

Characterization and Immunobiology of House Dust Mite Allergens

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Abstract

The examination of house dust mite extracts has indicated that over 30 different proteins can induce IgE antibody in patients allergic to the house dust mite. There are however dominant specificities especially the group 1 and 2 allergens which can account for much of the allergenicity of extracts. Of the 19 denominated allergens, the major IgE binding has been reported for the group 1, 2, 3, 9, 11, 14 and 15 allergens. The high-molecular-weight group 11, 14 and 15 allergens have only recently been described and although high IgE binding has been anticipated from immunoblotting, there is a need for considerable corroboration. Similarly, the study of the group 3 and 9 serine protease allergens has been incomplete. The group 4, 5, 7 and 8 allergens have shown intermediate IgE binding and the group 10 tropomyosins are of interest because of their potential cross-reactivity with allergen from disparate species. Although the progress with the production of recombinant group 1 allergens has been recent, many of the allergens can be produced as high IgE-binding polypeptides. The tertiary structure of the group 2 allergens has been determined

from recombinant proteins and they are an excellent model for the investigation of modified allergens. An unexpected property of the group 1, 2 and 3 allergens has been the high degree of polymorphism found by cDNA analysis. It has however been possible to identify sequences to represent the variation in the natural allergens. The group 7 and 14 allergens show secondary modifications which vary in different extracts creating batch variation. While some estimate of the importance of allergens can be obtained from IgE binding, few analyses of T-cell responses have been made and these regulate both the development of, and the protection from sensitization.

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House Dust Mite Extracts

House dust mites are complex organisms which produce thousands of different proteins and other macromolecules. House dust mite extracts are made from an aqueous extraction of variable mixture of whole mites, nymphs, faecal pellets, eggs and spent culture media. They have been proved to be potent in their ability to induce skin test reactivity and have high IgE binding in vitro tests. Much of this reactivity however would be expected from the high concentration of the major aller-

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gen group 1 and 2 allergens. The need to examine the importance of other possible allergens and how well they are represented in extracts is severalfold. It is known that at least 20% of adults allergic to mite have poor reactivity to the group 1 and group 2 allergens so, given that extracts are standardized by skin reactivity of unselected allergic subjects, the potency will not be appropriate for a large number of patients. The need to improve immunotherapy is however the main reason to identify the important mite allergens. Knowledge of the allergens can be used to standardize formulations so the important allergens are adequately represented or recombinant technology could be used to synthesize a balanced mixture of major specificities. Given that the concentration of allergens in extracts can differ by two orders of magnitude, it must be difficult to reach a therapeutic dose for a less abundant allergen without inducing anaphylactic side effects to others. It is possible that relatively few allergens are required for effective immunotherapy and this would facilitate the introduction of new strategies such as the use of peptides, adjuvants and modified allergens.

The group 1 and 2 allergens were identified as major allergens from a study of extracts [1, 2] and they are present in high concentrations in household dust [3, 4]. It is however possible that some allergens could be present in the environment but be poorly represented in extracts. This could be due to poor extraction, lability and other properties such as hydrophobicity. While stability in aqueous extracts would be a property which potentiates allergenicity, some proteins may be protected in particles or may be constantly renewed in the environment. The allergens are also compartmentalized. For example, an allergen found in fat bodies would not naturally be in prolonged contact with digestive enzymes. The studies of Tovey et al. [5] showed that each dung ball contains 0.15 ng of the group 1 allergen. If the mites produce an average of 20 dung balls per day, 100 mites would take 1 week to produce 2 µg of allergen. This is the amount associated with this number of mites and a level which induces sensitization [6]. It means that there must be a decay to maintain a steady concentration. The decay can be seen in the allergen levels of extracts made from different sources of mite material [7]. Extracts with high levels of faecal pellets and few mites had low levels of the group 1 allergen and the extracts with high levels of the group 1 allergen were low in faecal particles. A number of studies have been conducted to examine the spectrum of IgE antibody binding with techniques such as Western blotting [8, 9] and crossed radioimmuno-electrophoresis [2, 10]. Over 30 different IgE-binding bands were recognized by West-

ern blotting although only 7 bound with high frequency and strong immunostaining. The interpretation of the studies is however difficult because of variation from report to report, the poor dose-response characteristics of blotting and the lability of allergens. Recent studies have also shown that the group 3, 7 and 14 allergens are found with multiple bands which vary from extract to extract [11–13]. The use of recombinant techniques has provided a method of examining the allergens without relying on their abundance or stability in extracts. Many allergens have been detected directly by the screening of cDNA expression libraries for IgE binding with allergic sera [14, 15].

IgE Binding to Purified Allergens

Table 1 summarizes the biochemical and IgE-binding characterization of the known house dust mite allergens. The group 1 allergens bind IgE from mite-allergic subjects at a high frequency and strength. As originally described [1], Der p 1 bound IgE in the sera from all the allergic subjects tested in that study and remarkably 50–70% of the IgE to a *Dermatophagoides pteronyssinus* extract was directed to Der p 1. Absorption studies showed that depending on the sera, the anti-*D. pteronyssinus* specificities comprised from 6–21% of the total IgE. Lind [16] and van der Zee et al. [17] confirmed the high reactivity and a similar reactivity was reported for Der f 1 from *Dermatophagoides farinae* [18–20]. Der p 2 has a similar frequency and strength of IgE binding. Van der Zee et al. [17] showed that for 60% of patients 50–100% of the IgE reactivity to whole mite extracts was directed to Der p 1 and 2. Similar results were reported by Meyer et al. [21] who found that 80% of sera from mite-sensitive patients reacted with Der p 1 and 2 and that these allergens inhibited almost all the IgE anti-HDM reactivity of a pool of positive sera. Der p 1 and Der f 1 induce both cross-reacting and species-specific antibodies [19, 22], however, depending on the patient, 10- to 10,000-fold more of the heterologous allergens can be required for ELISA inhibition [19]. Der p 2 and Der f 2 have been reported to have almost complete cross-reactivity [23] although some difference has been noted [24]. IgE to the group 1 and 2 allergens appears to be very dominant in the allergic response of children. O'Brien and Thomas [25] showed that children attending an allergy clinic had strong IgE binding to Der p 1 and 2 and sporadic responses to 1–3 additional allergens. Adults presenting at a clinic for allergy diagnosis had lower IgE binding to Der p 1 and 2 but as demon-

