

ORIGINAL ARTICLE

Homology modeling and monoclonal antibody binding of the Der f 7 dust mite allergen

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The group 7 allergens are important allergenic specificities for mite-sensitive patients and may need to be incorporated into new diagnostic and therapeutic strategies. However, little is known about their biological and structural features. Position-specific iterative BLAST showed that they had strong ancestral homology to two related families of lipid-binding proteins, namely, the bactericidal permeability-increasing (BPI) proteins and the odorant-binding protein. A three-dimensional model of Der f 7 made with the Phyre and SWISS-MODEL homology-modeling servers showed a close match with the human BPI coordinates used for its construction. The binding of the monoclonal antibody HD12 known to block IgE binding could be blocked by the linear sequence (46GILDF50) with a critical role for L48 or F50. These hydrophobic residues were located on a surface loop of the model. The properties of Der f 7 that can be deduced from the model provide avenues for further characterizing these allergens, their IgE binding structures and biological properties that can enhance allergenicity.

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The house dust mites *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* are among the major causative agents of allergic diseases, including asthma, rhinitis and atopic dermatitis. Characterizing the allergens from both mite species is important for developing better prognostic tests and improving the treatment and understanding the pathophysiological mechanisms of these prevalent human disorders.

As analyzed for *D. pteronyssinus*, the most important house dust mite allergens are the group 1 cysteine protease and the group 2 ML-domain lipid-binding allergens.¹ Both Der p 1 and Der p 2 react with 50–65% of the anti-*D. pteronyssinus* IgE antibodies.² The Der p 4 amylase, and the Der p 5 and Der p 7 allergens of unknown function are the next most important allergens with each binding IgE in about 50% of allergic subjects. When present each binds to more than 10% of the total mite-specific IgE antibody and collectively they account for 30% of the total anti-mite IgE antibody of all HDM allergic subjects.^{1,2} Der p 21, which is a homolog of Der p 5, also binds at a similar level.³ From a quantitative perspective, most of the other allergens have low IgE binding, although the high-molecular-weight allergens, constituted by the group 11 paramyosin, the group 14 large lipid transfer protein, and group 15 and 17 chitinase-like allergens, need quantitative analysis.^{1,2} The quantitative importance of the IgE binding to the group 1, 2, 4, 5 and 7 allergens is consistent with the results of

studies using recombinant allergens to absorb out IgE binding to two-dimensional immunoblots of mite extract.⁴

Structural and functional studies of the group 1 and 2 allergens are advanced and the group 4 allergens are typical α -amylases. More information is required for the Der p 5 and the Der p 7 mite allergens. There has been recent progress on the group 5 allergens. Weghofer *et al.*⁵ localized Der p 5 to the secretory granules of the midgut epithelial cells in house dust mites. In addition, Der p 5 shares about 40% amino-acid sequence identity with a group 5 allergen from the mite *Blomia tropicalis* (Blo t 5).⁶ The three-dimensional (3D) structure of the major allergen Blo t 5 has been solved and it has a triple-helical bundle fold.^{7,8} IgE and monoclonal antibody (MoAb) recognition of Blo t 5 has also been characterized with nuclear magnetic resonance spectroscopy.^{7,8}

We have characterized the *Dermatophagoides* group 7 allergens by cDNA cloning.^{9,10} The recombinant polypeptides have molecular masses of about 22 kDa by SDS polyacrylamide gel electrophoresis. Owing to variable glycosylation, the natural allergens in mite extracts have molecular masses of 26, 30 and 31 kDa.^{11,12} Skin tests show reactivity to Der p 7 in 53% of mite-allergic subjects;⁹ however, until now, little has been known about the biological and structural features of the allergens. Cross-reactive and species-specific MoAbs against Der

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Table 1 Ancestral homology of group 7 allergens (psi-BLAST)

