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An update on allergens

House-dust-mite allergens

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Spectrum of house-dust-mite (HDM) allergens

Dermatophagoides pteronyssinus and *D. farinae* are the major source of HDM allergens, although *Euroglyphus maynei*, in temperate regions, and *Blomia tropicalis*, in tropical and subtropical areas, can be important. *D. microcerus* in Europe and *D. siboney* in the Caribbean have been occasionally studied. *Lepidoglyphus destructor* is the source of important occupational allergens, which in some locations can contribute to house-dust allergy (1). The results of immunoblotting studies of the allergens in extracts of the mites have differed in detail, but the broad picture shows both complexity and the existence of some dominant specificities. Western blotting using sera selected on the basis of high antimite IgE has revealed 32 different IgE-binding bands with molecular weights from 11 to over 100 K (2, 3). Most sera recognized a unique combination of 6–10 bands. Of the 32 bands, nine were recognized at high frequency. A subsequent study found that most sera reacted to 1–7 bands, although some did have a broader reactivity (4). This pattern is consistent with several similar studies using either *D. pteronyssinus* or *D. farinae* extracts (5–8). Although the blotting studies have provided a snapshot, their limitations should be recognized. The concentration of allergens varies from extract to extract, and blotting procedures can have sharp dose-response characteristics. Some allergens, such as Der p 2, have their IgE-binding activity enhanced by denaturation (9), while others, such as Der p 1, lose antigenicity (3). Finally, some allergens, such as Der p 7, show multiple bands (10).

Developmental and disease-related patterns of allergen reactivity

There are at least two situations where the number and the nature of the allergens recognized appear to be a function of the type of allergic subject. The first is multisensitivity, where patients have high total IgE immunoglobulin levels, skin test reactivity to many allergens, and poor responses to immunotherapy (11). Accordingly, their T cells produce high levels of interleukin (IL)-4 after mitogen stimulation. IgE antibodies from their sera bind more bands on Western blotting of mite extracts than IgE from monosensitive patients (11). Consequently, there may be fundamental differences between the type of stimulus required to induce immune responses in this group and others. The second situation is developmental. O'Brien & Thomas (12) showed that children attending an allergy clinic had strong IgE binding to Der p 1 and 2 and an additional 1–3 allergens. Adults presenting at a clinic for the first time had low IgE binding to Der p 1 and 2, but were shown, by Western blotting, to recognize a diverse array of allergens. As discussed in this paper, adults diagnosed in childhood had, like children, high anti-Der p 1 and 2 binding. The Western blotting of Shibasaki et al. showed a similar pattern, with bands equivalent to Der f 2 predominating in the very young group (13).

Der p 1 has poor IgE binding in Western blotting; therefore, it would be important to repeat the study with purified allergens. Consistent with these observations, adults have been reported to have more IgE binding to Der p 3 and 4 than children (14, 15).

Calkhoven et al. measured anti-Der p 1 and Der p 2 antibodies in sera from high-risk children to determine whether the first antimite antibodies measured by RAST coincided with the detection of anti-Der p 1 and 2 antibodies (16). This was the case for only 10/18 children. Thus, it appears that allergens other than Der p 1 and 2 induce early IgE antibodies and it could be important to identify them. The infants used in this study were the offspring of parents with a history of atopy; therefore, it would be of interest to measure similar responses in the broader population. Lin et al. described children with IgE to many bands on Western blotting, but their sera had IgE levels of over 1800 IU/ml, levels indicative of the multisensitive patients (6).

Thompson et al. reported that children with eczema had strong IgE reactivity to Der p 2, and those with asthma strong reactivity to Der p 1 (17). Despite the marked difference of the blotting pattern in their study, several other authors have not found any such association. Children have high IgE antibodies to Der p 1 and 2 regardless of the type of disease (12, 13, 16).

