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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.

be substituted with varying penetrance to LSLN respectively with Der p 2.0104 having all four substitutions. Der f 2 shows substitutions in residues 57, 58, 59 and 75, from SLDF in Der f 2.0101 to NINVY in Der f 2.0116, although there are substitutions in residues 88, 111 and 125 common to both divisions. The variants show a different geographical distribution with for *Dermatophagoides farinae*, Der f 2.0101-like sequences predominating in Korea[24] and probably Japan[24] and Der f 2.0116-like sequences predominating in Europe. Both types of Der f 2 have been reported in sequences from China with the Der f 2.0116-like sequence from Hainan and Guangzhou and Der f 2.0101-like sequences from elsewhere.[30] For *D. pteronyssinus* the Der p 2.0101 clones that predominate in Perth, Australia[20,29] have not been detected in Thailand[21] and constitute 50% of the sequences in Korea.[31]

The substitutions of Der p 2 and Der f 2 are in different regions of the molecule so the divergent patterns might only be from founder effects. The Der p 2 substitutions however cluster together in a loop region affecting monoclonal antibody[32] and IgE binding. Der p 2.0104 binds IgE on average at twice the titre of Der p 2.0101[30,33] including in Australia where it appears less abundant. Additionally Der p 2.0101 and Der p 2.0104 could inhibit IgE binding to Der p 2.0104 by sera from Australia but higher concentrations of Der p 2.0101 were required for sera from Thailand.[34] Although the lack of an effect of variants on basophil degranulation has been reported[35] this is not surprising for subjects with high antibody titres but immunotherapy based on IgG blocking antibodies would need to consider variants because, as shown in mice, they can induce antibodies with poor binding to other variants.[34] A caveat of these findings is that natural variants have yet to be examined.

Group 23 allergens

The peritrophin-like Der p 23 is part of the chitinous peritrophic membrane of the dung ball[4] thought to control digestion and protect the gut from digestive processes. It was found in low amounts in HDM extracts questioning its effects on diagnosis and showing the need to determine the amount in inhaled air. Given that it induces high IgE titres and it must induce substantial Th2 responses and hence at least pathological changes via cytokines such as IL-13 as found for subjects with high expression of periostin.[36]

Mid-tier allergens (groups 4, 5, 7, 21) (Table 2)

Group 4 allergens

Group 4 allergens are alpha amylases found in faeces.[37] They have high sequence conservation that could cause confounding cross reactivities. *D. pteronyssinus* has 86% identity with *D. farinae* and 65% with storage mites compared to a 30–40% for most other allergens. The identity is 50% with insect and mammalian amylases. High IgE binding has been found in indigenous Australians in the absence of antibody to Der p 1&2[38] and to Blo t 4 in China[39] in the absence of antibodies to the serodominant Blo t 5 allergen of *Blomia tropicalis*. In Australia there is evidence for high cross-reactions from present and past scabies infections.[40]

Groups 5&21 allergens

The group 5&21 allergens are homologous proteins unique to mites without known function. Der p 5 &21 only have 26% sequence identity while Blo t 5 &21 from *B. tropicalis* not only have over 40% sequence identity with each other but also with both Der p 5&21. There do not appear to be any motifs that differentiate group 5 and 21 sequences. There is only a low degree of cross reactivity between Der p 5 and 21[41] and between Der p 5 and Blo t 5[42] but Blo t 21 and Der p 21 cross react.[43] The structure of Der p 5 shows a monomeric bundle of coiled coils that can polymerise to create a 6-member cage with a hydrophobic cavity[44] It agrees with that solved by NMR for Blo t 5[45] and with minor differences with that of Chan *et al.*[46] and they closely resemble the structure of Blo t 21.[43] The group 5&21 allergens are the serodominant allergens of *B. tropicalis*.[8]

Group 7 allergens

The group 7 structures show these uniquely Arachnida proteins to be members of the LPS-binding/bactericidal permeability inducing proteins[47,48] with similarities to insect odorant binding proteins that include hormone receptors.[49] They have elongated structures with antiparallel beta-sheets wrapped around a helix to create a lipid-binding domain. Der p 7 and Der f 7 seem not to bind LPS but weakly bind the fungal lipopeptide polymyxin B. The natural group 7 allergens can be highly glycosylated (30%) and there are at least 3 different glycosylation isoforms found in varying degrees in different HDM extracts.[50]

Minor allergens (groups 3, 6, 8, 9, 10, 11, 13, 15, 16, 17, 18, 20) (Table 3)

Group 3, 6 &9 allergens

The group 3, 6 &9 allergens are the serine proteases trypsin, chymotrypsin and a collagenolytic enzyme respectively. Der p 3 has been reported to be low in body extracts but it is abundant and stable in spent mite media and Der f 3 has been demonstrated in faeces.[51]

Group 8 allergens

Although they have low IgE binding the group 8 glutathione-S-transferases of *D. pteronyssinus*[7,8,52] and *B. tropicalis*[7] could have importance because of their demonstrated cross reactivity with cockroach allergens[52] however only 6% of HDM-allergic subjects in

Table 2
Mid tier allergens.