BLAST target	Sequence category		
	(1) BPI (ca. 450 aa) ^a	(2) OBPDA (ca. 450 aa)	(3) OBPDA-L (ca. 220 aa)
Der f 7	Finch XP_002192464 9e-34 Dog XP_542993 1e-32 Panda EFB14835 2e-31 Human NP_001716 3e-27 Catfish AAX20011 5e-27 Squirt XP_002127451 5e-23 Anemone XP_001629157 1e-20 Oyster ACQ72919 1e-14 Poplar XP_002298841 3e-09 Moss XP_001765050 2e-05	Wasp XP_001606118 1e-28 ^c Culex XP_001863022 1e-28 ^c Aedes XP_001654113 2e-28 ^c Bee XP_395658 2e-27 ^c Aphid XP_001948728 1e-25 Tick XP_002406449 2e-21	Tick XP_002408892 8e-36 Aedes XP_001663945 7e-34 Anopheles XP_562422 2e-33 Beetle XP_976417 3e-32
Aca s 7	Human NP_001716 3e-32 Panda EFB14834.1 6e-31 Dog XP_542993 1e-29 Catfish AAX2001 7e-26 Squirt XP_002127451 5e-20 Anemone XP_001629157 4e-17 Oyster ACQ72918 4e-17 Poplar XP_002298841 8e-09 Moss XP_001765050 3e-05	Wasp XP_001606118 3e-29 Culex XP_001863022 5e-28 ^c Aedes XP_001654113 7e-28 ^c Bee XP_395658 6e-27 ^c Tick XP_002406449 4e-21	Anopheles XP_562422 7e-33 Aedes XP_001663945 7e-33 Tick XP_002408892 3e-32 Beetle XP_976417 3e-29
Tick XP_002408892	Toad NP_001089628 2e-40 Human NP_001716 2e-40 Panda EFB14835 1e-39	Anopheles XP_312626 2e-29 ^c Wasp XP_001606118 2e-28 ^c Beetle XP_972731 2e-27 Bee XP_395658 8e-27 ^c	Beetle XP_976338 4e-30 Anopheles 562422 2e-29 Beetle EFA03852 7e-29 Beetle XP_976417 1e-27
Aedes XP_001653919 C-terminal 251–445	Human FTP EAW75784 ^b 1e-40 Flounder ACV74252 3e-40 Panda EFB14835 5e-40 Finch XP_002192464 1e-39 Human NP_001716 2e-39	Aedes XP_001653919 1e-33 Anopheles XP_312626 3e-33 ^c Aedes XP_001654113 4e-33 ^c Culex XP_001863022 6e-33 ^c Wasp XP_001606118 1e-29 ^c Bee XP_395658 2e-29 ^c	Beetle EFA03852 3e-16 Beetle XP_976417 1e-17 Anopheles XP_562422 3e-16 Beetle XP_976338 1e-15 Beetle EFA03853 1e-11 Tick XP_002434263 1e-09

Abbreviations: aa, amino acid; BPI, bactericidal permeability-increasing proteins; OBPDA, odorant-binding protein domain-associated sequence; OBPDA-L, odorant-binding protein domain-associated sequence-like; psi-BLAST, position-specific iterative BLAST.

The accession numbers and accompanying expectation values are from the sixth iteration of a psi-BLAST. The significant matches belonged to three categories: (1) BPI proteins; (2) sequences that contained an N-terminal odorant-binding protein domain (OBPDA); and (3) sequences that on BLAST showed matches to the C-terminal OBPDA sequences (OBPDA-L). The boxes show the three most significant matches followed by sequences to illustrate the evolutionary diversity.

^aAll significant homologies for BPI sequences were to the N-terminal domain.

^bPhospholipid transfer protein.

^cThe highly significant homology to the OBPDA sequences was found on the C-terminal (non-odorant-binding protein) domains, but for sequences marked with a 'c' there was weaker homology with the N-terminal domain.

f 7 and Der p 7, which are useful tools for inhibition studies to map IgE binding, have also been generated.^{11–13}

Comparative protein homology modeling has been routinely used in constructing 3D models of functionally unknown proteins.¹⁴ This study demonstrates the ancestral homology of the group 7 allergens to the lipid-binding bactericidal permeability-increasing (BPI) family of proteins and related molecules. It presents a 3D model of the Der f 7 allergen constructed through homology modeling using the coordinates generated by X-ray diffraction of a human BPI (huBPI) protein as a template.¹⁵ The binding of a Der f 7-specific MoAb HD12 known to block IgE binding to Der f 7¹² was localized to a surface loop-like structure.

RESULTS

Structural homology and modeling

Comparative modeling of Der f 7 with structures of proteins in the protein database (PDB) showed the strongest homology to the N-terminal domain of huBPI protein (PDB 1bp1A). To further investigate this relationship, position-specific iterative BLAST (psi-BLAST) was performed with Der f 7 and with group 7 allergen sequences from

different mite species. The first iteration with Der f 7 showed the known identity among the different group 7 allergens and significant similarity to similarly sized hypothetical open reading frames from tick, beetle and mosquito species (OBPDA-L in Table 1). Further iterations revealed high position-specific homology with N-terminal domains of BPI proteins from a large number of species. As illustrated for iteration 6 with Der f 7 (Table 1), the high similarity was found not only with humans, birds and other mammals but also with highly divergent species including plants. Interestingly although BPI homologs from mollusks and other invertebrates were found, no such sequences were observed for insects or ticks. Psi-BLAST with the group 7 allergens from other species showed the same type of result, as shown for Aca s 7 from the acaridae mite *Acarus siro* (Table 1). Aca s 7 only has 23% sequence identity with Der f 7 in accord with the sequence disparity between the acaridae and pyroglyphidae family mites. Psi-BLAST with the tick homolog of Der f 7 (XP_002408892), which has 22% identity, gave similar results (Table 1). The sequence identity between Der f 7, Aca s 7 and tick (XP_002408892) is 12%. All the significant homologies found with the BPI sequences in all the searches were with the ca. 200 amino-acid N-terminal domain

