergens from different sources can be similar enough to produce a cross-reaction, and the careful identification and classification of allergens are critical to understanding the allergic response and treating allergic disease. Indeed, advances in the characterization of allergens through genomic, proteomic, and structural biochemistry methods have already led to more rapid development of specific immunotherapy (SIT) approaches to alleviate allergic disease caused by both environmental (e.g., cat dander) and food-borne (e.g., milk, egg) allergens [2].

House dust mites and storage mites are small arthropods that produce a number of allergens responsible for allergic diseases like atopic dermatitis, asthma, and rhinitis in many people worldwide [3]. Dust mites, including *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Euroglyphus maynei*, and other species in the family Pyroglyphidae, thrive in human residences [4]. Storage mites, predominantly of the superfamilies Acaroidea and Glycyphagoidea, are commonly found in grain, fishmeal, hay, dried fruit, and traditional Chinese medicine herbs; their allergens cause occupational respiratory allergies in farmers and other occupationally exposed individuals [5].

The prevalence of allergic disease resulting from mite allergens has resulted in great efforts to characterize them for the production of new and more efficacious specific immunotherapies against them [6, 7]. These efforts hinge on the understanding of epitopes, or binding sites, for immunoglobulin (Ig) E, the antibody that triggers the hypersensitive immune response resulting in allergic disease [8]. In this review, I will present the current knowledge of the mite allergen epitopes, particularly IgE-binding epitopes, and how this knowledge can advance the development of SIT for mite-derived allergic disease.

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IgE and the Allergic Response

IgE, a major histocompatibility complex antibody found only in mammals, binds to allergens to mediate type I hypersensitivity responses. Figure 1 depicts a detailed IgE response to allergens. First, allergens are processed by antigen-presenting cells for presentation to T lymphocytes. CD4⁺ helper T cells induce the production of allergen-specific IgE antibodies by B lymphocytes and produce Th2-type cytokines like interleukin-4. The release of cytokines and production of IgE antibodies occur on the first exposure to an allergenic substance; subsequent exposure invokes the type I hypersensitivity response, in which additional molecules, like histamines, are produced and trigger the symptoms of allergic disease [1].

An epitope is the portion of an antigen that binds with T and B cells. B cell epitopes are the allergenic regions of a protein recognized by IgE. In contrast, T cell epitopes can indirectly stimulate IgE production through a process mediated by T helper cells. Epitopes are classified as sequential epitopes and conformational epitopes [9]. Sequential, or linear, epitopes comprise a single continuous stretch of amino acids within a protein sequence that can react with anti-protein antibodies. T cell epitopes are often sequential. Conformational epitopes

comprise residues that are distantly separated in the sequence and brought into physical proximity by protein folding. Both conformational and sequential epitopes are important for the development of allergic reactions [10].

Methods for Identifying IgE-Binding Epitopes

Sequential Epitopes

Over the last 20 years, several methods for identifying IgE-binding sequential epitopes have been widely used. Conventionally, B cell epitopes have been localized by partial protease digestion or chemical cleavage at sites of specific amino acids, Western blotting using patient sera, and amino acid sequencing of IgE-binding fragments [10]. The solid-phase peptide (SPOT) membrane immunoassay was later introduced for mapping of allergen epitopes. In this system, overlapping peptides (Fig. 2) covering the primary amino acid sequence of allergens are synthesized in situ on a nitrocellulose membrane and subsequently incubated with patient sera. Peptides bound by the IgE antibody are detected by an anti-human IgE antibody labeled with peroxidase, which is then detected by a chemiluminescent

Fig. 1 The IgE response. Reprinted from [67] with permission by Nature Publishing Group