IgE responses to purified *Dermatophagoides* allergens

The first highly purified allergen to be examined for IgE binding was Der p 1 (18). It bound IgE in all sera from all the allergic subjects tested. Remarkably, 50–70% of the IgE to a *D. pteronyssinus* extract was directed to Der p 1 and the anti-*D. pteronyssinus* specificities comprised 6–21% of the total IgE. Lind (19) and van der Zee et al. (20) confirmed this high reactivity. Both also reported that Der p 2 had a similar frequency and strength of binding. van der Zee et al. showed that 60% of the patients reactive to these allergens produced anti-Der p 1 and 2 IgE levels equivalent to 50–100% of the reactivity to whole-mite extracts. Most remaining patients still had reactivities equivalent to 20–40%. Similar results were reported by Meyer et al., who found that 80% of sera from mite-sensitive patients reacted to Der p 1 and 2, and that these allergens inhibited almost all the anti-HDM reactivity of a pool of positive sera (21). Similar degrees of reactivity have been reported with the group 1 allergens from other *Dermatophagoides* mites (22–24).

Reports of IgE reactivity to the group 3 allergens have varied considerably, with frequencies reported as low as 16% (25) and as high as 100% with a potency similar to the groups 1 and 2 (26). Other reports show an intermediate (27) or variable degree of binding (28). The considerable variations may be attributed to differences in the purity of the allergen preparations or, as will be described below, to the presence of significant variants of the group 3 allergens.

The groups 5 and 7 react to IgE in 50% of sera but with a magnitude as high as the major allergens (29, 30). The binding of IgE to the chymotrypsin group 6 occurs at a frequency of 40% with intermediate potency (27), and the collagenolytic enzyme Der p 9 binds at a similar frequency to Der p 1 and 2 but at a slightly lower level (26). The designated allergens Der p 4 (15) and Der p 8 (31) react to IgE in 50% of sera from allergic subjects, but quantitative studies have not been reported. The binding of tropomyosin group 10 is controversial. Aki et al. showed that Der f 10 bound IgE in almost all sera of allergic subjects as strongly as Der p 1 (32), while work in Europe found that about 50% of subjects had antibodies to tropomyosin with a variable strength of binding (33). The amino-acid sequence of tropomyosin is evolutionarily conserved; therefore, the difference may be due to a cross-reactivity to nonmite allergens. Three of 31 people had high IgE to Der f 10 but no binding to Der f 1 or 2. As described below, the group 10 and some other highly reacting specificities are nonexistent or present in low concentrations in some extracts.

The allergen Mag 3 was identified from a partial cDNA clone which bound IgE with similar strength to Der f 2 (34). Several sera without IgE antibody to Der f 2 reacted strongly to Mag 3. Two other *D. farinae* allergens, a homologue of heat shock protein 70 (35), and a 39-kDa protein (Mag 1) (36), which have also been described by Aki et al., showed IgE-binding frequencies of 10% and 30%, respectively.

T-cell responses to purified *Dermatophagoides* allergens

Rawle et al. studied responses to Der p 1 and found that skin-test-positive subjects had higher proliferative responses than skin-test-negative subjects (37). The highest responders were subjects with either atopic dermatitis or asthma, and the lowest had rhinitis alone, but the system appeared to be suboptimal because many Der p 1-sensitive subjects did not respond. O'Brien et al. compared the T-cell proliferation to Der p 1 and 2 (38). The highest responders were subjects sensitive to mites; in particular, those with sensitivity to mites and other allergens. Again many HDM-allergic patients did

not respond. The responses correlated with total IgE levels. As found by Rawle et al., patients with asthma responded better than those with rhinitis alone. Responses to Der p 1 and 2 correlated closely. A further study compared the proliferation to Der p 1 and 2 to the specific IgE antibody levels. All subjects with IgE antibody had T-cell responses, but some subjects had T-cell proliferation and no IgE antibody. Cavaillon et al. found no differences in responses to Der f 1, but there was a trend to higher responses in allergic subjects, and most stimulations were low (39).

Studies using serum-free medium, which enhances responses to mite allergens, have shown quite different results. Proliferative responses could be induced in T cells from all allergic and nonallergic subjects (40, 41), but only the allergic patients produced Th2-type cytokines (42). Similarly, IL-4 mRNA production in response to purified Der p 2, in serum-containing cultures, correlated with asthma symptoms (43). In addition, over half the nonallergic subjects had positive interferon-gamma (IFN-γ) responses, showing responsive T cells.

The ability of recombinant Der f 2; Der f 10; and Mag 1, 3, and 29 to stimulate T-cell proliferation was compared (44). The responses to the highly IgE-binding allergens Der f 2 and Mag 3 were high, and the responses to the less reactive allergens Mag 1 and 29 low. Nonallergic subjects had low responses to all allergens. As in the results of O'Brien et al. (38), the responses to the major specificities correlated well. The T-cell responses to Der p 10 were low, a finding which was unexpected given its IgE-binding activity in the study population. Possibly, either the antitropomyosin IgE antibodies are the result of cross-reactivities which do not occur at the T-cell level, or recombinant tropomyosin is unsuitable for T-cell stimulation.