Table 1. House dust mite allergens

Group	Biochemical function	MW cDNA ¹ (SDS-PAGE)	Species ²	IgE binding ³
1	Cysteine protease	25,000	Dp, Df, Dm, Ds Em	80–100
2	Unknown (HE1 homologue)	14,000	Dp, Df, Ds, Em, Ld, Tp, Gd, As	80–100
3	Trypsin	25,000 (30,000)	Dp, Df, Ds, Em	16–100
4	α -Amylase	57,000	Dp, Em	40–46
5	Unknown	15,000	Dp, Bt, Ld	50–70
6	Chymotrypsin	25,000	Dp, Df	40
7	Unknown	25,000 (31,000, 29,000, 26,000)	Dp, Df, Ld	50
8	Glutathione-S-transferase	26,000	Dp	40
9	Collagenolytic serine protease	no cDNA, (30 000)	Dp	90
10	Tropomyosin	37,000	Dp, Df	50–95
11	Paramyosin	96,000 (92,000, 98,000)	Df, Bt	80
12	Unknown	14,000	Bt	50
13	Fatty acid-binding protein	15,000	Bt, Ld, As	10–23
14	Vitellogenin/apolipoprotein-like	177,000 (variable)	Df, Dp, Em	90
15	98,000 Chitinase	62,500 (98,000, 105,000)	Df	70
16	Gelsolin	55	Df	35
17	Ca-binding EF protein	30	Df	35
18	Chitinase	60,000	Df	60
19	Anti-microbial peptide	7,000	Bt	10

¹ MW calculated from cDNA (SDS-PAGE of natural allergen, if different).

² Allergen described for the species designated by initials: *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Euroglyphus maynei*, *Dermatophagoides siboney*, *Dermatophagoides microceus*, *Lepidoglyphus destructor*, *Blomia tropicalis*, *Tyrophagus putrescentiae*, *Glycophagus domesticus*, *Ascaris siro*.

³ Binding frequency (% patients, variation due to patient selection).

strated by Western blotting, recognized a diverse array of allergens. Adults diagnosed in childhood however had high anti-Der p 1 and 2 binding indicating a persistence of the early response. The Western blotting of Shibasaki et al. [26] showed a similar phenomenon with bands equivalent to Der f 2 predominating in the very young group. Der p 1 has poor IgE binding in Western blotting so it would be important to corroborate the observation with purified allergens. While the IgE binding to the group 1 and 2 allergens is high, the responses to other allergens must be considered. The report by van der Veen et al. [27] which showed that whole extract induced far stronger late reactions than a mixture of Der p 1 and 2, despite a similar to early response, is evidence for this. Additionally the affinity of the IgE can, as shown for Der p 2, vary over a 30-fold range and the ability to induce degranulation of basophils is proportional to the affinity [28].

Estimates of the IgE activity of the group 3 allergens have varied considerably with frequencies as low as 16% [29] and as high as 100% with a potency similar to the group 1 and 2 allergens [30]. Other reports show an intermediate [31] or variable degree of binding [32]. The con-

siderable 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. Standardized assays to determine the relative importance of this allergen are clearly required. A high frequency of IgE binding to Der p 3 has often been inferred from the frequent immunostaining of molecular weight (MW) 30,000 in Western blotting [33]. It is now known that binding to Der p 7, which also has a band at this size, may explain much of the staining. Antibodies affinity purified from the 30,000 MW fraction of mite extracts also reacted with a 25,000 MW band [34] which is indicative of the two known glycosylation products of Der p 7. A two-dimensional gel immunoblotting study found IgE binding to Der f 3 for 30% of sera. One study has compared the IgE binding of the serine protease allergens. Der p 9 had a high IgE-reactive similar to Der p 3 and more reactivity than the group 6 chymotrypsin [30]. The IgE reactivity to Der p 9 in individual sera correlated with the reactivity to the Der p 3 and 6 but there was little cross-reactivity.

The group 4 amylase allergen [35] was reported to bind IgE in 46% of adults and 25% of children allergic to house dust mites, although this analysis incorporated a Western blotting step which could affect the natural conformation of the protein. Subsequent studies with Der p 4 affinity purified with cyclodextrin showed 80% binding but the binding was extremely low and IgE from less than 40% of subjects bound IgE at >2 ng/ml [36]. The group 5 and 7 allergens react with IgE in 50% of sera often with a magnitude as high as the major allergens [37–39]. Der p 8 has been reported to bind IgE in 50% of sera from allergic subjects but quantitative studies have not been performed [40].

The binding of tropomyosin, the group 10 allergen, is variable. Aki et al. [41] found that Der f 10 bound IgE in almost all sera of allergic subjects as strongly as Der p 1, while work in Europe found about 50% of subjects had antibodies to tropomyosin with a variable strength of binding [42]. The amino acid sequence of tropomyosin is evolutionary conserved so the difference may be due to a cross-reactivity to non-mite allergens. A recent comparison has shown a high IgE binding of Der p 10 by sera from Africa and low binding in sera from Europe [43] suggesting cross-reactivity.

Der f 11 (paramyosin) has been shown to bind IgE at high frequency with a strong reactivity [44, 45]. This allergen therefore may be responsible for the high-frequency IgE-binding specificity at 90–100,000 reported in several Western blotting studies [8, 46, 47].

Der p 12 and 13 have only been described for storage mites and are minor specificities.

The high-molecular-weight group 14 allergens are high IgE-binding allergens. The original reports described IgE binding by two peptide fragments, Mag 1 and Mag 3, which were expressed from partial cDNA clones [48, 49]. The binding to the whole allergen, isolated by antibodies to Mag 3, was similar in strength and frequency as the binding to Der f 2 [49]. Several sera without IgE antibody to Der f 2 also reacted strongly to Der p 14. A recombinant fragment corresponding to residues 1–260 of Der p 14 has now been shown to bind IgE in 79% of mite-allergic sera [50], so it appears to be a highly significant allergen in two species and in different locations.

As shown by the IgE binding of Der f 15, the group 15 allergens may be important for both humans and pets. Der f 15 was found to bind IgE in almost 100% of dogs [51] and 73% of human sera [McCall, pers. commun.].

The group 16 and 17 allergens bind IgE in 35% of allergic sera [52] while the group 18 allergen, which is also a strong allergen of dogs, binds IgE in 60% of humans

[E. Weber, pers. commun.]. The designation of 19 has been made for a 7,000 MW allergen of *Blomia tropicalis* which bound IgE from 10% of subjects [Nge and Chua, pers. commun.].