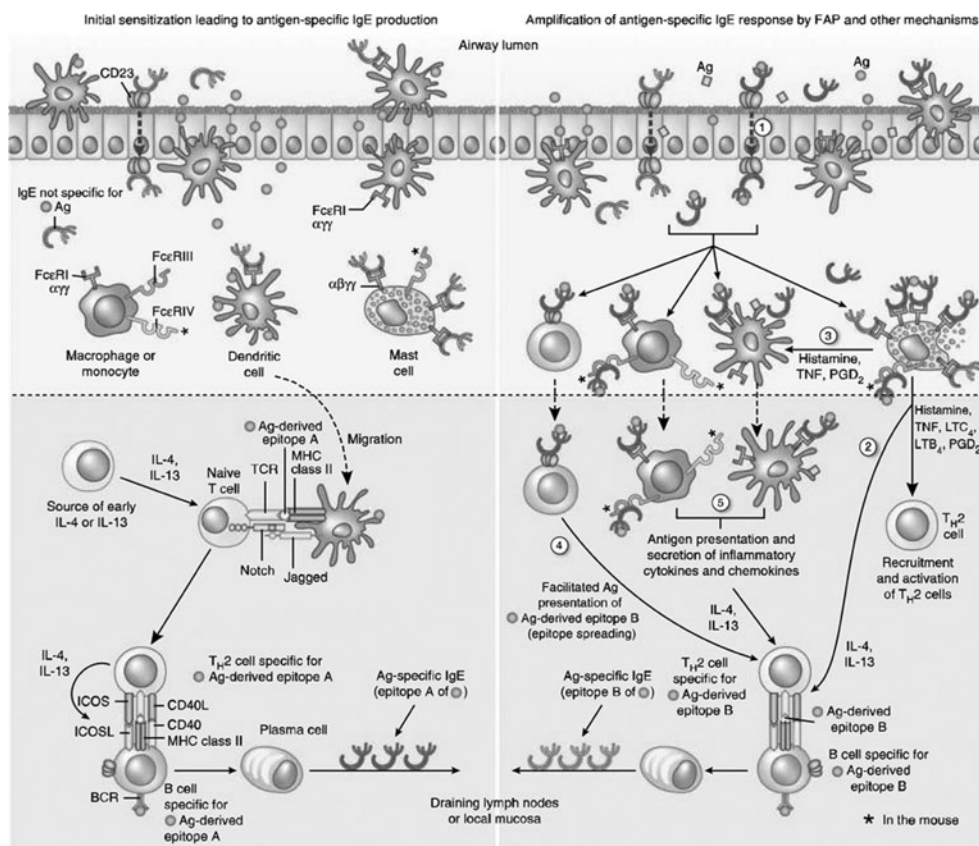




Fig. 2 Schematic of the strategy for generating overlapping peptides to map an epitope, using Der f 2 as example. In this figure, the overlapping fragments of 14 amino acids in length and 11 overlapping amino acids were designed and synthesized for the group 2 allergen of *D. farinae* (Der f 2)

with a total of 129 amino acid residues. If the peptides 2 (DVKDCANNEIKKVM) and 3 (DCANNEIKKVMVDG) are positive against sera from patients allergic to Der f 2, it is highly probable that an epitope structure exists in the overlapped amino acids (DCANNEIKKVM)

substrate and exposed to radiographic film [11]. To synthesize peptides by the traditional approach, many cycles of coupling, blocking, and deprotection must be performed. However, running so many reaction cycles causes by-products to accumulate, reducing the peptide specificity and increasing background noise [12]. This approach also requires prohibitive volumes of patient sera and does not offer a comprehensive selection of peptide targets. Despite these drawbacks, the SPOT method has succeeded in mapping many IgE sequential epitopes and is still used in epitope mapping.

Microarray immunoassay, first introduced for determining allergen epitopes by Shreffler et al. [13], was developed to determine IgE-binding epitopes of the major peanut allergens—Ara h 1, Ara h 2, and Ara h 3. This assay identified the same epitopes as the SPOT assay. Further, this approach offers some advantages over SPOT synthesis, since small volumes of patient sera can be used to screen thousands of

peptides. In contrast to SPOT, the commercial synthesis of microarray peptides is controlled to ensure purity and specificity. Additionally, the use of fluorescent antibodies to detect the target peptides on a scanner improves visualization over radiographic techniques. Finally, qualitative results can be produced for multiple allergens in the same assay system [12]. The development of this system for epitope mapping can allow experiments to be completed more rapidly and with higher quality results than those produced by the SPOT assay.