Dermatophagoides allergens in the environment and extracts

Der p 1 and Der f 1 are usually present at 100–10 000 ng/g of dust (45, 46). Levels of 100 ng are associated with sensitization, and the risk increases with increasing doses. These allergens are found in high concentrations in dung balls as well as mite extracts and spent mite medium (47). Group 2 allergens have also been measured, and the levels of allergen and the number of mites correlate well (48). The group 2 concentrations were similar to the group 1 (48–50), even though they have been reported to be present at about a fivefold lower concentration than the group 1 allergens in mite extracts, and in higher concentration in body extracts than spent mite medium (25, 51).

The concentration of other allergens has been scarcely studied. Li et al. reported that, in Taipei, Taiwan, where mites are abundant, Der p 5 was usually under 100 ng/g dust (52). Witteman et al. found that tropomyosin was as abundant as Der p 1 and 2, but the assay used detected tropomyosin from other arthropods as well as the group 10. Nevertheless, it showed that tropomyosin can accumulate to high levels (50). Group 10 (32) as well as groups 3, 5, and 7 allergens are present in low concentration in mite extracts (52–54). There is no information on environmental exposure, but the allergens do induce high titres of IgE antibody. Group 3 allergens are predominantly found in faecal preparations, and only low concentrations are present in body extracts (53).

If we take the measurements of Der p 1 (47) as a marker for faecal allergens, and if we assume the production of 10–20 faeces a day per mite and a life span of 30 days, 90% of the allergen in the environment would be faecal; and if we assume a steady state of allergen production, 15% would be fresh. Care in measuring labile allergens needs to be taken because the current methods of sampling typically store the dust for extended periods. The degree of allergen exposure is important in considering the contribution of the different allergens to disease. For example, many subjects are high responders to Der p 5 and 7, but is the exposure to these allergens sufficient to cause disease?

Species specificity

IgE antibodies from mite-allergic patients cross-react with several diverse species, as well as containing specificities unique to the sensitizing mite (55–59). Only some investigations have used purified allergens and quantitative inhibition to assess the cross-reactivity accurately.

Der p 1 and Der f 1 induce both cross-reacting and species-specific antibodies (23, 60); however, 10–10 000-fold more of the heterologous allergen, depending on the patient, is required for ELISA inhibition (23). Der p 2 and Der f 2 have been reported to have almost complete cross-reactivity (61), although some difference has been noted (59). The group 3 allergens have not been directly compared, but the reactivities of different sera to Der p 3 and Der f 3 do not correlate as well as the interspecies reactivities to the groups 1 and 2 (27). The group 10 tropomyosins with their sequence conservation and cross-reactivity with other invertebrates should be highly cross-reactive (32, 33).

The reactivity of recombinant Blo t 5 from *B. tropicalis* and Der p 5 has been compared (62). Sera from England and the northern USA usually reacted only to Der p 5, while sera from the

southern USA and Brazil, where both species thrive, reacted to both or exclusively to Blo t 5 or Der p 5. This demonstrates a considerable antigenic difference which correlates with the 57% sequence disparity.

Recombinant Lep d 2 has limited IgE cross-reactivity to the homologous allergens in *Dermatophagoides* (63) and *B. tropicalis* (56). The amino-acid sequence identity of Der p 2 and Lep d 2 is only 40% (64), which is consistent with low cross-reactivity. The reactivity to *B. tropicalis* is of considerable interest because this is the best evidence of group 2 allergens being produced by this species. A 39-kDa *L. destructor* allergen cross-reactive with *D. pteronyssinus*-specific antibodies has also been described (65).