T-Cell Responses to Purified Allergens

Despite the requirement to establish dose-response curves and allergen specificity, most studies on mite allergy have used mite extracts. The recent study with mite ferritin showed that a non-allergenic protein induced high proliferative responses and both Th1 and Th2 cytokines from the T cells of allergic and non-allergic subjects [53]. There is thus a need to distinguish between the responses to allergens and other proteins. Rawle et al. [54] were the first to report proliferative responses to Der p 1 by peripheral blood mononuclear cells. The conclusions were that skin-test-positive people had higher proliferative responses than skin-test-negative ones. However, while this study did find low responses from cells of non-allergic subjects, the cells from many people with IgE to Der p 1 were also unresponsive. This included most subjects with asthma and rhinitis. The subjects who responded were almost entirely patients with atopic dermatitis and there was no correlation with IgE antibody titres. In a later study, O'Brien et al. [55] found more consistent responses. In a comparison of T-cell proliferation to Der p 1 and 2, the highest responders were subjects who were sensitive to mites plus other non-mite allergens. As a group, the responses of subjects without house mite allergy were lower, but overlapped the responses of allergic subjects. Responses to Der p 1 and 2 correlated closely. In a further study, the proliferation to Der p 1 and 2 was compared to the specific IgE antibody levels. All subjects with antibody had T-cell responses but some subjects had T-cell proliferation and no IgE antibody [25]. Cavaillon et al. [56] found no differences in responses of allergic and non-allergic subjects to Der f 1, but most stimulations were low.

Studies using media supplemented with growth factors have revealed proliferative responses to Der p 1 from T-cells from most allergic and non-allergic subjects [57, 58], but only the allergic patients produce Th2-type cytokines [59, 60]. Similarly, IL-4 mRNA production in response to Der p 2, correlated with asthma symptoms [61] and with the results of immunotherapy [62]. Over half the non-allergic subjects had positive T-cell responses as shown by the induction of mRNA for IFN- γ . Studies with peptides representing Der p 2 have also revealed strong responses of cells from non-allergic subjects [63]. The ability of Der

p 1 [59, 60, 64] and Der p 2 [65, 66] to induce the release of Th2 cytokines from peripheral blood cells of allergic subject is now established, and this agrees with responses reported for T-cell lines stimulated with Der p 1 [67] and Der p 2 [68, 69]. The allergens however do not induce higher IFN- γ release from the peripheral blood cells of non-allergic subjects. This is now recognized as the normal profile of cytokine release in the absence of extended T-cell culture. Similar profiles have been found for work with extracts [70–73]. As well as inducing CD4 T-cell responses, Der p 1 has been shown to stimulate CD8 responses which can release IFN- γ and are increased in allergic subjects [64].

There are few reports of T-cell responses to allergens other than group 1 and 2. The ability of recombinant Der f 2, Der f 10 and Mag 1, 3 (fragments of Der f 14) to stimulate T-cell proliferation was compared [74]. The responses to the high IgE-binding allergens Der f 2 and Mag 3 peptide were high and the responses to Mag 1 and Der f 10 were low. The responses to Mag 3 and Der f 2 were correlated and higher in mite-allergic patients. The lack of response to Der f 10 was interesting because the population studied has been reported to have a high IgE-binding activity. It is possible that this is evidence for a cross-reactive antibody response without shared T-cell epitopes. Epton et al. [75] compared the T-cell proliferation induced by Der p 2 and the peptide 1310–1650 of Der p 14 (equivalent to Mag 1). The responses to the Mag 1 were higher than to Der p 2 and significant responses to both allergens were found for cells from allergic and non-allergic subjects. Although the data was not presented, the allergens were reported to induce Th2 cytokines in allergic subjects.

A comparison of T-cell responses from peripheral blood cells was made for Der p 1 and Der p 7 [59]. Although Der p 7 did not bind IgE at the same frequency as Der p 1, all mite-allergic subjects responded to the allergen and produced the Th2 cytokine IL-5. It appears therefore that allergens can contribute to allergic responses even in the absence of IgE. Other Th2 cytokines were not measured in this study but would be of interest. A further significant result was that Der p 7 induced higher IFN- γ responses than Der p 1, particularly in non-allergic subjects. Thus the Th1 responses which protect from the development of allergy may be induced to allergens other than the major specificities. A comparison of responses of the allergens from *D. farinae* and *D. pteronyssinus* showed that they were equally stimulatory for proliferation and cytokine production. Given the study was conducted in a location known to have few *D. farinae*, the responses to

this mite are likely to be cross-reactivities, even though the allergens have sequence differences of 20%. Responses to synthetic peptides in contrast frequently showed species specificity [58].

Group 1 Allergens

The first major mite allergen described was Der p 1 from *D. pteronyssinus* [1]. Der f 1 was later characterized by several investigators [18, 19, 76]. Der p 1 was also the first allergen to be analysed by cDNA cloning [77]. cDNA sequences of the group 1 allergens revealed that they were cysteine proteases in the same family as papain and actinidin and had 222 or 223 residues with a calculated MW of 25,000 [77–79]. The X-ray crystallographic structures of the other cysteine proteases [80, 81] have been used to model Der p 1 [82, 83]. It has two globular domains formed independently by the N- and C-terminal sequences. The substrate binding and catalytic residues are in the cleft between the domains and domains are connected by a flexible outside loop. The sequence in the loop is the most divergent region of molecule. Der p 1 and Der f 1 [79] show a sequence identity of 80%. The divergence is predominantly in the N-terminal residues 1–20 (45%), the C-terminal 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. Studies by Greene et al. [84] found that species-specific antigenic determinants were in the variable central loop. The sequence of Eur m 1 from *Euroglyphus maynei* has also been determined. A partial sequence was obtained from a genomic PCR amplification product [85] and full-length cDNA clones have been described [86]. Surprisingly the divergence of the sequence of Eur m 1 was the same as the divergence between Der p 1 and Der f 1. The sequence of all the group 1 allergens contained an N-glycosylation site from residues 53–55, which is consistent with the report of carbohydrates in the purified natural allergen [1].

Studies with T-cell lines [87] and the stimulation of peripheral blood cells [58] showed that the most frequent and strongest stimulating peptides were in the central loop. More recently the study of Kircher et al. [88] confirmed the high stimulatory activity of peptide 101–131 in this region and found it was associated with an expansion of cells with a restricted T-cell receptor usage. The strong responses induced by the loop structure may be an important determinant for the high allergenicity of this molecule. Some studies have been conducted with Eur

m 1 peptides. Responses to peptides in this region were detected in 8/12 people, but stronger responses were found to other epitopes [58].