In addition to experimental approaches, epitopes can be identified *in silico*. Many allergens have similar sequences and structures; homology search and alignment guide the identification of specific epitopes. Going beyond BLAST, in the last few years, a number of bioinformatics tools and network resources have been developed for prediction of the sequential epitopes based on physicochemical properties like hydrophilicity, flexibility, accessibility, and turns, such as DNASTAR Lasergene

(DNASTAR Inc., Madison, WI, USA). For a more detailed discussion of bioinformatic analysis and characterization of allergens, see my recent review on mite allergens [14]. Bioinformatic analyses provide a great starting point to guide future experimental studies of epitopes.

Conformational Epitopes

Conformational epitopes are more significant for the study of mite allergens than for food-derived allergens because three-dimensional structures of food-derived allergens may be damaged during preparation and/or digestion, altering protein stability [10]. Thus, a number of approaches have been developed to characterize these three-dimensional structures.

One approach, site-directed mutagenesis, introduces changes to the amino acid sequence at specific sites to produce a recombinant allergen. The recombinant allergen is then compared with the native allergen in patient sera, to identify differences in IgE binding capacity. A reduced binding capacity signals that a particular amino acid is critical to the allergenicity of the protein [15], likely because it is important for some aspect of the epitope conformation.

A relatively new technique, phage display, creates a mimic of a natural epitope, called a mimotope, to permit its characterization [16]. Phage display libraries are based on random peptides that can be used to mimic the binding site for an antibody. A phage display library screens peptides with purified allergen-specific IgE. Subsequently, *in silico* tools can be used to map the mimotope onto the allergen structure. Mimotopes vary in length from 6 to 20 amino acids. Interestingly, the linear sequence of the allergen of interest may be different from the mimotope; indeed, the peptides can represent not only conformational epitopes, but also sequential epitopes and non-protein epitopes [17].

Although mutagenesis and phage display do provide useful insight to potential epitopes, conformational epitopes must be verified by X-ray crystallography or nuclear magnetic resonance spectroscopy. These two classical approaches provide higher resolution and more detailed information, though they are technically more challenging.

Epitopes of Mite Allergens

House dust mites and storage mites produce allergens inducing specific IgE-mediated sensitization in susceptible individuals. Because of their importance to allergic disease, the IgE-binding epitopes of these allergens must be well understood. Mite allergens are grouped based on similarity and are named using the first three letters of the genus, the first letter of the species name, and a number designating the order in which the allergen was isolated or the number for other already-characterized allergens it matches in homology and molecular weight. For example, group 1 allergens from the house dust mite *D. farinae* are

termed Der f 1. The International Union of Immunological Societies (IUIS) nomenclature database [18] lists 24 different mite allergens; 22 have been described from *Dermatophagoides* spp., and 14 have been described from storage mites. Table 1 summarizes the known allergens for house dust mite and storage mite species. Known epitopes for some of these allergens are described in detail below and summarized in Table 2.

Epitopes of the Group 1 Allergens

Mite allergens belonging to group 1 are cysteine proteases in the same family as the plant proteins papain and cathepsin L [19]. Der p 1 and Der f 1, from *D. pteronyssinus* and *D. farinae*, respectively, have a similar primary structure of 222 amino acids (aa) and a molecular mass of 25 kDa. They display 81 % sequence homology, as deduced from cDNA clones, and elicit IgE responses in 80 % of mite-allergic subjects. Initial epitope characterization using five mouse monoclonal antibodies (mAbs) with different specificities against *D. farinae* extract identified two different epitopes and two partly overlapping determinants in Der f 1 [20]. Subsequent studies by Chapman et al. [19] assessed the binding of six ¹²⁵I-labeled mAbs to solid-phase allergen to define four non-repeated, non-overlapping epitopes on Der p 1 (10B9, 5H8C12, 5H8D8, and C4.1), a single species-specific epitope on Der f 1 (6A8), and a cross-reacting epitope (4C1) present on each allergen. Similarly, Le Mao et al. [21] reported the presence of at least four non-overlapping, non-repeated epitopes on Der f 1, i.e., sites A, B, C, and D, which were recognized by the selected mAb (4C1B8, MAF1-4, MAF-5-7, and MAF 8-9) obtained from BALB/c mice immunized with purified Der f 1. Together, these studies revealed the similarities in epitopes and IgE binding for the group 1 allergens.