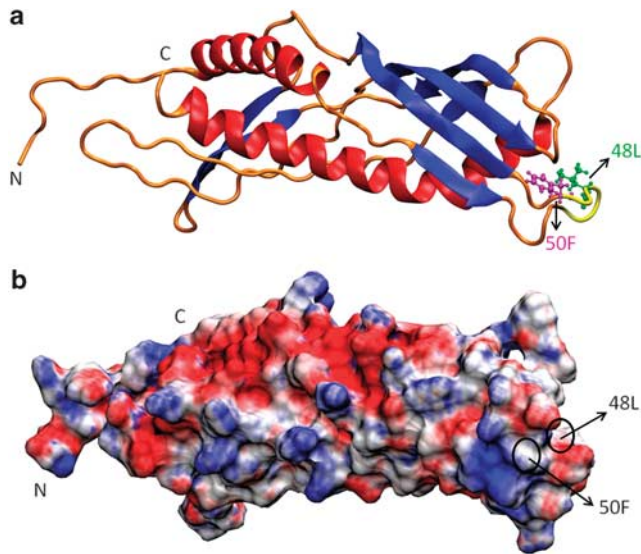


Figure 1 (a) A ribbon diagram representing the fold of Der f 7 allergen. The linear epitope ⁴⁶GILDF⁵⁰ and the two critical core amino-acid residues (Leu48 and Phe50) recognized by MoAb HD12 are colored in yellow, green and magenta, respectively. (b) An electrostatically charged surface representation of a 3D model of Der f 7 allergen.

despite the fact that these molecules contain a structurally similar C-terminal domain.

Besides BPI proteins, the other sequences that showed significant homology with the group 7 allergens were two types of hypothetical proteins found in insects and ticks. The largest type of these have been called odorant-binding protein domain-associated sequences (OBPDA) in Table 1 because they have a ca. 200 amino-acid domain in their N-terminal (DUF 233) that is homologous to the insect odorant-binding proteins (OBPs). The highly significant sequence homology with the group 7 allergens was however with the associated C-terminal (ca. 200 amino acid) domain. The N-terminal DUF233 domain also showed homology, but with over 10 orders of magnitude less significance. The smaller type of homologs were about 250 amino acids long and had homology to the OBPDA sequences, designated as OBPDA-L. As illustrated by psi-BLAST with the tick (XP_002408892) sequence, these homologs showed the same ancestral relationship with BPI and OBPDA as the group 7 allergens (Table 1), although their similarity to the DUF233 domain-containing proteins is apparent from a simple BLAST (not shown). As will be discussed, the sequences of the OBP domains share a very similar and distinctive structure with BPI proteins.

Psi-BLAST with the C-terminal (OBPDA region) of *Aedes* XP_001653919 (Table 1) showed the same results as the other analyses except that strong homologies were also found to human (Human FTP EAW75784) and other phospholipid transfer proteins, which are similar to BPIs. In addition, not all the OBPDA-L proteins showed homology including those with significant BLAST scores against OBPDA proteins.

A 3D model of Der f 7 was constructed using the coordinates of strongest PDB match, and highly significant ancestral homolog, huBPI protein, (PDB 1bp1A) as template (Figure 1a). huBPI has two similar domains. The modeling was made with the N-terminal domain because this had the strongest match. The structural elements show a short α -helix, a five-stranded antiparallel β -sheet barrel and a long helix closing a gap in the β -sheet (Figure 1a). These are very similar to

Table 2 Sequences of synthetic overlapping peptides of Der f 7

Name	Sequences
Df7-1	DIPIHYDKITEEINKA
Df7-2	EINKAIDDAIAAIEQ
Df7-3	AAIEQSETIDPMKVP
Df7-4	PMKVDPHADKFERHV
Df7-5	FERHVGILDFKGELA
Df7-6	KGELAMRNIEARGLK
Df7-7	ARGLKQMKRQGDANV
Df7-8	GDANVKGEEGIVKAH
Df7-9	IVKAHLIGVHDDIV
Df7-10	HDDIVSMEYDLAYKL
Df7-11	LAYKLGLDLPHTTHVI
Df7-12	TTHVISDIQDFWVAL
Df7-13	FVVALSLEISDEGNI
Df7-14	DEGNITMTSFEVRQF
Df7-15	EVRQFANVVNHIGGL
Df7-16	HIGGLSILDPIFGVL
Df7-17	IFGVLSVDLTAIFQD
Df7-18	AIFQDTRVKEMTKVL
Df7-19	MTKLAPAFKRELEK
Df7-20	RELEKN

the folds of the NH₂-terminal domain of the BPI protein and the model had a low, 1.21 Å, root mean square deviation value.