Since molecular shape is more conserved than primary amino-acid sequence, T cells may be more discriminatory than antibody. T-cell proliferation to peptides representing the group 1 allergens of *D. pteronyssinus*, *D. farinae*, and *E. maynei* have been measured in the same subjects (41). Peptides from the most abundant allergen in the study environment, Der p 1, elicited the most responses. There were only a few cross-reactivities with the equivalent peptides of Der f 1 and, consistent with the absence of *D. farinae*, almost no independent sensitization to Der f 1 epitopes. *E. maynei* was present in the study environment, and 3/17 mite responders recognized multiple Eur m 1 peptides with only minor reactivity to Der p 1 peptides. A further two had their highest responses to Eur m 1. Thus, the responses to T-cell epitopes could identify sensitizing species. Unpublished results have shown that T-cell responses to the whole Der p 1 and Der f 1 molecules were, in contrast to the peptides, indistinguishable. This is possibly due to the additive effects of the cross-reactions and minor epitopes or reactivity to variants of Der p 1 found in the environment.

Allelic polymorphisms

The groups 1, 2, and 3 allergen genes are present as single loci in the mite genome, but the alleles show an unusually high degree of polymorphism. The pattern of polymorphism observed for groups 1 and 2 is quite similar, with very few sequences being identical and with most containing unique combinations of a set of defined substitutions. In contrast, the group 3 polymorphisms show a distinctly different pattern, with most sequences being conserved but with some being quite divergent. The polymorphisms will be described in greater detail in later sections on specific allergens.

Such frequent polymorphism is very rare; therefore, it is intriguing to ask why it occurs in the major allergens. It is possible that the variation is a fundamental property which predisposes a protein to become a major allergen. The variation will firstly increase the range of epitopes which bind MHC molecules and thus increase the pool of responders to the allergens. Secondly, low-affinity interactions of antigens with the MHC and T-cell antigen receptor have been shown to default to Th2-type T-cell responses; thus, exposure to a mixture of variant epitopes could facilitate sensitization (66–68). The variation also means there may be regional differences in mite allergens and differences between commercial cultures and the mites in the environment. There is evidence that the amino-acid substitutions affect immune responses to allergens (69). T-cell responses to peptide 45–60 of Der p 1 are markedly affected by the residue at position 50. One-third of responders to Der p 1 peptides recognized peptide 45–60 but only when residue 50 was tyrosine, and not histidine. Correspondingly, tyrosine was the only residue found at position 50 in mites isolated from homes.

Molecular properties of defined allergens

Group 1 allergens

The immunochemical properties of Der p 1 (18) and Der f 1 have been described by several investigators (22, 23, 51). The cDNA analysis showed that they are cysteine proteases in the same family as papain and actinidin and have 222 or 223 residues with a calculated MW of 25 K (70–72). The X-ray crystallographic structures of the other cysteine proteases (73, 74) have been used to model Der p 1 (75, 76). It has two globular domains formed independently by the N- and C-terminal halves. They are connected by a flexible outside loop formed by the central residues of the sequence and with interactions from the N- and C-terminal regions. The substrate binding and catalytic residues are in a cleft formed by the juxtaposition of the domains. Although the overall homology of the group 1 allergens and the other cysteine proteases is about 25–30%, the residues surrounding the active cysteine 34 and histidine 171 and the substrate-binding asparagine-serine-tryptophan sequence 190–192 are highly conserved. Disulphide bonds form at cysteines 4 and 117, 31–71, and 65–71. Studies with T-cell lines (77) and peripheral blood cells (41) showed the most frequent and strongest stimulating peptides to be in the central domain connecting region. Stimulatory Eur m 1 peptides were found in this region in 8/12 subjects, but stronger responses were found to epitopes in other regions (41).

Like other cysteine proteases, Der p 1 and Der f 1 have 19 residue leader and 79 residue proenzyme sequences (72, 78), The natural allergens isolated from mites have cysteine protease activity (79, 80), but further study has revealed that Der p 1 exhibits a unique mixed cysteine and serine protease activity, even though it has only one active site (81). The preferred cleavage site for the cysteine protease activity was glutamate, and it was arginine for the serine protease activity. Der f 1, Der p 1, and Eur m 1 (GenBank AF047610) contain a glutamate at residue-1 of the proenzyme sequence, suggesting the possibility of autocleavage.

Der p 1 and Der f 1 (72) show a sequence identity of 80%. The divergence is predominantly in the N-terminal residues 1–20 (45%), the C-terminal residues 201–222 (31%), and a central region 91–130 (30%). The sequences 21–90 and 131–200, which form the two globular domains, show 6% and 14% divergence, respectively. The central and terminal residues form more flexible structures connecting the domains which contain most of the conserved hydrophobic core residues. Of the substitutions between Der p 1 and Der f 1, 27/41 were homologous. The regions containing divergent sequences corresponded to regions containing antigenic differences found by immunoscreening random fragment cDNA libraries (82).