Further work identified five IgE-binding determinants of Der p 1 by the reactivity of peptide fusions expressed in *Escherichia coli* using serum with high reactivity to Der p 1 [22]. Four (15–33, 60–80, 81–94, and 101–111 aa) are located in the N-terminal region and one (155–187 aa) is located in the C-terminus. Peptides 81–94 and 101–111, which demonstrated strong reactivity, are located on a flexible loop connecting the domains. Predictive algorithms regarding accessibility, mobility, hydrophathy, and flexibility identified four other peptides (52–71, 117–133, 176–187, 188–199) as sequential epitopes of Der p 1; homology modeling from actinidin and papain confirmed these potential epitopes [23]. Studies indicated that only sera from patients allergic to *D. pteronyssinus* react with these four peptides, more frequently to 52–71 and 117–133 aa; the authors therefore underlined the possible epitopes in these synthetic Der p 1-derived peptides [23].

Dai et al. [24] used a hybridoma-based antibody (W108) producing Der p-specific IgG2b to identify two peptide fragments (151–197 and 286–320 aa) in Der p 1 precursor that belong to the inter-connecting loops located in the substrate-

Table 1 Mite allergens listed in the IUIS Allergen Nomenclature [18] as of October 6, 2013

	Species names	Allergens identified
House dust mites	<i>Dermatophagoides farinae</i>	Der f 1, Der f 2, Der f 3, Der f 6, Der f 7, Der f 10, Der f 11, Der f 13, Der f 14, Der f 15, Der f 16, Der f 17, Der f 18, Der f 22, Der f 24
	<i>D. microceras</i>	Der m 1
	<i>D. pteronyssinus</i>	Der p 1, Der p 2, Der p 3, Der p 4, Der p 5, Der p 6, Der p 7, Der p 8, Der p 9, Der p 10, Der p 11, Der p 14, Der p 15, Der p 18, Der p 20, Der p 21, Der p 23
	<i>Euroglyphus maynei</i>	Eur m 1, Eur m 2, Eur m 3, Eur m 4, Eur m 14
Storage mites	<i>Acarus siro</i>	Aca s 13
	<i>Blomia tropicalis</i>	Blo t 1, Blo t 2, Blo t 3, Blo t 4, Blo t 5, Blo t 6, Blo t 10, Blo t 11, Blo t 12, Blo t 13, Blo t 19, Blo t 21
	<i>Glycyphagus domesticus</i>	Gly d 2
	<i>Lepidoglyphus destructor</i>	Lep d 2, Lep d 5, Lep d 7, Lep d 10, Lep d 13
	<i>Tyrophagus putrescentiae</i>	Tyr p 2, Tyr p 3, Tyr p 10, Tyr p 13, Tyr p 24

binding cleft and on the surface of the domain comprising mainly β -sheets. Further, these fragments bound to specific IgE antibodies from pooled sera of Der p-sensitized patients [24]. In contrast, SPOT synthesis of 31 peptides containing 15 aa each, which covered the full 222 aa of Der p 1 protein sequence, revealed three strong IgE-binding epitopes (85–99, 106–120, and 190–204 aa) [25]. Although, together, these studies indicate that the group 1 allergens contain sequential epitopes, some have concluded that the specific binding epitopes of the house dust mite allergens Der p 1 and Der f 1 are discontinuous in nature [26].

A Der p 1 conformational epitope was identified by Furmonaviciene et al. [27] using a mouse monoclonal anti-Der p 1 antibody (mAb 2C7). This antibody was chosen to represent a component of the human IgE anti-Der p 1 response. The identified epitope, Leu147-Gln160, was recognized from both non-constrained (M13, 15mer) and constrained (T7, 9mer) phage-display peptides in the native Der p 1 sequence [27]. These mimotopes were placed onto three-dimensional computational matching to map them to the allergen structure [28]. Further work by Chruszcz et al. [29], who used fragment antigen-binding (Fab) and identification of mAb and IgE interaction with allergens, revealed a unique cross-reactive epitope between Der p 1 and Der f 1 that is associated with IgE recognition.