Location of HD12 epitope

To locate the HD12 binding site, a series of overlapping synthetic peptides spanning the Der f 7 sequence (Table 2) were used to block the binding of HD12 to Der f 7 in a dot-blot inhibition assay. Only peptide Df7-5 produced inhibition, essentially removing all detectable binding to Der f 7 (Figure 2). The fungal control peptide did not produce inhibition and nor did Der p 7. In addition, peptide Df7-5 did not inhibit the binding of Der f 7 to the anti-Der f 7 MoAbs, HD2 and HD4, and the anti-Der p 7 cross-reactive antibodies, WH9 and HD19 (Figure 3). These antibodies have been shown not to inhibit the binding of HD12.^{12,13} The binding to HD10, which does inhibit the binding of HD12¹² was however inhibited, suggesting it may in fact be identical to HD12. These results show that the inhibitory activity of the Df7-5 peptides was highly specific for HD12 and the related or identical HD10 MoAbs. As the overlapping Df7-4 and Df7-6 peptides showed no inhibitory activity, it appears that nonoverlapping region ⁴⁶GILDF⁵⁰ was important for the inhibition. In addition, given that Der p 7 (U37044), which has the residues ⁴⁶GIIDL⁵⁰, does not react with HD12,¹² Leu48 and/or Phe50 might be important. As expected from this, the Der p 7 peptide equivalent to Df-5 did not inhibit (DP7-5 in Figure 2) and the only substitutions in the overlapping region were Leu48Ile and Phe50Leu.

DISCUSSION

After the initial search of the PDB showed the homology of Der f 7 to huBPI protein, a wider search of sequences accessible from GenBank was made by psi-BLAST. Strong ancestral homology, which is detected by this method, extended across BPI-like proteins from a wide range of species from mammals to plants. It could be equally shown with the group 7 sequences of mite species from different families despite their low (25%) identity. The results demonstrate that the high significance was not due to a fortuitous alignment for Der f 7. The ancestral BPI homology could also be demonstrated for tick and insect homologs of

Inhibitors	—	Df7-4	Df7-5	Df7-6	DP7-5	control peptide
Der f 7 dot immunoassay	●	●	●	●	●	●

Figure 2 Immunodot blot inhibition of MoAb HD12-binding against rDer f 7 by overlapping synthetic peptides Df7-4, Df7-5 and Df7-6 as listed in Table 1.

Inhibitor (Df7-5)	MoAbs					
	HD2	HD4	HD10	HD12	HD19	WH9
—	●	●	●	●	●	●
+	●	●	●	●	●	●

Figure 3 Inhibitory activity of peptide Df7-5 to the binding of different MoAbs to Der f 7. The results show that only the binding to HD12 and the related¹² HD10 antibodies were inhibited.

the group 7 allergens. As will be discussed below, these have similarities to OBPs and the odorant-binding domain, which share a similar and highly distinctive structure with BPI proteins.

A 3D model of Der f 7 was constructed by comparative protein homology modeling, using 1bp1A as a template. The 1bp1A depicts the structure of huBPI protein, which is an antimicrobial protein of 456 residues.^{15,16} It is a boomerang-shaped molecule consisting of two domains (residues 1–229 and residues 251–456) with the same fold connected with a proline-rich region (residues 230–250).¹⁵ Each domain contains three structural elements, including a short α -helix, a five-stranded antiparallel β -sheet barrel and a long helix closing a gap in the β -sheet.¹⁵ In addition, each domain also contains an apolar binding pocket that may bind the acyl chains of lipid molecules.¹⁵ However, the two domains display only 13% sequence identity and have distinct functions.^{15,16} The N-terminal domain is potentially antibacterial and anti-endotoxin,^{16–18} whereas the C-terminal domain has been implicated in opsonization of Gram-negative bacteria.^{16,19} The approximate two-fold symmetry and bifunctional BPI protein is a member of a structurally and functionally related family of lipid-interactive proteins, including lipopolysaccharide-binding protein, cholesterol ester transfer protein and phospholipid transfer protein.^{15,20,21} The ancestral homologies found here, which encompassed BPIs across animals and plants, were all to the N-terminal and not to the C-terminal domain as per the 3D model demonstrating a specific structure. The amino-acid sequence identity between Der f 7 and huBPI is 21% (41 of 196 residues of Der f 7). It is not unusual for structural analysis to show strong homology of proteins with low sequence identity. Indeed, at least 119 pairs of proteins with <30% sequence identity but with similar structure folds have been found.²²