Group	Biochemical denomination	IgE binding†	Biological activity	Location in mite	Molecular weight
4	Amylase	40% 5–50 IU/mL	glycoside hydrolase	Faecal	56 000
5	Unknown	30–40% 5–50 IU/mL	Hydrophobic binding proposed from structure	Faecal	14 000 subunits form hexameric bundle
7	Similar to LPS-binding protein/ bactericidal permeability increasing family	40% 5–50 IU/mL	Hydrophobic binding proposed	Unknown	Polypeptide 21 000 with glycovariants of 24&29 000
21	Unknown group 5 homologue (paralogue)	30% 5–100 IU.mL	Hydrophobic binding proposed	Gut	14 000 subunits form hexameric bundle

† Numbers illustrative.

Table 3
Minor allergens.

Group	Biochemical denomination	Biological activity (predicted)
3	Trypsin	Serine protease
6	Chymotrypsin	Serine protease
8	Glutathione-S -transferase	Glutathione transferase
9	Collagenolytic protease	Serine protease
10	Tropomyosin	Muscle protein
11	Paramyosin	Muscle protein
13	Fatty acid binding protein	Lipid transfer
15	Chitinase	Family 18 of glycosyl hydrolases
16	Gelsolin	lipid-binding Microtubule regulator
17	EF hand protein	Undetermined
18	Chitin binding	Non-catalytic chitinase-like
20	Arginine kinase	ATP maintenance

Korea,[53] where cockroach allergy is prevalent, had anti-Der f 8 antibodies.

Group 10 allergens

The group 10 tropomyosins were first reported as prevalent high-titre IgE binding proteins in Japan[54] but recent analysis with a quantitative assay revealed little IgE binding with sera from patients in the same prefecture[55] in agreement with the low binding found in Australia,[7] Europe,[56] and Korea.[53] Tropomyosins are famous for their conserved amino acid sequences with HDM tropomyosins being 75% identical to other arthropod tropomyosins and thus being implicated in cross reactivity. While it does occur with shrimp[57,58] and cockroach[59] the significance has been overestimated. There is only low-level sensitisation to both cockroach[59] and HDM tropomyosin and subjects with both HDM and shrimp allergy do not necessarily have IgE antibody binding to Der p 10 even when they bind shrimp tropomyosin. More interestingly the 15% of HDM-allergic subjects responding to tropomyosin had higher levels of total IgE and IgE antibodies to a diverse spectrum of HDM allergens.[56] They did not however have more clinical symptoms and IgE to Der p 10 was not only found in low titres but was a poor elicitor of basophil degranulation.

Group 11 allergens

Paramyosins that constitute the group 11 allergens are components of muscles but can be found in the teguments of helminthic parasites where they are often prominent antigens. They are less conserved than tropomyosin with Der p 11 showing 95% identity to *B. tropicalis* and scabies but only 60% to insects and 50% to helminth proteins. The specificity of published IgE assays with degraded proteins is questionable especially since one of them showed far stronger IgE binding signals to Der p 11 than those to Der p 2.[60] Recently Banajee *et al.* noted degradation and aggregation in natural and recombinant Der p 11 preparations but with CD spectra showing the expected secondary structure.[61] Sera of subjects with respiratory HDM allergy examined with this preparation only showed 6% IgE binding prevalence but it was 60% for atopic dermatitis patients.