The group 1 allergens, like other cysteine proteases, have 19-residue leader and a 79-residue proenzyme sequences [79, 86, 89]. The Der p 1 allergen isolated from mites was first shown to have cysteine protease activity [90, 91] but further study revealed that it exhibits a unique mixed cysteine and serine protease activity, even though it only has one active site [92]. The preferred cleavage site was glutamate for the cysteine protease activity and arginine for the serine protease activity. Der f 1, Der p 1 and Eur m 1 contain a glutamate at residue 1 of the proenzyme sequence suggesting the possibility of autocleavage. Several studies have suggested that proteolytic activity of the allergen may provide an adjuvant activity. It was shown to cleave CD23 [93, 94] and CD25 [95] which are important in the regulation of IgE responses, and it has been shown that an inhibitor of proteolytic activity, E64, reduced the ability of Der p 1 to induce IgE in mice [96]. An adjuvant activity for a bystander allergen has also been described [97] and the proteolytic activity has been shown to enhance Th2 cytokine release from human T cells [98]. While these effects are plausible, the IgE enhancement in mice was not marked and the induction of the IgE still required alum adjuvant. Other investigators have shown that Der p 1 can loosen tight junctions in the respiratory epithelium [99] and can induce inflammatory cytokine release from epithelial cell cultures [100]. Again, the effects are plausible but whether they occur in vivo and their importance remains still has to be demonstrated. One indication that the proteolytic effect may not be a dominant influence is that dogs respond to mite with chitinase as the major allergen, not the group 1 proteins [101, 102]. Additionally, even though cysteine proteases are common enzymes, there is no evidence for a major group-1-type allergen in storage mites.

A feature of Der p 1 is its extraordinary high degree of polymorphism. Der p 1 only has one gene [78] so the changes must be allelic. Initially it was found that cDNA from 5 clones constructed from *D. pteronyssinus* obtained from CSL Ltd all varied from each other by 1–4 residues, usually with conservative changes [89]. The variation was the result of different combinations of single substitutions in 5 positions without any apparent linkage [89]. Sequences from 12 environmental isolates from Perth, Western Australia, have now been described as well as 3 sequences from Sydney and 1 from the UK [103]. The pattern of sporadic and frequent substitution of residues was continued. For the 20 clones available, all except 2 of

the substitutions have only been found in only 1 or 2 molecules and there has been no suggestion of an inherited pattern. The first exception was at residue 124 which was equally alanine or valine, and the second was at residue 50 where the histidine in the first published sequence has not been found in any environmental isolates, which instead have tyrosine. The alanine to valine substitution at residue 124 is in the immunogenic loop so it could be useful to ensure allergen preparations contain both isoforms. It also has a major effect on the responses to mice, where high responder H-2^b mice preferentially respond to the alanine form [104]. Except for the tyrosine 50 found in environmental mites, the other changes are so varied that individually they could only have a minor influence. It is possible that the unusual polymorphism may be connected with the high allergenicity, for example by allowing optimal MHC binding of peptide, but evidence is lacking. According to the model of Topham et al. [83], only the rare substitution found in residues 136 and 138 are in solvent-inaccessible regions and are likely to affect the core structure. There was also no tendency for the substitutions to be in regions of divergence between species.

Der p 1 and Der f 1 are frequently found in high concentrations in house dust, from 100 to 10,000 ng/g of dust [3, 105]. 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 in mite extracts and spent mite medium. [5]. Studies by De Lucca et al. [106] have shown that when collected by the filtration of inhaled air, the Der p 1 is associated with a range of particles and not just faecal material.

Group 2 Allergens

The group 2 allergens were first characterized as 14,000–18,000 MW allergens with a high IgE-binding activity [16, 29, 76, 107, 108]. The cDNA sequences of Der p 2 [15, 109] and Der f 2 [110, 111] showed that the allergens had 129 residues, a calculated MW of 14,000 and no N-glycosylation sites. Der p 2 and Der f 2 had 12% amino acid divergence which was evenly distributed throughout the sequence. This identity is significantly higher than the group 1 allergens, and substitutions are more conservative. Only one third of the substitutions were non-conservative compared with the opposite ratio for the group 1 allergens. The Eur m 2 [86] has 82% sequence identity to both Der p 2 and Der f 2 with no preferential similarity to either allergen.

The biochemical function of the group 2 allergens is still unknown. There was some speculation that Der p 2 may be a mite lysozyme, but it is clear that it is not [112], particularly now that the tertiary structure has been solved [113, 114]. Anti-group 2 antibodies have been reported to show reactivity associated with the gut and other unidentified structures [115, 116]. Recently the group 2 allergens have been shown to have similarity in sequence, size and distribution of cysteine residues with a family of epididymal proteins [117]. These include the human HE1 [118] and EPI-1 from chimpanzees [119]. These proteins like the group 2 allergens form disulphide bonds between the cysteines at positions 1 and 6, 2 and 3 and 4 and 5 [120]. The possibility that the group 2 allergens could be an epididymal secretion is intriguing and it should be noted that the reproductive tract which can form a large part of the mass of male mites and the HE-1 and EPI-1 comprise 20% of the protein in epididymal fluid. The HE1-type protein has however been associated with other tissues and functions. The bovine homologue EPV20 [121] is found in the kidney, liver, spleen and mammary glands but not the testis. A transcript from the tobacco hornworm *ers16* gene which is in the same structural family is found in high levels in the epithelial cells of the tracheae surrounding the central nervous system [122] and is associated with pupal ecdysis [123]. More recently, the HE1 protein has been identified as a gene affected in the human Niemann-Pick-type C2 disease [124]. This is characterized by a lack of production of the HE1 protein in fibroblasts and a defect in the lysosomal transport of cholesterol. This is not inconsistent with its concentration in epididymal fluid which is rich in lysosomal proteins. Moreover, HE1 has been shown to specifically bind cholesterol [125]. In this context it is of interest that the IgE-binding activity of Der p 2 greatly increases following incubation in mild detergent [126] which suggests the possibility of a major structural rearrangement.