The structure shared by the two BPI domains defines a unique superwrap fold in which the α -helix wrapped in a curved antiparallel β -sheets.¹⁵ It is very similar to that found in OBP family, which includes the receptor for insect juvenile hormone.²³ The BPIs and OBPs bind to lipid in a similar manner so the dual ancestral homology of the group 7 allergens to the BPI and OBP sequences by psi-BLAST is good evidence for the veracity of the model. It is of interest that homologs with a similar size to BPI had a similar molecular organization with two BPI-type domains, one of which was more closely related to OBP domain and the other was closer to the BPI. This may be related to the observation that despite being found across the

animal and plant kingdoms, the extensive genome sequences of insects and ticks have not identified sequences as BPIs. Der f 7 differs from BPI in only having one BPI domain. Single-domain homologs were identified here in insects and additionally the mammalian PLUNC or parotid secretory molecules are single domain homologs.²⁴

There is considerable interest in possible properties of allergens that could promote interactions with the pattern recognition receptors of the innate immune system and thus enhance allergenicity. The potential lipid-binding property of Der f 7, which is a very common property found among major allergens,²⁵ could have this function, in accord with evidence showing that lipopolysaccharide binding by the MD-2-like Der p 2 allergen can enhance its allergenicity.²⁶ The model here shows that the apolar pocket required for the lipid binding of BPI is conserved in Der f 7. The binding to lipid is also known to induce conformational changes²³ so that the allergen may have a variable structure, although it is possible to completely absorb out IgE reactivity to natural Der p 7 with recombinant Der p 7.⁹

The antibody binding results provide evidence for a linear epitope ⁴⁶GILDF⁵⁰ with Leu48 and Phe50 as critical amino-acid residues for the interaction between Der f 7 and the MoAb HD12. The corresponding amino acids on Der p 7, which does not react with HD12, are Ile48 and Leu50, respectively. Thus, a slight difference in the side chain structures of Leu and Ile has a large effect on antibody binding. In addition, the aromatic ring of Phe50 may provide a hydrophobic interaction of Der f 7 with MoAb HD12. According to the 3D model of Der f 7, the Leu48 and Phe50 required for HD12 binding are, despite being hydrophobic, located on the surface within a loop-like structure. The surface location is expected from first principles and from other monoclonal and IgE antibody-binding studies^{27–30} and supports the authenticity of the 3D model. As a small study has shown that HD12 can inhibit up to 26% of the IgE binding,¹² the region may be one of the sites for engineering a hypoallergen.

Antibodies bind to the surface of antigens by a diversity of side chain interactions that differ even for antibodies that cast an almost identical footprint on the antigen. The importance of the binding HD-12 antibody is not to define the binding interactions to specific side chains, but to provide a topographical marker for the study of IgE anti-Der f 7 binding. Inhibition of IgE binding by MoAbs can define regions of IgE binding, which can, in the case of the mite allergen Der p 2, be spread about the surface,³¹ or for other allergens, such as dominant birch and grass allergens, Bet v 1 and Phl p 1 are concentrated in restricted areas.^{32,33} These inhibition studies can also be conducted in a more direct format in which the allergen is immobilized on a solid phase by a standard concentration of different inhibitory MoAbs.³⁴ To date, binding studies with anti-Der f 7 MoAbs have shown four non-cross-inhibitory groups of antibodies all of which can inhibit IgE binding.^{12,13} As the binding site of only one MoAb has been located, more extensive studies are required and these will be worthwhile now that the epitypic regions can be placed on a model for Der f 7. Antibody-binding studies with the classic lysozyme system have shown that although steric hindrance and remote conformational changes can be produced by antibody binding, concurrent binding by antibodies with close contact residues, <10Å° apart, readily occurs.³⁵ One of the techniques that can be used to locate

antibody binding is the use of the MoAbs for biopanning of peptide libraries to obtain mimotopes. The mimotopes not only have potential for vaccination but their sequence and the allergen structure can be used in algorithms to define the surface determinants that they represent.^{36,37} Other studies have located the regions of MoAb binding by X-ray crystallography of the antibody–allergen complex,^{34,38} although crystals cannot be produced for all complexes and it is an elaborate procedure.

In conclusion, a 3D model showing potential lipid-binding activity of Der f 7 was constructed by protein homology modeling. A linear epitope with surface-exposed hydrophobic side chains as critical core residues was bound by the MoAb HD12. Following the submission of this paper, an X-ray crystallographic structure of Der p 7 fused to maltose-binding protein was presented.³⁹ It had the BPI-like structure modeled here for unfused Der f 7. Modeling of Der f 7 on the coordinates of the Der p 7 maltose-binding protein fusion construct (PDB code 3H4Z) revealed a very similar structure to the model constructed here with a root mean square deviation value of 2.29 Å, indicative of a very reliable fit. Leu48 and Phe50 in the HD12 epitope remained as surface exposed residues on the loop as shown for the model in Figure 1. The biological activity that can be inferred from these structures points to possible interactions with the innate immune system that can enhance its allergenicity.

