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Hierarchy and molecular properties of house dust mite allergens

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Abbreviations:
CD, circular dichroism; nDer, prefix for natural *Dermatophagoides* allergen; rDer, prefix for recombinant *Dermatophagoides* allergen; ELISA, enzyme linked immunosorbent assay; HDM, house dust mitel; MD-2, Myeloid differentiation antigen 2; ML-domain, MD-2-like domain; LPS, lipopolysaccharide; TLR4, toll-like receptor 4

ABSTRACT

The allergenic load of house dust mite allergy is largely constituted by a few proteins with a hierarchical pattern of allergenicity. The serodominant specificities are the group 1&2 and the group 23 faecal allergens. The collective IgE binding to the group 1&2 allergens can measure unequivocal HDM sensitisation better than HDM extracts although discrepancies have been found in regions with complex acarofauna suggesting a need to investigate the specificity with allergen components. The group 4, 5, 7&21 allergens that each induce responses in about 40% of subjects are mid-tier allergens accounting for most of the remaining IgE binding. Their titres are proportional to the concomitant responses to Der p1&2. Group 2 allergen variants have different antibody binding. Body proteins only occasionally induce sensitisation although a higher prevalence of binding by atopic dermatitis patients provides a new avenue of research. A broad spectrum of IgE binding has been associated with diverse symptoms but not with the severity of asthma which is associated with low IgG antibody. Some allergens such as the group 14 large lipid binding proteins and the recently described proteins Der f 24–33, need further investigation but with the cognoscence that other denominated allergens have been found to be minor sensitisers by comparative quantitative analyses. Scabies is a confounder for diagnosis with extracts, inducing cross-reactive antibodies with Der p 4&20 as is seafood allergy with cross reactivity to Der p 10 a minor HDM allergen. The HDM genome sequence can now be used to verify allelic and paralogous variations.

Introduction

The dominant house dust mite (HDM) allergen Der p 1 is found at concentrations of 0.05–0.2 ng/m^3 in inhalable indoor air, mostly on large particles, and contrary to dogma the lowest exposure is found in bed.[1] Pollen and cat allergens are found at the higher levels of 1–5 ng/m^3 and 20 ng/m^3 respectively.[2] Most HDM allergy can be accounted for by responses to a small number of proteins. The immunodominant group 1&2 allergens are both about the 40th most abundant proteins produced by HDM[3] and the recently recognized potent sensitiser Der p 23[4] has only been found in minute quantities, although in faeces. Each of the HDM allergen components has a characteristic propensity to induce sensitisation providing a resource for investigating the events underpinning sensitisation. Knowledge from this can assist in the development of new types of immunotherapy, improved diagnoses, standardised measurements and the identification of confounding cross-reactions.

Spectrum of HDM allergens

Initial electrophoretic analyses of HDM extracts indicated a complex pattern of IgE binding. These assays however accentuate low titre antibody binding as well as disregarding the variable concentrations of components, the presence of degradation products and variations in glycosylation. Since IgE responses to one allergen can promote responses to bystander antigens, collateral responses to otherwise non-allergic components would be expected imposing a need for quantitation to identify the main allergens that drive sensitisation. Absolute (gravimetric) and comparative titrations are thus required. The term serodominant has been used here to refer to allergens that quantitatively make the most important contribution to the IgE responses in contrast to the term major used by the IUIS allergen nomenclature that only refers to prevalence.

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Most studies have been conducted with *Dermatophagoides pteronyssinus* where Der p 1&2 have long been recognized as serodominant specificities. Trombone *et al.*[5] showed that 95% of subjects with greater than 2 IU/mL of anti-HDM IgE bound one and usually both of these specificities with the combined titres closely correlating with anti-HDM titres and accounting for 60% of antibodies. Following studies showing that absorption of sera with nDer p 1 and rDer p 2, 5, 7, 8&10 removed a median of 80% of the IgE binding of sera to HDM, and the binding to their natural counterparts,[6] a quantitative study was conducted with this panel expanded to include nDer p 3 and 4 and enzymatically active rDer p 20.[7] Der p 1&2 accounted for 50–65% of the antibodies of each subject and although only recognized by 40% of subjects each of Der p 4, 5 and 7 accounted for 10–15% of the total binding when present and collectively most of the non-Der 1&2 binding. The titres to Der p 4, 5&7 in different subjects strongly correlated with their titres to Der p1&2. IgE binding to Der p 3 only accounted for about 2% of antibody with few subjects producing more than a 1–2 IU/mL. Binding to Der p 8, 10 and 20 was infrequent only occasionally reaching significant titres. The IgG binding for each allergen mirrored the IgE binding although the correlation of IgE and IgG for individuals was poor. Titres obtained with a larger 13-member panel showed much the same pattern for Singaporean children.[8] It provided additional data showing mid-tier responses for Der p 21, low responses to Der p 13&15 and sporadic responses to peptides representing Der p 14. Titres to rDer p 4 were low but since it was produced in *Escherichia coli* this would be expected from its poor folding in other studies. Similar binding prevalences for Der p 1, 2, 4, 5, 7, 8, 10 and a Der p 14 peptide were found for subjects from several European countries.[9]