Group 13 allergens

The group 13 allergens are fatty acid binding proteins that transport fatty acids and other lipids such as eicosanoids and retinoids. Recombinant Der f 13 has the expected structure and its binding to fatty acid and other lipid ligands has been demonstrated.[62] They however only occasionally bind IgE despite being one of the most abundant HDM proteins.[3]

Group 15&18 allergens

rDer p 15 and rDer p 18 representing a chitinase and a chitinase-like chitin-binding protein can be produced in *Pichia pastoris* with structures resembling the natural proteins[17] but are weakly allergenic. A key point is that Der f 15 was discovered as the dominant allergen for HDM-allergic dogs but it is now known that the IgE binding was entirely directed to its large carbohydrate moiety either from N- or O-glycosylation.[16] An intriguing aspect of the responses to Der p 15&18 is despite the disparate sequences (24%) and lack of cross reactivity their titres in individuals correlate highly but not to titres to Der p1, 2, 5&7.[17]

Group 16&17 allergens

The group 16&17 allergens correspond to little-investigated gelsolin and EF-hand proteins of *D. farinae*, allergens reported to show weak binding IgE in 50% and a small percentage of allergic subjects respectively.[63]

Group 20 allergens

The moth mealworm arginine kinase allergen was found to bind IgE in the sera of HDM-allergic subjects[64] consistent with their 75 and 80% identity to insect and crustaceans homologues. They are abundant proteins similar to creatine kinase of mammals that generate energy in muscles. Despite being a minor HDM allergen[7,8] Der p 20 exhibits high binding to IgE and IgG from the sera of people with active scabies.[40]

Allergens of unknown importance (groups 14, 22, 24–33) (Table 4)

Group 14 allergens

The group 14 allergens were first represented by two cDNA clones that encoded residues 1310–1650 (Mag −1) and 891–1239 (Mag-3) of Der f 14. The peptides that are not natural products bound IgE in 30 and 70% of sera from allergic subjects. Antibodies produced against Mag-3 showed it to be part of a 170 000 MW protein that degraded in extracts to many IgE-binding products.[65] Complete cDNA[66] showed it was constituted by 1650 amino acids of MW 189370 with 90% identity between pyroglyphid mites and 43% with *B. tropicalis*, homologous to the large lipid transfer protein (LLTP) family that includes vitellogenins of eggs and type I and II apoliphorins that transport retinoids and other lipids. Antibodies produced against Der p 14 reacted with mite eggs and male and female bodies indicating a protein or proteins with both functions.[67] Although full-length proteins have been reported for

Table 4
Allergens of unknown importance.

Group	Biochemical denomination
14	Large lipid transfer protein
22	MD-2 like protein
24	Ubiquinol-cytochrome c reductase binding protein
25	Triosephosphate isomerase
26	Myosin alkali light chain
27	Serpin
28	Heat Shock Protein
29	Cyclophilin
30	Ferritin
31	Cofilin
32	Pyrophosphatase
33	Alpha-tubulin

homologues of other species, HDM studies have only been conducted with peptides. IgE-binding to peptide 1–260 representing the N-terminal domain was infrequent[9] while binding with peptide 1310–1650 in Australia at a median of 7 IU/mL suggested potential importance[68] as has binding to peptide 891–1239 in Singapore.[8] It was also shown that the equivalent peptide of scabies bound IgE at 10 fold higher levels for scabies infested but not HDM-allergic subjects.[68] A recent European study found that Der p 14 rarely bound IgE in the sera of asthmatics but bound IgE in 67% of atopic dermatitis patients although at low titre.[61] Vitellogenin-like allergens have now been reported in bee venom[69] and they the major allergens for people allergic to fish roe.[70]

Group 22 allergens

Der f 22 was recorded by the IUIS nomenclature subcommittee following the submission of sequence and allergenicity information. It is an ML-domain protein similar to the group 2 allergens but only has 42% amino acid identity. Its allergenicity has not been published but it is in the *D. farinae* genome.[71]

Group 24 allergens

rDer f 24, a ubiquinol-cytochrome c reductase binding protein (UQCRB)-like protein, has been reported to produce skin test reactions in 50% of HDM allergic subjects and by ELISA to bind IgE in sera from 18/18 patients.[71] The IgE responses have yet to be quantitated or compared with other allergens.

Newly denominated allergens

2-D immunoblotting has revealed IgE binding by Der f 25 (triosephosphate isomerase), Der f 26 (myosin alkali light chain), Der f 27 (serpin), Der f 28 (heat shock protein), Der f 29 (cyclophilin), Der f 30 (ferritin) Der f 31 (cofilin), Der f 32 (pyrophosphatase) and Der f 33 (alpha-tubulin).[72] Allergenicity was further examined with chromatographically isolated proteins although the profiles show a requirement for further purification and then quantitation and the exclusion of cross-reactive carbohydrate binding. Little IgE binding to myosin alkali light-chain protein, pyrophosphatase and ferritin has been found to recombinant proteins[71,73] in other studies and ferritin has been found not to induce Th2-biased cytokine responses.[73]

Biological activity and allergenicity

It has been speculated that biological effects of allergens on barrier and homeostatic functions and their interactions with pathogen and danger associated molecular patterns are important determinants of allergenicity.[74] Knowledge of this could have considerable application for the development of pharmaceutical interventions and the development of mouse models with some semblance to the pathophysiology of HDM sensitisation.