METHODS

Der f 7 constructs

The cDNA used was derived from the clone reported by Shen *et al.*¹⁰ (accession number of S80655). It was noted that it had an apparent mutation of ¹⁴²ttg¹⁴⁴ instead of ¹⁴²gtg¹⁴⁴ and encoded a polypeptide with Leu 48 instead of Val48. When site-directed mutagenesis was used to restore the Val48, the recombinant polypeptide no longer reacted with the MoAb HD12 prepared by immunizing mice with recombinant Der f 7 expressed from the clone.¹² The MoAb also reacts with natural Der f 7 in two-dimensional blot analysis,¹² and so it was concluded that the original assignment was a sequencing error. The sequence of the cDNA used is accordingly the same as that described by others (ACK76298) with Leu48.

Peptide synthesis and immunodot blot inhibition

Twenty overlapping peptides covering the complete sequence of the mature Der f 7 were custom synthesized (Genemed Synthesis Inc, San Antonio, TX, USA) (Table 2). The synthetic peptides were 15 amino acids long and offset by 5 amino acids. Immunoreactivity of the peptides with MoAb HD12 was determined by immunodot blot inhibition assay. Briefly, 1 µg of rDer f 7 was dotted onto nitrocellulose membranes (0.2 µm, Bio-Rad Laboratories, Hercules, CA, USA) using a Bio-Dot microfiltration apparatus (Bio-Rad) according to the manufacturer's instructions. The membrane was blocked with 2% skimmed milk. For inhibition assays, the hybridoma culture supernatant containing MoAb HD12 was preincubated individually, at room temperature for 2.5 h, with individual or various combinations of synthetic peptides (50 µg of each peptide) before immunodot blot analysis. These pretreated MoAb HD12 supernatants were then incubated individually, at room temperature for 1 h, with nitrocellulose membranes dotted with rDer f 7. HD12 reactivity against rDer f 7 was detected with horseradish peroxidase-labeled goat anti-mouse IgG (H+L) (Jackson ImmunoResearch Laboratories, Inc, West Grove, PA, USA) and developed with enzyme substrates as previously described.¹¹ As a control, the HD12 hybridoma culture supernatant was preincubated with 50 µg of a synthetic peptide covering the N-terminal amino-acid sequence of the mature Asp f 13 allergen (^NCGLTTQKSAPWGLGSISHKG^C) before dot-blot immunoassay. Each experimental condition was performed in triplicate and repeated at least three different times.

Comparative protein homology modeling of Der f 7

Comparative protein homology modeling¹⁴ was performed to construct a 3D model for Der f 7. To search for the homology templates, a database search

using the Phyre server (<http://www.sbg.bio.ic.ac.uk/phyre/html/index.html>)⁴⁰ was performed to identify which proteins of known structures were related to the Der f 7 sequence. A structure-based sequence alignment of the sequences from the templates and the Der f 7 was carried out. Subsequently, a homology model of Der f 7 was generated using the SWISS-MODEL (ExPASy proteomics server) according to the sequence alignment result of the Phyre program. The model structure was optimized by 500 steps of conjugate gradients and molecular dynamics with simulated annealing to optimize the spatial restraints. The PROCHECK program^{41,42} was then used to access the stereochemical quality of the model. Model building and molecular visualization was carried out using VMD software (VMD 1.8.6, <http://www.ks.uiuc.edu/Research/vmd/>). The accuracy was estimated by the root mean square deviation.

Psi-BLAST⁴³ was performed with BLOSUM62 matrix against NCBI non-redundant sequences (nr) (<http://www.ncbi.nlm.nih.gov>) without *Drosophila* sp. sequences (although similar results were obtained when these were retained). All BLAST results were screened with a significance threshold of 0.005 and all matching sequences were used for further iterations.