Epitopes of the Group 2 Allergens

Group 2 allergens are categorized as ML domain lipid-binding proteins [30]. Epitope mapping using SPOT synthesis with 14 peptides of 15 aa and spanning the entire sequence of Der p 2 identified one sequential epitope comprising residues 65–78 [31]. Similarly, SPOT for Der p 2 in polyclonal T cells isolated from sensitized individuals recognized peptides 61–86 and 78–104 aa in a majority, 11–50 aa in half, and 81–96 and 91–101 aa in some individuals; the authors concluded that the

T cell epitopes of Der p 2 located at 11–50 and 61–104 aa are immunodominant [32]. Another panel for Der p 2 was applied to polyclonal Der p 2-specific T cell lines prepared from the peripheral blood of five atopic patients. The panel reacted with ten different epitopes at residues 1–15, 11–24, 20–33, 29–42, 38–51, 47–60, 56–69, 92–105, 101–114, and 116–129. Additionally, the specificity of the T cell response to Der p 2 was studied in one atopic patient using a short-term polyclonal T cell line that strongly reacted to one single peptide (116–129) of the allergen [33]. Further, Verhoef et al. [34] identified four distinct T cell epitopes located at residues 16–31, 22–40, 82–100, and 111–129 by using overlapping synthetic peptides of Der p 2. Serological inhibition studies showed that recognition of peptides 16–31 and 111–129 is restricted by HLA-DQ (DQB1*0301), whereas peptide 82–100 is recognized in association with HLA-DR (DRB1*1101), and peptide 22–40 is presented by both HLA-DRB1*1101 and -DQB1*0301 class II molecules [34].

T cell determinants within the Der p 2 molecule were also identified by assays using a series of overlapping peptides (15- to 19-mers) spanning the entire protein. Most sensitized patients responded to at least one peptide; 66.7 % of patients responded to the peptide spanning residues 111–129. Other peptides were each recognized by between 8 and 25 % of individuals [35]. To identify such epitopes, mice were immunized with rDer p 2 and separated for T cells, and a universal epitope was recognized encompassing residues 21–35 that was also suggested as a suitable candidate for T cell anergy induction [36]. Further study by Kobayashi et al. [37] revealed that IgE and IgG4 antibodies against Der p 2 recognize different epitopes. In most patient sera, IgE antibodies bound to full-length Der p 2 (1–129) as well as one or more of fragments 41–80, 64–105, or 81–129 but not to 1–40 or 20–63 [37]. However, in sera where IgE antibodies bound to Der p 2 fragments, few had IgG4 antibodies reactive to the Der p 2 fragments. Thus, IgG4 antibodies possess a different binding spectrum against Der p 2

Table 2 Epitopes of mite allergens (partial)