The sequence of a polymerase chain reaction (PCR) amplification of genomic Eur m 1 (83) was reported, and a full-length cDNA clone was isolated by Smith, Hazel, Mills, Hart, and Thomas (GenBank AF047610). There was 20% divergence from both Der p 1 and Der f 1. Of 223 amino-acid residues, 170 were invariant and 16 were unique to Eur m 1, with only four of these varying in all sequences. Of the remaining 30 residues, the Eur m 1 resembled Der p 1 for half and Der f 1 for half. The variation was again found in the central region at 90–125 and the N-terminal 20 residues. The overall identity between the three species was 76%, while the comparison with both Der p 1 and Der f 1 was 84%. The sequences of Der p 1, Der f 1, and Eur m 1 all contained an N-glycosylation site from residues 53–55, a finding which is consistent with the report of carbohydrates in the purified natural allergen (18).

cDNA from five clones constructed from *D. pteronyssinus* obtained from CSL Ltd all varied from each other by 1–4 residues, usually with conservative changes (78). The variation was the result of different combinations of single substitutions in five positions. Thus, position 50 could be tyrosine or histidine, position 81 glutamate or lysine, position 124 alanine or valine, position 136 serine or threonine, and position 215 glutamate or glutamine. The clones had a variety of combinations of these substitutions without any apparent linkage of particular substitutions (78). The genomic sequences from eight environmental isolates from different regions of Australia had the same substitutions in only two of the five positions found in commercial mites but also had a further three in different positions (69). A UK sequence has also shown two unique substitutions. In all, there have been substitutions reported in 10 positions of Der p 1. Clearly, studies on the variation and regional difference in the allergens are required. The substitutions usually occurred in surface-exposed residues (76), but the recently described aspartate substitution at position 130 has been modelled to occur as a solvent-inaccessible part of a helix.

Group 2 allergens

The 14-kDa group 2 allergens were first characterized by immunochemical studies (19, 25, 51, 84, 85). The cDNA sequences of Der p 2 (86, 87) and Der f 2 (88, 89) showed that each had 129 residues, a calculated MW of 14 K, and no N-glycosylation sites. They had a 12% amino-acid divergence, which was evenly distributed throughout the sequence. This is significantly higher than the group allergens, a fact also reflected in the finding that only 5/15 of the substitutions were nonconservative compared with the opposite ratio for the group 1. The Eur m 2 from *E. maynei* described by Smith, Hazell, Mills, Hart, and Thomas (GenBank AF047613) had 18% sequence disparity to both Der p 2 and Der f 2. Where there was disparity in the sequence of Der p 2 and Der f 2, the Eur m 2 had the residue of Der p 2 seven times, and Der f 2 five times, indicating no preference.

Anti-group 2 antibodies have been reported to show reactivity associated with the gut and other unidentified structures (90). In the past, there has been speculation that the group 2 allergens are lysozymes, but it is now clear that they are not (91–93). However, they are similar in sequence, size, and distribution of cysteine residues to a family of epididymal proteins (64). These include the human HE1 (94) and chimpanzee EPI-1 (95). Another homologue is bovine EPV20 (96), which is found in the kidney, liver, spleen, and mammary glands, but not the testis. The disulphides in these proteins, like the group 2 allergens (97), form between the cysteines at positions 1 and 6, 2–3, and 4–5. A transcript from the tobacco hornworm *ers*16 gene, which is in the same structural family, is found in high levels in the epithelial cells of the trachea surrounding the central nervous system (98) and is associated with pupal ecdysis (99). These proteins may be involved in defence against bacteria (like

lysozyme), because binding of Der f 2 to the surface of *Escherichia coli* has been demonstrated (93). The possibility that the group 2 allergens are an epididymal secretion suggests an interesting type of dissemination. The reproductive tract can form a large part of the mass of male mites, and HE-1 and EPI-1 comprise 20% of the protein in epididymal fluid. The tertiary structure of Der p 2 (92) and Der f 2 (93), recently determined by nuclear magnetic resonance, has shown a protein consisting entirely of β-sheets in a single-domain immunoglobulin fold. It has close structural homology to the third and fourth domains of transglutaminase coagulation factor XIII.