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- 1 Thomas WR, Heinrich TK, Smith WA, Hales BJ. Pyrolytic house dust mite allergens. *Protein Pept Lett* 2007; **14**: 943–953.
- 2 Hales BJ, Martin AC, Pearce LJ, Laing IA, Hayden CM, Goldblatt J *et al*. IgE and IgG anti-house dust mite specificities in allergic disease. *J Allergy Clin Immunol* 2006; **118**: 361–367.
- 3 Weghofer M, Dall'Antonia Y, Grote M, Stocklinger A, Kneidinger M, Balic N *et al*. Characterization of Der p 21, a new important allergen derived from the gut of house dust mites. *Allergy* 2008; **63**: 758–767.
- 4 Weghofer M, Thomas WR, Pittner G, Horak F, Valenta R, Vrtala S. Comparison of purified *Dermatophagoides pteronyssinus* allergens and extract by two-dimensional immunoblotting and quantitative immunoglobulin E inhibitions. *Clin Exp Allergy* 2005; **35**: 1384–1391.
- 5 Weghofer M, Grote M, Dall'Antonia Y, Fernández-Caldas E, Krauth MT, van Hage M *et al*. Characterization of folded recombinant Der p 5, a potential diagnostic marker allergen for house dust mite allergy. *Int Arch Allergy Immunol* 2008; **147**: 101–109.
- 6 Arruda LK, Fernandez-Caldas E, Naspić CK, Montealegre F, Vailes LD, Chapman MD. Identification of *Blomia tropicalis* allergen Blo t 5 by cDNA cloning. *Int Arch Allergy Immunol* 1995; **107**: 456–457.
- 7 Chan SL, Ong TC, Gao YF, Tiong YS, Wang de Y, Chew FT *et al*. Nuclear magnetic resonance structure and IgE epitopes of Blo t 5, a major dust mite allergen. *J Immunol* 2008; **181**: 2586–2596.
- 8 Naik MT, Chang CF, Kuo IC, Kung CC, Yi FC, Chua KY *et al*. Roles of structure and structural dynamics in the antibody recognition of the allergen proteins: an NMR study on *Blomia tropicalis* major allergen. *Structure* 2008; **16**: 125–136.
- 9 Shen HD, Chua KY, Lin KL, Hsieh KH, Thomas WR. Molecular cloning of a house dust mite allergen with common antibody binding specificities with multiple components in mite extracts. *Clin Exp Allergy* 1993; **23**: 934–940.
- 10 Shen HD, Chua KY, Lin WL, Hsieh IM, Thomas WR. Molecular cloning and immunological characterization of the house dust mite allergen Der f 7. *Clin Exp Allergy* 1995; **25**: 1000–1006.
- 11 Shen HD, Chua KY, Lin WL, Hsieh KH, Thomas WR. Characterization of the house dust mite allergen Der p 7 by monoclonal antibodies. *Clin Exp Allergy* 1995; **25**: 416–422.
- 12 Shen HD, Lin WL, Tsai LC, Tam MF, Chua KY, Chen HL *et al*. Characterization of the allergen Der f 7 from house dust mite extracts by species-specific and cross-reactive monoclonal antibodies. *Clin Exp Allergy* 1997; **27**: 824–832.
- 13 Shen HD, Chua KY, Lin WL, Chen HL, Hsieh KH, Thomas WR. IgE and monoclonal antibody binding by the mite allergen Der p 7. *Clin Exp Allergy* 1996; **26**: 308–315.
- 14 Sali A, Blundell TL. Comparative protein modelling by satisfaction of spatial restraints. *J Mol Biol* 1993; **234**: 779–815.
- 15 Beamer LJ, Carroll SF, Eisenberg D. Crystal structure of human BPI and two bound phospholipids at 2.4 angstrom resolution. *Science* 1997; **276**: 1861–1864.
- 16 Elsbach P, Weiss J. Role of the bactericidal/permeability-increasing protein in host defense. *Curr Opin Immunol* 1998; **10**: 45–49.
- 17 Ooi CE, Weiss J, Elsbach P, Frangione B, Mannion B. A 25-kDa NH2-terminal fragment carries all the antibacterial activities of the human neutrophil 60-kDa bactericidal/permeability-increasing protein. *J Biol Chem* 1987; **262**: 14891–14894.
- 18 Ooi CE, Weiss J, Doerfler ME, Elsbach P. Endotoxin-neutralizing properties of the 25 kD N-terminal fragment and a newly isolated 30 kD C-terminal fragment of the 55–60 kD bactericidal/permeability-increasing protein of human neutrophils. *J Exp Med* 1991; **174**: 649–655.