Allergens	Epitope (corresponding amino acid position)	Epitope type	Method(s) for identification of epitopes	References
Der p 1	15–33, 60–80, 81–94, 101–111, 155–187	Linear	Overlapping peptides	[22]
	52–71, 117–133, 176–187, 188–199	Linear	In silico	[23]
	151–197, 286–320	Linear	Overlapping peptides	[24]
	85–99, 106–120, 190–204	Linear	Overlapping peptides	[25]
	147–160	Conformational	Peptide display library	[27]
	181–182–183–180–179–178–13–12–11, 162–211–150–146–147–151–152–153–155, 200–25–189–190–150–196–195–194–199, 209–215–212–143–211–150–196–195–194, 156–155–198–199–200–25–193–194–195	Conformational	Peptide display library	[28]
	14, 18, 19, 21, 157, 186, 199, 204,	Conformational	Site-directed mutagenesis	[29]
	144–169, 21–49, 78–100, 110–131, 197–212	Linear	Overlapping peptides	[60]
	110–131, 110–119, 107–119	Linear	Overlapping peptides	[66]
	90–89–37–36–35–34–33–94–92, 91–92–35–33–94–95–96–97–98, 15–39–88–89–37–36–92–35–91, 66–67–68–91–35–34–33–94–92,	Conformational	Peptide display library	[28]
Der p 2	65–78	Linear	Overlapping peptides	[31]
	11–50, 61–104	Linear	Overlapping peptides	[32]
	1–15, 11–24, 20–33, 29–42, 38–51, 47–60, 56–69, 92–105, 101–114, 116–129	Linear	Overlapping peptides	[33]
	16–31, 22–40, 82–100, 111–129	Linear	Overlapping peptides	[34]
	111–129	Linear	Overlapping peptides	[35]
	21–35	Linear	Overlapping peptides	[36]
	41–80, 64–105, 81–129	Linear	Overlapping peptides	[37]
	101–120	Linear	Overlapping peptides	[38]
	14, 18, 157, 186, 199, 204	Conformational	Site-directed mutagenesis	[29]
	67–90	Linear	Deletion mutants	[40]
Der f 2	69, 71, 74, 128, 129	Conformational	NMR and site-directed mutagenesis	[41]
Der f 7	46–50	Linear	Overlapping peptides	[45]
	156–160	Linear	Overlapping peptides	[46]
Der f 13	41, 63, 91, 103	Conformational	Site-directed mutagenesis	[49]
Der f 15	411–429, 480–503	Conformational	Peptide display library	[50]
Blo t 5	76–91	Linear	Overlapping peptides	[43]
Blot 10	1–134, 203–284	Linear	Overlapping peptides	[47]
Blo t 11	336–424, 448–557	Linear	Overlapping peptides	[48]
Blo t 12	30–49, 73–92, 111–130	Linear	Overlapping peptides	[52]
Blo t 21	74, 79, 84, 89, 96	Conformational	NMR and site-directed mutagenesis	[51]
Lep d 2	11–25, 61–75	Linear	Overlapping peptides	[42]

fragments [37]. Within one pool of Der p 2 peptides, the 20-mer peptide (101–120) elicited the highest and most frequent response, which contained T cell epitopes restricted through several alleles, including HLA-DPB1*01 and HLA-DRB1*01 [38].

Der f 2 has been investigated in detail as well. By introducing artificial mutations into Der f 2 protein, the two disulfide-forming regions of Der f 2 (Cys8-Cys119 and Cys73-Cys78) were concluded to be associated with major epitope structures recognized by human IgE antibodies [39]. The B cell epitope of Der f 2 was analyzed using deletion mutants expressed in *E. coli*. IgE epitopes of Der f 2 are highly dependent on the

integrity of conformational structure; sequences 1–24, 25–29, and 121–123 are the minimum N- and C-terminal sequences required for IgE binding; and the cores of the epitopes for murine anti-Der f 2 mAbs in the peptide comprise 24 amino acids, residues 67–90 [40]. Using nuclear magnetic resonance to study Der f 2, Ichikawa et al. [41] detected the conformational epitopes for mAbs 15E11 and 13A4 as two separate surface patches with possible residues at D19, D69, N71, and H74 for 15E11 and D19, R128, and D129 for 13A4 [41].

The storage mite *Lepidoglyphus destructor* is an important resource of indoor allergens, especially in farming environments.

There is a significant difference in T cell response between the two recombinant isoforms of Lep d 2, the Lep d 2.6Cys mutant, and peptides of Lep d 2 in allergic and non-allergic individuals [42]. Furthermore, amino acid residues 11–25 and 61–75 are the most frequently recognized parts of Lep d 2 and most likely contain the immunodominant T cell epitopes [42]. However, additional investigation is needed for this major allergen.

Epitopes for Other Groups of Mite Allergens

Group 5 allergens have been isolated from several species of dust mites, including *Blomia tropicalis* (Blo t 5) and *D. pteronyssinus* (Der p 5). Blo t 5 contains a 17-residue signal peptide and 117-residue mature protein. Using overlapping peptides, a major putative linear IgE epitope of Blo t 5 was identified from residues 76–91 (ELKPTDLNILERFNYE) comprising four charged residues, Glu76, Asp81, Glu86, and Glu91 near the turn region connecting helices α 2 and α 3 [43].