The Der f 2 gene has a single locus. The cDNA sequences of Der f 2 showed polymorphisms in residues 76, 88, 111, and 125 (88), as also occurred in the PCR products reported by others (14, 89). A further four genomic sequences from the mite cultures showed the same substitutions, although in different combinations (100). The variant proteins can be detected in mite extracts and be distinguished with a monoclonal antibody (101).

Variants of Der p 2 have been described (102). Of three cDNA clones from commercial mites and four genomic sequences from the environment, only two were identical; one genomic sequence from Sydney, Australia, and one from Taipei, Taiwan. The variants had different combinations of substitutions, usually conservative, which occurred at five positions such that the sequences differed from each other by 1–4 residues. A cluster of divergence was found at the C-terminal residues 111, 114, and 127 in a region frequently recognized by T cells (103, 104). The closely spaced residues at positions 40 and 47 were leucine and serine in 3/4 of the environmental sequences, but this combination was not found in commercial sequences. As for Der f 2, variant-specific monoclonal antibodies have been described (105).

Group 2 allergens (initially called Lep d 1) were described for *L. destructor* from both cDNA and protein sequences (63, 106). They show the same resemblance to the HE-1 family, and variants have been found by protein and cDNA analysis.

Group 3 allergens

Der f 3 was determined to be a 30-kDa protein by SDS–PAGE (25); its N-terminal sequence and a homologous sequence from *D. pteronyssinus* (107) were similar to a serine protease. Stewart et al. used benzamidine for affinity purification of a 30-kDa allergen with the same N-terminal sequence, demonstrating that Der p 3 is a trypsin-like molecule (108). This was further shown by substrate specificities (28).

The cDNA sequence (109) showed Der p 3 to have 233 residues with a calculated MW of 25 K and to contain the catalytic and substrate-binding sites of trypsin. It has no N-glycosylation sites, and, interestingly, the recombinant polypeptide also migrates as a 30-kDa molecule in SDS–PAGE. By its homology with trypsin, Der p 3 contains disulphide bonds at positions 42–58, 168–182, and 191–220 and an unpaired cysteine at residue 12.

The Der f 3 cDNA sequences (110, 111) were found to have 81% identity with Der p 3. Exactly 28/37 substitutions were nonconservative, a figure similar to that of group 1. Der f 3 also contained the unpaired cysteine 12 residue. The regions of sequence variation between Der p 3 and Der f 3 could be matched to structures in three-dimensional models of serine proteases (112). Most nonconservative variations were found in residues 65–79, 80–84, 94–99, 150–166, and 193–195. Residues 65–79 and 94–99 (8–96 and 110–115 by bovine chymotrypsin numbering) corresponded to regions of serine proteases which are structurally conserved but, because they are surface exposed, have reduced structural constraints. Residues 80–84, 150–156, and 193–195 (chymotrypsin 97–101, 166–179, 203–206) are joining loops with divergent structures and sequences. Region 85–92 (chymotrypsin 102–109) is conserved in structure and sequence. It contains aspartate 85 of the catalytic triad and the hydrophobic core residues for the N-terminal hemisphere of trypsins and two of the residues connecting the interface of the hemispheres (113).

Eur m 3 (GenBank AF047615) had 81% identity to Der p 3 and Der f 3. Of the residues, 75% were the same for all three molecules. Where the molecules differed, 18 of the Eur m 3 residues were Der p-like and 19 Der f-like. Therefore, there was no trend toward a Der p- or Der f-like sequence. There was, however, an indication that the Eur m 3 has more unique residues than Eur m 1. Twenty-three of the substitutions were found only in Eur m 3, and the residues at 11 of the positions were different in all species. The variations in Eur m 3 occurred in the same regions previously described for Der p 3 and Der f 3.