Other possibilities have been suggested for the biochemical function. The ability of Der f 2 to bind to the surface of some *Escherichia coli* has been demonstrated [114], suggesting a function in defence. The tertiary structure of Der p 2 [113] and Der f 2 [114], has shown a protein consisting of β -sheets folded into a single immunoglobulin domain. It has structural similarity to the third and fourth domain of the transglutaminase coagulation factor XIII.

Sequence polymorphism for the group 2 allergens was first described for Der f 2 which is encoded by a single locus. The cDNA sequences showed polymorphisms in residues 76, 88, 111 and 125 [110] and substitutions in

PCR products were reported by others [111, 127]. A further 4 genomic sequences from the mite cultures found the same substitutions, although in different combinations [128]. The variant proteins can be detected in mite extracts and can be distinguished with a monoclonal antibody [129].

Studies with Der p 2 revealed that the pattern of polymorphism was distinct from the sporadic changes described for Der p 1. The variants of Der p 2 were first described by Chua et al. [130] but have now been analysed in light of further information [103]. Der p 2 showed a clear pattern of divergence. This could be identified by key sequence changes extended across mites from Perth, Western Australia, Taiwan and from a commercial culture. The most abundant sequence was the variant Der p 2.0101 which was the first sequence described for Der p 2. About half the clones had this sequence. Most changes to the sequence involved a pattern of substitution where residues 40, 47, 111 and 114 were substituted from VTMD in Der p 2.0101 to LSLN in Der p 2.0104. The change in these amino acids was accompanied by a series of silent mutations showing a common origin of genetic diversity. Natural Der p 2 would be best represented by a mixture of the sequence Der p 2.0101 and Der p 2.0104 which contains all of the common substitutions. The residues 40, 47, 111 and 114 all cluster close to each other in the tertiary sequence. Residues 47 and 114 are in adjacent loops and 111 is at the end of a β -strand while residue 40 is within a β strand. It is possible to distinguish the variants with monoclonal antibodies [112] and the residue 114 has been shown critical in this regard. The region of reactivity to the monoclonal antibodies was determined by hydrogen exchange and it corresponded to the cluster of sequence changes [131]. There is evidence for a small effect on IgE binding. Smith et al. [132] found that the binding of Der p 2.0101 correlated less well with natural Der p 2 than the responses to other variants and a direct comparison of the IgE between the different recombinant variants found the same difference [66].

From the study of O'Hehir et al. [63], the regions of Der p 2 most frequently recognized by T-cell responses were within residues 61–86 and 78–104 and would not be affected by the substitutions. A major region of recognition was however found with peptide 111–129 which induced proliferation in cells from 16/24 subjects [133] and this may be affected by the close changes at positions 111 and 114. A study of proliferative responses induced with recombinant variants found that they all were all stimulatory [103] but some differences could be detected especially when cytokine production was measured [66]. The most abundant Der p 2.0101 and the most divergent

Der p 2.01014 were highly stimulatory and induced a Th2 pattern of cytokine from allergic subjects. Rare variants with only single amino substitutions had modified cytokine responses showing subtle changes could be important, even when a polyclonal response to a whole antigen was measured. An important result for the study however was that a mixture of the main variants Der p 2.0101 and Der p 2.0104 would provide a good representation of responses to natural Der p 2.

Group 2 allergens (initially called Lep d 1) were described from storage mites *Lepidoglyphus destructor* from both cDNA and protein sequences [134, 135]. The allergens have now been analysed for the other storage mites *Glycophagus domesticus* and *Tyrophagus putrescentiae*. Although Lep d 2, Tyr p 2 and Gly d 2 cross-react, the cross-reactivity with Der p 2 is minimal [136]. The cross-reactivity of Der p 2, Der f 2 and Eur m 2 can be seen by the conservation surface residues while the Lep d 2 and Tyr p 2 show multiple substitution across the surface [132]. Similarly, Der p 2 had little ability to inhibit IgE binding to extracts of *B. tropicalis* but while there is some evidence for the existence of a Blo t 2, it has not been unequivocally demonstrated [137].

The level of group 2 allergen and the number of mites in house dust correlates well [138]. The concentrations of group 2 were surprisingly similar to group 1 [4, 138, 139] even though the group 2 allergens have been reported to be found at about a 5-fold lower concentration in mite extracts and in higher concentration in body extracts than spent mite medium [29, 76].

Group 3 Allergens

Der f 3 was described as a protein migrating as a 30,000 MW band by SDS-PAGE [29] with an N-terminal sequence similar to a serine protease. A similar but smaller molecule, probably a degradation product, was then isolated from *D. pteronyssinus* [11]. It was subsequently found that benzamidine could be used to affinity purify an allergen which migrated in SDS-PAGE as a 30,000 MW protein and had the same N-terminal sequence. This was evidence that Der p 3 was a trypsin-like molecule [140] and this was further shown, for Der f 3, by a substrate specificity [32].

cDNA sequencing [141] showed that Der p 3 had 233 residues with a calculated MW of 25,000 and contained the catalytic and substrate-binding sites of trypsin. It had no N-glycosylation sites and interestingly the recombinant polypeptide also migrated as a 30,000 MW molecule

by SDS-PAGE. By its homology with trypsin, Der p 3 contains disulphide bonds between positions 42–58, 168–182 and 191–220 and an unpaired cysteine at residue 12.

The Der f 3 cDNA [142, 143] had 81% sequence identity with Der p 3. 28/37 of the substitutions were non-conservative, which is similar to the conservation of the substitutions in the group 1 allergens. Der f 3 also contained the unpaired cysteine 12 residue. The regions of variation between the species can be matched to structures in three-dimensional models of serine proteases [144]. Residues 80–84, 150–156 and 193–195 (chymotrypsin 97–101, 166–179, 203–206) are joining loops with divergent structures and sequences. The 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 [145].

Eur m 3 (accession No. AF047615) has 81% identity to Der p 3 and Der f 3. 75% of the residues are the same for all three molecules. Where the molecules differed, 18 of the Eur m 1 residues were Der p-like and 19 were Der f-like. There was therefore no trend towards a Der p- or Der f-like sequence. There was however an indication that the Eur m 3 had more unique residues than Eur m 1. Twenty-three of the substitutions were only found in Eur m 1 and the residues at 11 of the positions were different in all the group 3 sequences. The variations in Eur m occurred in the same regions previously described for Der p 3 and Der f 3. Der s 3 has been partially characterized as a major allergen of *Dermatophagoides siboney* [146].