The group 7 mite allergens induce IgE responses in about 50 % of asthmatic patients allergic to dust mite extract, and Der f 7 and Der p 7 share 86 % amino acid sequence identity [44]. To locate the binding site of the mAb prepared by immunizing mice with recombinant Der f 7, 20 overlapping peptides covering the complete sequence of the mature Der f 7 were synthesized with 15 amino acids offset by 5 amino acids, and one linear sequence (46GILDF50) with a critical role for L48 or F50 was determined by immunodot blot inhibition assay [45]. Meanwhile, by immunodot blot inhibition using synthetic overlapping peptides, allergen mutants, and a Der f 7 structural model, one antigenic determinant ($^{151}\text{HIGGLSILDPIFGVL}^{165}$) was identified and narrowed for $^{156}\text{SILDP}^{160}$ on Der f 7; IgE antibodies recognize residues Ile-157, Leu-158, and Asp-159 [46]. Furthermore, the corresponding Der p 7 peptide ($^{151}\text{HIGGLSILDPIFAVL}^{165}$) also inhibited IgE-binding against Der f 7, and Asp-159 is a key residue contributing to IgE cross-reactivity between Der f 7 and Der p 7 [46].

Overlapping polymerase chain reaction-derived cDNA molecules were generated and expressed as glutathione-S-transferase recombinant proteins in *E. coli* to identify shared and unique IgE epitopes of Blo t 10 and Der p 10. IgE epitope mapping of Blo t 10 revealed that the epitopes are mainly located at N- and C-termini of the molecule, and ELISA inhibition assays of overlapping recombinant fragments indicated that the unique IgE epitopes of Blo t 10 are located at the C-terminus [47].

Blo t 11 is an important allergen with an in vitro IgE reactivity of approximately 52 % among sera from patients with asthma [46]. Based on secondary structure predictions using the PHD secondary structure prediction program (pbil.ibcp.fr), overlapping fragments for Blo t 11 were used for epitope mapping. Initial IgE immunodominant peptide mapping revealed a significant reactivity of the 36 sera tested to two synthesized peptides at amino acid positions 305–447 (86 %) and 425–647 (83 %) [48]. Further IgE peptide

mapping using smaller Blo t 11 fragments showed significant reactivity to two short peptides at amino acid positions 336–424 (78 %) and 448–557 (75 %) [48].

The residues Glu-41, Lys-63, Lys-91, and Lys-103 were identified to be critical for IgE binding on Der f 13 [49]. Employing random heptapeptide T17 and random 12-mer M13 phage libraries, the amino acid regions, 411–429 and 480–503 of Der f 15 allergen, appeared to be the main epitope clusters binding to mAbs [50].

In *B. tropicalis*, the most prevalent and potent allergens are Blo t 21, Blo t 5, and Blo t 7 [51]. Using synthetic overlapping peptides, three IgE-binding regions were detected for Blo t 12 by means of ELISA inhibition: 30–49, 73–92, and 111–130 [52]. Using site-directed mutagenesis and specific IgE-binding ELISA, Blo t 21 was found to contain both conserved and unique charged IgE epitope residues at the L2 loop region and on helix α 3, and the four identified IgE epitope residues (Asp-79, Glu-84, Glu-89, and Asp-96) and Glu-74 (one of the identified IgE epitope of Blo t 5) are arranged in two separate patches, with Glu-74, Asp-79, and Glu-84 on one side and Glu-89 and Asp-96 on the other side of the protein based on the structure of Blo t 21 [51].

Application of IgE-Binding Epitopes in the Diagnosis and Immunotherapy of Mite-Allergic Diseases

Recently, studies have suggested a correlation between IgE-binding epitope diversity and patients' allergy severity or persistence. Further, studies of food allergens like peanut, milk, and egg have shown a potential role of IgE-binding epitopes as biomarkers for characterizing various phenotypes [53–55]. Thus, an emerging technique, referred to as peptide-ELISA or epitope-ELISA, has the potential to offer rapid advances in clinical diagnosis of allergic disease [56]. This technique treats the short peptide synthesized based on epitope information as a coat antigen in ELISA. Although the approach is not used widely yet, it has successfully been used to identify antibodies for certain infections, like H1N1 flu [57]. The application of this approach to diagnosis of disease related to mite allergens should be explored.