The polymorphism of the group 3 allergens showed a different pattern from the groups 1 and 2. For Der p 3, 4/6 sequences from a commercial source were the same, with the other two differing at positions 98, 105, and 116 and at positions 98, 105, 145, and 150 (114). Therefore, it appears that, although the variation is less frequent, allergens with a combination of several nonconservative substitutions occur. The same was apparent for Der f 3. Two Der f 3 sequences from commercial mites in Australia differed only at position 5 with either

valine or aspartate residues (110). Two sequences from Japan (111) contained a valine at position 5 and differed from the Australian sequences by substitutions at positions 89, 108, and 192. A further five sequences contained an aspartate at position 5 and the same variations at the other positions. However, an eighth sequence with valine at position 5, differed at positions 8, 52, 57, 89, 108, and 192. The changes at 89, 108, and 192 were found in the Australian mite sequences. Thus, again, the occurrence of variants was less frequent but involved multiple amino-acid substitutions. Unlike for group 1, for group 3, the substitutions were in positions where the serine protease structure has variation.

Group 4 allergens

Der p 4 was isolated as amylase by its substrate affinity (15). It bound IgE in 46% of adults and 25% of children. It had a mass of 56–63 kDa determined by analysis in reducing and nonreducing SDS–PAGE gels. The 19-residue N-terminal sequence had some homology with other amylases, as has now been confirmed by unpublished DNA sequence data.

Group 5 allergens

Tovey et al. (115) isolated a cDNA clone which reacted to IgE in 40% of allergic sera. IgE antibody affinity purified from the recombinant protein reacted to a 14-kDa band in mite extracts. The sequence was translated into a 17-kDa polypeptide with no N-glycosylation sites and no cysteines, but, because the sequence was truncated, no further analysis could be made. Full-length cDNA revealed a 132-residue polypeptide, with the first 19 residues comprising a proposed leader sequence (116). The mature protein had a calculated molecular weight of 15 K. In the study of Lin et al. (116), the N-terminal of the protein from mite extracts has now been determined, showing the mature polypeptide to have 112 residues, beginning at the aspartate-designated residue 2, and a molecular weight of 13 K. Blo t 5 from *B. tropicalis* reacts to 70% of sera (62) and has 43% sequence identity to Der p 5.

There have been five clones of Der p 5 sequenced from commercial mites, and, in complete contrast to the major allergens, only a single amino-acid variation has been found, an alanine to aspartate at position 61 (116).

Group 6 allergens

The group 6 allergens were isolated as 25-kDa proteins produced by *D. farinae* and *D. pteronyssinus* (27). They have the substrate specificity of chymotrypsins and are serologically unrelated to the group 3 trypsins. A cDNA clone showed that Der p 6 contains the catalytic charge relay residues of serine proteases and that position 189 has a serine, the residue essential for the substrate specificity of chymotrypsins (118). The 231-residue mature protein has a calculated MW of 25 K and three disulphide bonds based on the structure of bovine chymotrypsin. The amino-acid sequence had 37% identity with Der p 3. This is consistent with the poor antigenic relationship of Der p 6 to Der p 3 and is similar to the sequence identity between bovine trypsin and bovine chymotrypsin.

Group 7 allergens

Der p 7 was identified as a recombinant polypeptide which bound IgE in 50% of sera from allergic people. The cDNA encoded 215 residues with no cysteines, a single N-glycosylation site, and a predicted 17-residue leader peptide (10). The predicted mature 198-residue polypeptide had a molecular weight of 22 K. The N-terminus has been confirmed by protein sequence (54).

Antibodies to recombinant Der p 7 reacted to components of 29, 27, and 24 kDa in mite extracts (119). All of these are larger than the polypeptide encoded by the cDNA sequence. Absorption of sera from allergic patients removed IgE reactivity to bands of 29, 27, 13, and 11 kDa. The 29-, 27-, and 24-kDa bands (estimated at 31, 30, and 26 kDa in further reports) were confirmed with several monoclonal antibodies, indicating that they were not merely incidental cross-reactivity. Der f 7 showed 86% identity to Der p 7 (120) as well as to the multiple bands. One monoclonal antibody also reacted to lower molecular weight bands indicative of degradation products (54). N-glycosidase treatment of extracts shifted the 31- and 30-kDa reactivities to the 26-kDa band; therefore, the high-molecular-weight species were due to glycosylation (119). The multiple Der p 7 bands also explain the report that antibodies affinity purified from the 30-kDa fraction of mite extracts reacted to a 25-kDa band (121). Recently, it was found that in some extracts the most abundant molecular species were in the 31–30 kDa fractions and in other extracts in the 26-kDa fraction (54). A thorough investigation in commercial extracts is warranted.