The group 3 allergens are also polymorphic and have shown 2 patterns of linked inheritance of 3–6 amino acid non-conservative substitutions [147]. Unpublished studies with cDNA from mites in the house dust of Perth have revealed that 50% of the Der p 3 sequence were a third type with multiple substitutions poorly represented in the commercial cultures. The same was apparent for Der f 3. Two Der f 3 sequences from commercial mites in Australia differed from each other in only one position [142] but Der f 3 sequences from Japan [143] differed from this and fell in two groups with multiple changes. The variation therefore was less frequent than the group 1 and 2 allergens but involved multiple substitutions. Unlike for the group 1 and 2 allergens, the substitutions occurred in regions where serine proteases typically show structural variation and where the sequences of the allergen from different species vary. Der p 3 has been shown to be encoded by a single gene so the polymorphisms are allelic [147]. A range of serine proteases with structural similari-

ties should however be expected including the chymotrypsin group 6 and the less well defined group 9.

As for Der p 1, it has been proposed that the proteolytic activity may have some effect on allergenicity. The group 3 allergens have been shown to produce anaphylatoxins C3a and C5a from the proteolytic cleavage of complement components C3 and C5 [148]. Both Der p 3 and Der p 9 have been shown to trigger a signalling pathway, for the pro-inflammatory cytokines GM-CSF and eotaxin, by the activation of protease-activated receptor-2 on lung epithelial cells [149].

The group 3 allergens are known to be major constituents of mite faeces but they are often present in a low concentration in body extracts [150]. It is possible that the allergen is highly susceptible to degradation as shown by the isolation of low molecular products from mite extracts [11]. The concentration in the environment is not known and reagents for double site monoclonal antibody assays do not exist.

Group 4 Allergens

Der p 4 was isolated as amylase by its substrate affinity to oyster glycogen [35]. It had a MW of 56,000–63,000 determined by analysis in reducing and non-reducing SDS-PAGE gels. The 19-residue N-terminal sequence however only had weak homology to other amylases because this is a region of sequence variation. cDNA cloning of Der p 4 and Eur m 4 revealed that the allergens were 496-amino-acid residue α -amylases with a calculated MW of 57,000 [151]. The sequence contained all of the conserved regions known to be important for amylase structure, calcium binding, substrate and catalytic activity. Overall, the sequences were 50% identical to insect and mammalian α -amylases and contained the cysteines for conserved amylase disulphide bonds. Both Der p 4 and Eur m 4 contained N-glycosylation sites but at different positions, 10–12 and 147–149 respectively. Three partial clones containing the C-terminal half of the open reading frame of Der p 4 did not reveal any polymorphisms. The sequences of Eur m 4 and Der p 4 were 90% identical, which is greater similarity than that found for the other allergens. A recombinant Der p 4 polypeptide produced in *E. coli* bound IgE from 3/10 of sera tested although in this sample IgE from only 6/10 bound Der p 2. No reactivity was found for Eur m 4 despite its strong identity. A larger study with Der p 4 expressed in *Pichia pastoris* has shown approximately 40% IgE binding [36].

Group 5 Allergens

Der p 5 was identified from a partial cDNA clone which produced a polypeptide reactive with IgE in 40% of allergic sera [39]. The sequence encoded a 17,000 MW polypeptide with no N-glycosylation sites or cysteines, but IgE antibody affinity purified from the recombinant protein reacted with a 14,000 MW band in mite extracts. Full-length cDNA revealed a 132-residue polypeptide with the first 19-residue constituting a putative leader sequence [152]. The mature protein had a calculated MW of 15,000. The N-terminal amino acid of the natural protein has now been determined [153] and has shown that the mature polypeptide has 112 residues beginning at aspartate designated residue 2 in the study of Lin et al. and a MW of 13,415. Blo t 5 from *B. tropicalis* appears to be a very important allergen for this species because it reacts with 70% of sera [154]. It has 43% sequence identity to Der p 5 and little cross-reactivity [155]. A partial cDNA clone of Lep d 5 has been reported [156].

There have been 5 clones of Der p 5 sequenced and, unlike the major allergens, only a single amino acid variation has been found, an alanine to aspartate at position 61 [152].

Der p 5 does not appear to be abundant in house dust although double monoclonal antibodies have not been performed. In Taipei, where mites are abundant, Der p 5 was usually under 100 ng/g dust [157] and the concentration in extracts was low.

Group 6 Allergens

The group 6 allergens have been described as 25,000 MW proteins purified from extracts of *D. farinae* and *D. pteronyssinus* [31]. They had the substrate specificity of chymotrypsins and were not serologically cross-reactive with the group 3 trypsins. King et al. [30] found a similar result with IgE and RAST inhibition. A cDNA encoding Der p 6 contained the characteristic catalytic residues of serine proteases and position 189 had a serine which determines the substrate specificity of chymotrypsins [158]. The 231-residue mature protein had a calculated MW of 25,000 and had three disulphide bonds based on the structure of bovine chymotrypsin. The amino acid sequence had 37% identity with Der p 3 consistent with the lack of cross-reactivity. The cDNA sequence of Der f 6 showed it had 75% identity with Der p 6 [159].

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 [12]. The mature 198-residue polypeptide had a MW of 22,000. The N-terminus has been confirmed from the natural protein [160].

Antibodies which were affinity purified from recombinant Der p 7 reacted with components of 29,000, 27,000 and 24,000 MW in mite extracts [161]. All of these are larger than the polypeptide encoded by the cDNA sequence. Correspondingly, absorption of sera from allergic patients removed IgE reactivity to 29,000, 27,000, 13,000 and 11,000 bands in extracts indicating the presence of 4 isoforms of the allergen. The 29,000, 27,000 and 24,000 bands (estimated at 31,000, 30,000 and 26,000 in further reports) were confirmed to be Der p 7 with monoclonal antibodies. One monoclonal antibody also reacted with lower MW bands indicative of degradation products [160]. Der f 7 showed 86% identity to Der p 7 [162] and the same multiple bands. N-glycosidase treatment of extracts shifted the 31,000 and 30,000 reactivities to the 26,000 band so 16% of molecule is carbohydrate [161]. The multiple Der p 7 bands also explain the report that antibodies affinity purified from the 30,000 fraction of mite extracts reacted with a 25,000 band [34]. The modifications were however variable. In some extracts the most abundant molecular species were in the 31,000/30,000 fractions and in other extracts in the 26,000 fraction [160]. The group 7 allergen Lep d 7 has been detected by cDNA cloning and showed reactivity with IgE in 67% of allergic sera [156].