Conventional immunotherapy for allergic disease has used the paradigm of desensitization: increasing amounts of an allergen are introduced to a patient over time, leading to a weakened immune response to that allergen and, thereby, lessening symptoms [1]. To reduce adverse effects, such as anaphylactic shock, and to help standardize treatment approaches, immunotherapy is moving toward the use of hypoallergenic mutants devoid of IgE-binding epitopes rather than native allergens [58]. Current approaches focus on the development of recombinant allergens with modified B cell epitopes that will prevent IgE binding but with intact T cell epitopes to produce the immunotherapeutic benefit [59].

This approach has been employed for mite allergens to some extent. Preclinical studies in animal models are aiding in the translation of allergen immunotherapies to those that will be safe and useful in humans. Hoyne et al. [60] developed recombinant Der p 1 allergens with amino acid substitutions in residues important to IgE binding. Recombinant allergens were administered to mice, and the residue-222 Der p 1 allergen produced T cells sensitized to the dominant epitopes encompassed in peptides 21–49, 78–100, 110–131, and 197–212 [60]. In an intriguing approach, Suzuki and colleagues expressed T cell epitopes of recombinant Der p 1 in transgenic rice seeds to alleviate allergen sensitivity in mice [61]. Such an approach has yet to be attempted in humans [62]. Some work on Der p 2 allergen therapy in mouse models has also shown promise. A recombinant Der p 2 with a truncated peptide was administered to mice in milk, producing reduced airways inflammation [63]. Interestingly, transgenic mice were generated using T cell receptor (TCR)- $\alpha\beta$ derived from a CD4⁺ T cell hybridoma reactive with Der p 1 residues p 110–131; this provides a model for investigating the pathophysiological mechanisms of pulmonary inflammation following sensitization by exposure of the airways to allergen and can also be used to investigate the mode of action and efficacy of novel immunotherapeutics [64]. These types of preclinical studies should lead to novel therapies in the clinic more quickly.

Some clinical trials in humans have been initiated in recent years to high light improved safety and efficacy of more specific recombinant mite allergen therapies. For example, administration of a recombinant allergen based on a short peptide of Der p 1 that contained B cell epitopes was associated with reduced symptoms, particularly skin and conjunctival reactions, in individuals with dust mite allergies [65]. A triple mutant of Der f 13 (E41A_K63_K91A) was generated and found to have significantly reduced IgE binding and histamine release in skin prick tests on patients allergic to group 13 dust mite allergens. The triple mutant was also able to induce peripheral blood mononuclear cell proliferation in allergic patients with indices similar to those of wild-type Der f 13 and shift the secretion of cytokines from a Th2 to Th1 pattern, indicating potential for the triple mutant as a hypoallergen for specific immunotherapy [47]. A search of clinicaltrials.gov (October 2013) identified seven clinical studies that are currently or soon to be recruiting to investigate specific immunotherapy to dust mite allergens. More studies can be anticipated as the characterization of mite allergen epitopes and preclinical studies of recombinant allergens in animal models advance the field.

Conclusions

Much effort has focused on identifying the epitopes of mite allergens, but particularly for the major (groups 1 and 2) allergens in *Dermatophagoides* spp. Future work should seek to identify

the epitopes from other allergens, like groups 4, 5, and 7 with mid-potency specificities. Continuing identification of allergens in other mite species, including storage mites, remains important.

The availability of more sensitive technologies offers an opportunity for more rapid discovery of novel therapeutic and diagnostic approaches. Microarray immunoassay permits the identification of mite allergens for large-scale epitope mapping. Peptide-ELISA is a sensitive and highly specific method that should be employed more widely for sero-diagnosis of allergic diseases. Finally, genetically engineered allergens with modified B cell epitopes and synthetic peptides representing immunodominant T cell epitopes of major allergens are novel immunotherapeutics with improved safety and efficacy profiles. By applying these techniques, it is possible to discover how the mounting allergen epitope data can be exploited in the diagnosis and immunotherapy of mite allergic disorders.

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