- 19 Iovine NM, Elsbach P, Weiss J. An opsonic function of the neutrophil bactericidal/permeability-increasing protein depends on both its N- and C-terminal domains. *Proc Natl Acad Sci USA* 1997; **94**: 10973–10978.
- 20 Tall A. Plasma lipid transfer proteins. *Annu Rev Biochem* 1995; **64**: 235–257.
- 21 Canny G, Levy O. Bactericidal/permeability-increasing protein (BPI) and BPI homologs at mucosal sites. *Trends Immunol* 2008; **29**: 541–547.
- 22 Rice DW, Eisenberg D. A 3D-1D substitution matrix for protein fold recognition that includes predicted secondary structure of the sequence. *J Mol Biol* 1997; **267**: 1026–1038.
- 23 Kolodziejczyk R, Bujacz G, Jakob M, Ozyhar A, Jaskolski M, Kochman M. Insect juvenile hormone binding protein shows ancestral fold present in human lipid-binding proteins. *J Mol Biol* 2008; **377**: 870–881.
- 24 Bingle L, Barnes FA, Lunn H, Musa M, Webster S, Douglas CW *et al*. Characterisation and expression of SPLUNC2, the human orthologue of rodent parotid secretory protein. *Histochem Cell Biol* 2009; **132**: 339–349.
- 25 Thomas WR, Hales BJ, Smith WA. Structural biology of allergens. *Curr Allergy Asthma Rep* 2005; **5**: 388–393.
- 26 Trompette A, Divanovic S, Visintin A, Blanchard C, Hegde RS, Madan R *et al*. Allergenicity resulting from functional mimicry of a Toll-like receptor complex protein. *Nature* 2009; **457**: 585–588.
- 27 Lai HY, Tam MF, Chou H, Lee SS, Tai HY, Shen HD. Molecular and structural analysis of IgE-binding epitopes of Pen ch 13, an alkaline serine protease major allergen from *Penicillium chrysogenum*. *Clin Exp Allergy* 2004; **34**: 1926–1933.
- 28 Padavattan S, Schirmer T, Schmidt M, Akdis C, Valenta R, Mittermann I *et al*. Identification of a B-cell epitope of hyaluronidase, a major bee venom allergen, from its crystal structure in complex with a specific Fab. *J Mol Biol* 2007; **368**: 742–752.
- 29 Chruszcz M, Chapman MD, Vailes LD, Stura EA, Saint-Remy JM, Minor W *et al*. Crystal structures of mite allergens Der f 1 and Der p 1 reveal differences in surface-exposed residues that may influence antibody binding. *J Mol Biol* 2009; **386**: 520–530.
- 30 Skov LK, Seppälä U, Coen JJ, Crickmore N, King TP, Monsalve R *et al*. Structure of recombinant Ves v 2 at 2.0 Å resolution: structural analysis of an allergenic hyaluronidase from wasp venom. *Acta Crystallogr D Biol Crystallogr* 2006; **62**: 595–604.
- 31 Mueller GA, Smith AM, Chapman MD, Rule GS, Benjamin DC. Hydrogen exchange nuclear magnetic resonance spectroscopy mapping of antibody epitopes on the house dust mite allergen Der p 2. *J Biol Chem* 2001; **276**: 9359–9365.
- 32 Mirza O, Henriksen A, Ipsen H, Larsen JN, Wissenbach M, Spangfort MD *et al*. Dominant epitopes and allergic cross-reactivity: complex formation between a Fab fragment of a monoclonal murine IgG antibody and the major allergen from birch pollen Bet v 1. *J Immunol* 2000; **165**: 331–338.
- 33 Flicker S, Steinberger P, Ball T, Krauth MT, Verdino P, Valent P *et al*. Spatial clustering of the IgE epitopes on the major timothy grass pollen allergen Phl p 1: importance for allergenic activity. *J Allergy Clin Immunol* 2006; **117**: 1336–1343.
- 34 Bernard H, Drumare MF, Guillon B, Paty E, Scheinmann P, Wal JM. Immunochemical characterisation of structure and allergenicity of peanut 2S albumins using different formats of immunoassays. *Anal Bioanal Chem* 2009; **395**: 139–146.
- 35 Smith-Gill SJ, Lavoie TB, Mainhart CR. Antigenic regions defined by monoclonal antibodies correspond to structural domains of avian lysozyme. *J Immunol* 1984; **133**: 384–393.
- 36 Suphioglu C, Schappi G, Kenrick J, Levy D, Davies JM, O’Hehir RE. A novel grass pollen allergen mimotope identified by phage display peptide library inhibits allergen-human IgE antibody interaction. *FEBS Lett* 2001; **502**: 46–52.
- 37 Szalai K, Fuhrmann J, Pavkov T, Scheidl M, Wallmann J, Bramswig KH *et al*. Mimotopes identify conformational B-cell epitopes on the two major house dust mite allergens Der p 1 and Der p 2. *Mol Immunol* 2008; **45**: 1308–1317.
- 38 Li M, Gustchina A, Alexandratos J, Wlodawer A, Wunschmann S, Kepley CL *et al*. Crystal structure of a dimerized cockroach allergen Bla g 2 complexed with a monoclonal antibody. *J Biol Chem* 2008; **283**: 22806–22814.
- 39 Mueller GA, Edwards LL, Aloor JJ, Fessler MB, Glesner J, Pomes A *et al*. The structure of the dust mite allergen Der p 7 reveals similarities to innate immune proteins. *J Allergy Clin Immunol* 2010; **125**: 909–917.
- 40 Kelley LA, Sternberg MJ. Protein structure prediction on the Web: a case study using the Phyre server. *Nat Protoc* 2009; **4**: 363–371.
- 41 Morris AL, MacArthur MW, Hutchinson EG, Thornton JM. Stereochemical quality of protein structure coordinates. *Proteins* 1992; **12**: 345–364.
- 42 Laskowski RA, MacArthur MW, Moss DS, Thornton JM. PROCHECK: a program to check the stereochemical quality of protein structures. *J Appl Cryst* 1993; **26**: 283–291.
- 43 Jones DT, Swindells MB. Getting the most from psi-BLAST. *Trends Biochem Sci* 2002; **27**: 161–164.