Der f 7 allergens have been shown to be present in low concentration in mite extracts [160] and no data from house dust has been reported.

Group 8 Allergens

Der p 8 was identified from a cDNA clone which produced a 209-residue polypeptide reacting with IgE in 40% of sera [40]. It is homologous to a glutathione-S-transferase. The predicted MW was 25,000. The cDNA had no leader sequence 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. Unpublished studies have revealed a clone with several different amino acids to the published se-

quence. Cockroach glutathione-S-transferase, Bla g 5, is an important allergen which binds IgE in 75% of allergic subjects. 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 were extremely low [163] and unpublished.

Group 9 Allergens

Der p 9 is a serine protease with a collagenolytic activity distinguishable from Der p 3 and Der p 6. It migrated as a 28,000 MW band in SDS-PAGE and had a MW of 24,000 by mass spectroscopy [30]. The N-terminal amino acid sequence showed 7/18 differences to Der p 3. It has, along with Der p 3, been shown to induce GM-CSF and eotaxin by the activation of protease-activated receptor-2 on lung epithelial cells [149]. This allergen appears to an important but largely uncharacterized specificity.

Group 10 Allergens

Tropomyosins form filaments in the actin groove of muscle and regulate contraction. They are of special interest because the sequence is highly conserved between disparate species and have the potential for cross-reactivity with the major tropomyosin allergens of other invertebrates. Perhaps relatedly, the IgE-binding activity has been shown to vary with the geographical location. cDNA for Der f 10 encoded a 284-amino-acid molecule which had a MW of 33,000 [41]. Natural tropomyosin had a MW of 37,000 by SDS-PAGE. The cross-reactivity of the group 10 allergens to invertebrate tropomyosins has been demonstrated [42]. It reflects the 75% identity of the mite sequence to other arthropod tropomyosins and 60% to mammalian tropomyosins. Trypsins, for comparison, show 45 and 44% identity and chymotrypsin 32 and 34% respectively. Der p 10 [164] and Der f 10 show only 3 amino acid differences in the entire sequence to give 98% identity compared to the 80% of the other allergens. There was however a 2-residue difference between the Der p 10 sequence reported by Asturias et al. [164] and that obtained by Smith, Mills and Thomas (GenBank AF016278).

Wittman et al. [139] found that the tropomyosin was as abundant as Der p 1 and 2 in house dust but the assay

used also reacted with tropomyosin from other arthropods as well as mites. It nevertheless showed that these allergens can accumulate to high levels. The group 10 allergen was present in low concentration in mite extracts [41].

Group 11 Allergens

Paramyosin is a structural muscle protein of invertebrates. The 98,000 MW paramyosin of *D. farinae* has been shown to bind IgE at high frequency with a strong reactivity [44, 45]. It may be the high-frequency IgE-binding band at 90,000–100,000 reported in several Western blotting studies [8, 46, 47]. Blo t 11 from *B. tropicalis* has recently been examined. The 875-residue recombinant protein bound IgE in 50% of allergic sera [165].

Group 12 and 13 Allergens

The group 12 and 13 allergens have only been described by cDNA analysis of glycophagoid storage mites. Blo t 12, which bound IgE in the sera of 50% of allergic subjects, had a mature sequence with 124 amino acids and a MW of 14,000. Recombinant [166] Blo t 13 bound IgE in only 10% of sera. It is a 130-amino-acid protein with a MW of 14,800. The sequence is highly homologous to cytosolic fatty acid-binding proteins found in many species [167]. *Asa s 13* has been reported for the *Acarus siro* and also has a low, 23%, IgE-binding frequency [168].

Group 14 Allergens

Recent data shows that the group 14 allergens may be major specificities and abundant mite proteins. The peptide, Mag 3, which was an arbitrary fragment expressed from a partial cDNA clone from *D. farinae*, was shown to be a part of a 177,000 MW allergen with the same strength of reactivity as Der f 1 and 2 in both IgE-binding and T-cell assays [49, 74]. The high-molecular-weight protein did not contain lipid or carbohydrate moieties and the staining of histological section showed it was distributed throughout the body [49]. Western blotting of extracts with anti-Mag 3 revealed many bands which varied with different extracts [13] although bands of over 70,000 MW were prominent. The storage of the extract led to the proteolytic breakdown of the 177,000 MW allergen. When

IgE binding was analysed it was concluded that the fragments were more allergenic than the whole protein. The large protein has now been identified by sequence homology as a vitellogenin or apolipoprotein-like protein [169]. These molecules are lipid transport or lipid storage molecules which may explain the instability in aqueous extracts. Further, vitellogenins are typically processed into several polypeptides which are modified by phosphorylation, glycosylation and lipidation. Western blotting of mite eggs with antisera to a recombinant fragment of Der p 14 has revealed three major bands which would be consistent with a vitellogenin being processed to vitellin [170]. The sequence of the group 14 allergen was first determined for a cDNA clone of *E. maynei*. It was then recognized that the fragment Mag 3 and another fragment Mag 1 were located in the C-terminal of a 1650-amino-acid molecule. The biochemical homology of the allergen was only recognized when the N-terminal sequence was determined. It was not possible to obtain clones encoding the entire sequence of Der p 14 but it has now been determined by PCR from mite cDNA (AF373221). It appears that the group 14 allergens are important high-molecular-weight allergens. The natural form of the allergen encountered in the environment may be complex and variable. The presence in extracts is known to be low, presumably due to stability because it is a very abundant protein in mite bodies [49].

Group 15 Allergens

The group 15 allergens are potentially very significant because they are the major allergens recognized by dogs and cats, and because they are highly glycosylated, consisting of almost 50% carbohydrate. The allergen which has been described from *D. farinae* is homologous to insect chitinases [51]. It is located in the gut suggesting it has a function in digestion rather than in moulting. It is extensively O-glycosylated and thus will have multiple carbohydrate determinants which may provide an opportunity for cross-linking of IgE on mast cell receptors. The allergen corresponds to bands of 98,000/105,000 MW on Western blotting of mite extracts but the polypeptide accounts for 63,200. As well as reacting with IgE in almost all mite-allergic dogs, the allergen binds IgE in the sera of 70% of humans allergic to mites [McCall et al., entry to IUIS database <http://www.allergen.org>].