Group 8 allergens

Der p 8 was identified from a cDNA clone which produced a 209-residue polypeptide reacting to IgE in 40% of sera (31). It is a glutathione-S-transferase with strong sequence homology to the enzyme in

other species. The predicted molecular weight was 25 kDa. There was no leader sequence, as is typical of cytosolic proteins and glutathione-S-transferases. Since there are gene families of glutathione-S-transferases in other species, it could be a family of allergens. Cockroach glutathione-S-transferase, Bla g 5, is an important allergen which binds IgE in 75% of allergic subjects (122). The overall sequence of identity of 25% is not indicative of cross-reactivity with the mite, and there are no special blocks of identity. Although both the cockroach and the mite allergens have been isolated from extracts by affinity chromatography with glutathione-coupled Sepharose, the yields are extremely low (Arruda et al. [122] and Smith [unpublished]).

Group 9 allergens

Der p 9 is a serine protease which migrates as a 28-kDa band in SDS–PAGE and has a molecular weight of 24 kDa, as determined by electrospray mass spectroscopy (26). The frequency of IgE reactivity is similar to Der p 3, but the level of IgE binding was less. The anti-Der p 9 reactivity of IgE in individual sera correlated with the reactivity to Der p 3 and 6, but there was little cross-reactivity by RAST inhibition. Der p 9 differs from Der p 3 and 6 by its collagenolytic activity, and the N-terminal amino-acid sequence showed 7/18 differences from Der p 3, with only one of the differences being found in Der f 3.

Group 10 allergens

Tropomyosin forms filaments in the actin groove of muscle which function in regulating contraction. Der f 10 was isolated by IgE screening of a cDNA expression library (32). The cDNA encoded a 284-amino-acid molecule which had a molecular weight of 33 K and was highly homologous to tropomyosin. The purified natural tropomyosin had a molecular weight of 37 kDa by SDS–PAGE. The cross-reactivity of the group 10 allergens to invertebrate tropomyosins (33) is matched by its sequence, which shows about 75% identity to other arthropod tropomyosins and 60% to mammalian tropomyosins. Trypsins, by comparison, show only 45% and 44% identity and chymotrypsin 32% and 34%, respectively. Der p 10 and Der f 10 show only three amino-acid differences in the entire sequence. This is 98.5% identity compared to the 80% of the other allergens. There are, however, two differences between the Der p 10 sequence obtained by Asturias et al. (123) and that obtained by Smith, Mills, and Thomas (GenBank AF016278).

Group 11 allergens

Paramyosin is a structural muscle protein of invertebrates. The 98-kDa paramyosin of *D. farinae* has recently been shown to bind IgE at very high frequency and with strong potency (124). It may be the high-frequency IgE-binding specificity at 90–100 kDa reported in several Western blotting studies (2, 6, 7).

Groups 12 and 13 allergens

The groups 12 and 13 designations have been given to allergens from *B. tropicalis* identified by screening cDNA expression libraries for IgE-binding polypeptides. Blo t 12, which bound IgE in the sera of 50% of allergic subjects, had a mature sequence with 124 amino acids and a molecular weight of 14 kDa (125). Blo t 13 bound IgE in only 10% of sera. It is a 130-amino-acid protein with a molecular weight of 14.8 kDa. The sequence is highly homologous to cytosolic fatty acid-binding proteins found in many species (126).

Undesignated allergens

The allergen designated Mag 3 from a cDNA fragment from *D. farinae* has the same reactivity as Der f 1 and 2 in both IgE-binding and T-cell assays (34, 44). The cDNA fragment encoded a 349-residue polypeptide with a molecular weight of 40.5 K which lacked an initiation and a termination codon. Antibodies affinity purified from the clone were used to isolate a 177-kDa mite protein which did not contain lipid or carbohydrate moieties (34). Further characterization of Mag 3 would seem to be a priority.

Two other allergens have similarly been described from *D. farinae* cDNA libraries, Mag 29 and Mag 1. The former is a 67-kDa homologue of the heat-shock protein, *hsp* 70, reacting to IgE from 10% of patients (35), and Mag 1 reacted to IgE in 30% of patients (36). The 1114-bp nucleotide sequence of Mag 1 encoded a 341-residue C-terminal polypeptide fragment. IgE antibodies affinity purified from the recombinant polypeptide reacted primarily to a 39-kDa band in mite extracts, but antibodies from some patients reacted to higher molecular weight bands.

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