IgE binding to the group 11 paramyosin allergens has been difficult to appraise because of the instability of the recombinant and natural proteins and the lack of specificity controls and quantitation. A recent study of structurally-validated paramyosin found that it bound IgE in 5% of sera from patients with asthma but 60% subjects with atopic dermatitis.[10] The same study showed that the prevalence of IgE antibodies to Der p 10, 14&18 was also higher although titres were low.

Der p 23, found in the chitinous membrane of the mite faecal ball, has been discovered to bind IgE with titres similar to Der p 1&2 and have high activity in basophil degranulation tests.[4] The titres showed a strong concordance with binding to Der p 1&2 although some subjects had high IgE binding to Der p 23 without binding to Der p 1 or 2.

The IgE binding to serine proteases is an issue because historically some studies have indicated prevalent binding for group 3 trypsin allergens but comparisons with gravimetric estimations have found low titres titres to group 3[7,8,10,11] allergens and lower titres to the group 6 chymotrypsin.[10] Reponses to the collagenolytic group 9 serine protease were even lower.[12] Recently similar low binding was reported for natural and functional rDer p 3 but a non-catalytic mutant showed higher binding for some sera providing a valuable reagent for further study.[13]

The Der f 15[14]&18[15] allergens were discovered as important allergens of HDM-allergic dogs having primary structures similar to chitinase enzymes. It is however now known that the highly reactive and heavily glycosylated Der f 15 bound dog IgE via carbohydrate determinants[16] and that the IgE binding by humans found in 40% of allergic subjects is usually of low titre.[17]

Pyroglyphid house dust mite homologues of the *Blomia tropicalis* chitin-domain containing peptide Blo t 12 and the anti-microbial peptide Blo t 19 have not been found and will not be considered here.

Serodominant allergens (groups 1, 2 and 23) (Table 1)

Group 1 allergens

X-ray crystallography of rDer p 1[18] and nDer f 1[19] has shown the expected cysteine protease structures and Der p 1 and Der f 1, which have 81% sequence identity, show subtle differences in the contact residues for binding cross-reactive monoclonal antibodies. Der p 1 cDNA from different environments and pharmaceutical cultures show sporadic amino acid changes throughout their sequences with most cDNAs differing from each other by 1–3 residues.[20,21] Only one substitution, position 124 valine/alanine. showed a regular allelic exchange and this has importance for T-cell responses of mice[22] and some humans.[20] Der p 1.0102 and Der p 1.0105 could be used for recombinant allergens to accommodate these substitutions noting that the histidine-50 of the first-cloned Der p 1.0101 is rare. Der f 1 in contrast showed little variation[21] as also found with genomic DNA from Pakistan[23] and some commercially cultured mites. A smaller similarly-obtained sample of *D. pteronyssinus* corroborated the exchanges found in Der p 1. The sporadic changes might only be significant in special circumstances but it should be noted that error rates from reverse transcription and PCR do not account for their frequency and they are not found in parallel analyses of group 2 allergens. A different level of confidence should be placed in other results without this discrimination but they did find common substitutions.[24]

Group 2 allergens

The structure solved for Der p 2[25] and Der f 2[26,27] defined the ML (MD-2 like lipid binding) domain proteins. It consists of a raft of beta sheets folded over like a clam to form an internal lipid-binding cavity leaving two clusters of connecting loops opposed to each other. The binding of an unknown lipid in the cavity of recombinant allergens has been demonstrated and from NMR the clam appears flexible being able to accommodate different ligands. Der f 2 can bind lipopolysaccharide (LPS) at high affinity with structural changes similar to those occurring for its MD-2 homologue.[28]

Allelic variants of Der p 2[20,21,24,29] and Der f 2[21,24,30] show a pattern indicating different genetic lineages that might be important for allergy. For *D. pteronyssinus* the amino acids 40, 47, 111 and 114 VTMD (in single letter amino acid notation) in Der p 2.0101 can

Table 1
Serodominant allergens.

Group	Biochemical	IgE binding[†]	Biological activity	Location in mite	Molecular weight
1	Cysteine protease	80–100% 25–200 IU/mL	Proteolysis	Faecal	25 000
2	ML domain protein	80–100% 25–200 IU/mL	Lipid binding including LPS	Faecal	14 000
23	Peritrophin	74% Similar to Der p 1&2 by ISAC array	Chitin binding to stabilise dung ball (proposed)	Faecal	8000

[†] Numbers illustrative.