Table 2. Recombinant allergens

Allergen group	Yield	Bioactivity compared to natural
1	High	Natural enzymatic and IgE-binding activity
2	High	Natural IgE binding
3	Low (fusion) ¹	High IgE binding
4	Moderate	Natural amylase activity but reduced IgE binding
5	High	IgE binding high and crystallizable but no natural protein tested
6	Low (fusion)	High IgE binding
7	High	70–90% of natural IgE-binding activity
8	Unclear	IgE binding high, binds glutathione substrate but no natural protein tested
9	No cDNA	No cDNA
10	High (fusion)	Lower IgE than natural
11	Moderate (fusion)	Lower IgE than natural
12	Unknown	IgE binding in phage assay
13	Moderate	IgE binding high but natural allergen not tested
14	Only peptide fragments expressed	IgE binding detectable
15–19	Insufficient information	Insufficient information

¹ 'Fusion' if protein has only been reported to be expressed a fusion, e.g. with glutathione-S-transferase from pGEX.

Group 16, 17, 18 and 19 Allergens

The allergens in this section have recently been entered into the IUIS allergen database but the details have not been widely published. The designations of 16 and 17 have been given for gelsolin and an EF calcium-binding protein, both of which bind IgE in 35% of sera from mite-allergic patients [52]. Der f 18 is a 60,000 MW chitinase which is distinct from Der f 15 and is a strong allergen for dogs and also reacts with 60% of humans [E. Weber, IUIS database]. The designation of 19 has been made for an allergen of *B. tropicalis* which is homologous to an antimicrobial peptide [Nge and Chua, IUIS database]. It only had reactivity with 10% of subjects but this is judged only from recombinant material. The small size of 7,000 MW may be of interest.

Recombinant Mite Allergens

Table 2 summarizes the ability to express recombinant mite allergens. They have the potential to provide standardized reagents and open avenues for new type of immunotherapy. The ability to faithfully reproduce the natural molecule can however be variable. For the mites, the most obvious problem has been the production of the group 1 allergens. The original experiments with allergens expressed in *E. coli* showed that fusion polypeptides had about 50% of the IgE-binding activity of the natural mole-

cule [171, 172]. Expression in *Saccharomyces cerevisiae* produced a more allergenic product [173] but it was still antigenically deficient. Recent studies have concentrated on the production of these proteins from the proenzyme sequence on the understanding that the proenzyme is required for folding. While the proenzyme does permit the production of high levels of protein, a problem of correctly removing the large pro sequence has remained. The Der f 1 proenzyme has been produced in insect cells [174] and in *P. pastoris* [175–177]. The pro sequences are either removed by the host organism [176], or conditions of in vitro incubation have been devised to induce self-cleavage. The allergens have shown the natural IgE-binding activity. Mutants made to remove the N-glycosylation site and prevent hyperglycosylation in *P. pastoris* have retained the normal IgE-binding activity [177]. The allergen has also been produced as a high IgE-binding polypeptide in the yeast *Aspergillus oryzae* [178]. The production of recombinant Der p 1 has not been as advanced but has now been expressed from the proenzyme in insect cells [179] and in *P. pastoris* [180]. The expression in *P. pastoris* has been found to produce a high yield of protein with similar properties to the natural allergen. Mutation could also be used to prevent hyperglycosylation.

In contrast to the group 1 allergens, the group 2 proteins have been easy to produce as recombinant allergens in *E. coli* in fused [181, 182] and unfused forms [113, 183], although the latter must be renatured. Der p 2 can be secreted from *S. cerevisiae* in a form essentially indis-

tinguishable from the natural allergen [184]. The group 2 allergens are ideal for structural studies and the study of mutants and modified allergens. A number of amino acid substitutions have been shown to substantially reduce IgE binding to Der p 2 [185–188] and Der f 2 [189–191]. As shown for Der p 2 [181, 192] and Der f 2 [193, 194], the IgE binding is highly dependent on the tertiary structure.

The production of recombinant serine protease allergens has not been extensively examined. Both Der p 3 and Der f 3 have been produced as fusion proteins with glutathione-S-transferase from the pGEX vectors and show high IgE-binding activity [143, 195]. The yields of soluble protein were however low. The Der f 3 was produced as a proenzyme fusion, renatured and was then hydrolysed to produce a mature protein by cleavage at an introduced enzyme site [143]. The group 6 chymotrypsin allergens have been difficult to produce. Der f 6 expressed as a pGEX fusion was insoluble although some IgE-reactive fragments were obtained after thrombin digestion of the renatured polypeptide [159]. cDNA encoding the Der p 9 allergen has not been cloned.

Der p 4 has been secreted from *P. pastoris* in good yield as an active enzyme with IgE-binding activity [36]. The recombinant polypeptide of Der p 5 has high IgE-binding activity but the natural protein has never been isolated for comparison. The recombinant protein has been crystallized indicating a natural structure [196]. Der p 7 and Der f 7 can be produced in high yield as soluble fusion protein from pGEX vectors and as unfused polypeptides from a variety of other vectors [present authors, unpubl. data]. The recombinant allergen was shown to have 70–90% of the reactivity of the normal protein depending on the

serum tested [37]. Der p 8 has been produced as an unfused polypeptide in *E. coli* and could be isolated by substrate affinity chromatography on glutathione agarose indicating a natural conformation [40]. The binding activity has however not been measured in a quantitative assay or been compared to the natural allergen. The group 10 tropomyosin allergen has only been reported to be produced as a pGEX fusion, which had a significantly lower IgE-binding activity than the natural allergen. It is however possible to produce recombinant tropomyosins with biological activity provided a hydrophobic N-terminal is available for polymerization [197]. A partial cDNA sequence of Der f 11, paramyosin was expressed as a fusion in a pGEX system and shown to bind IgE in 50% of sera and induce skin test reactivity in 60% of patients [45]. This is less than the over 80% described for the natural protein but the cDNA was truncated so no direct comparison can be made. The production of the group 14 allergens is likely to be complex because it is a very large molecule and because its homology to vitellogenin indicates that the natural allergen will be processed by proteolytic cleavage and be highly substituted with lipid and other moieties. The C-terminal fragments Mag 1 [48] and Mag 3 [49] have some IgE-binding activity and the recombinant peptide 1–260 was found to bind IgE in 80% of sera [50]. This region was produced because it corresponded to the evolutionary conserved domain typical of vitellogenin-like proteins and thus may provide a surrogate allergen. The expression of the polypeptide of Der f 15 has not been reported and given the high carbohydrate content of the natural allergen, the IgE binding of the recombinant polypeptide would be of interest.

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