The cysteine protease group 1 allergens hydrolyse enumerable proteins that might affect allergic responses[75] and the E64 catalytic inhibitor has been reported to reduce their sensitising ability in alum-adjuvanted mouse models.[76,77] Investigations conducted with the homologue papain have shown that it induces chronic Th2-type inhalation allergy in mice without adjuvant and stimulates responses to bystander antigens in an E64 inhibitable manner.[78,79] There are however concerns about the biological significance since IL-33 release, shown to occur in papain sensitisation,[80] from damaged cells activates type 2 innate lymphoid cells to unleash their Th2 promoting activity. As documented elsewhere[2] cysteine proteases are highly susceptible to oxidative inactivation, and no activity of cysteine proteases has been demonstrable in HDM extracts without adding reducing agents that would not be encountered in the oxidising extracellular environment.[2]

Cysteine proteases are rarely allergens. Their enzymatic activity can be readily demonstrated in extracts of storage mites[81] that preferentially respond to other allergens including *Lepidoglyphus destructor*,[82] *Glycyphagus domesticus*[83] and *Chortoglyphus arcuatus*.[84] When measured in calibrated comparative assays Blo t 1 from *B. tropicalis*, in contrast to early assays, shows little IgE binding compared with Blo t 5&21, the serodominant allergens.[9] While the cysteine protease activities described in cultures of *Lepidoglyphus destructor*, *Glycyphagus domesticus* were half those of *Dermatophagoides* spp. and 15% for *B. tropicalis*, these depend on the nutrients and the stage of culture and Der p 1 levels in homes of sensitised subjects vary over this range.[85] Monoclonal antibody detection reported Blo t 1 to be 0.5 μg/mg of protein in *B. tropicalis* extracts close to the 1–3% reported for Der p 1.[86]

Cysteine proteases are abundant in the cell wall of birch and ragweed pollen but only induce IgE antibody in a small percentage of the most allergic subjects[87] although a cysteine protease from a more extractable fraction of ragweed pollen showed allergenicity.[88] It bound IgE from 60% of allergic subjects and induced basophil activation but to a lesser extent than Amb a 1.[88] Quantitative IgE binding and T-cell studies will provide interesting data to compare with the well established the immunodominant allergen. The most commonly encountered non-mite cysteine protease allergen is actinidin, Act d 1, from kiwifruit that comprises 50% of the protein in the fruit pulp. The presence of IgE antibody to Act d 1 in kiwifruit allergic subjects varies geographically from 11% in Western and Central Europe, 32% in Iceland[89] and reported as unimportant in England.[90] The most prevalent kiwifruit allergens are the pollen related PR-10 and profilin proteins and although IgE to Act d 1 is more prevalent in subjects without pollen allergy it was only found in 40% of this subgroup.[89]

Experiments in mice showing that Der p 2 might functionally mimic MD-2 by binding LPS and then loading it and itself onto TLR-4 suggested a mechanism that could enhance its allergenicity[91] and indeed Der f 2 was shown to bind LPS with high affinity in similar manner to MD-2.[28] The transient sensitisation shown in MD-2 deficient mice has however not been shown to be able to be sustained and no evidence for humans has been reported. It is not known if LPS could displace the natural group 2 ligand and how MD-2-like binding would help the responses of subjects already replete in MD-2. Indeed epicutaneous non-adjuvanted Der p 2 can induce prolonged Th2 responses by TLR4 independent mechanisms.[92] The same study also showed that LPS binding might have an important function of protecting Der p 2 from protease.

The potency of Der p 23 suggests a delivery system that could include chitin-mediated adjuvant activity. Chitin can interact with the TLR-2 and Dectin 1 innate immune receptors[93] as well as mammalian chitotriosidase[94] that in pulmonary cryptococcal infection can cleave inflammatory products from chitin to initiate harmful Th2 responses. The ability of chitin binding to enhance Th2 responses has been demonstrated in mice showing chitin increased Th2 responses to the chitin-binding-domain peptide Blo t 12.[95] Although they are minor allergens, the concordance of the IgE titres to the chitinase-like Der p 15&18 and their discordance with other allergens[17] indicates that they are regulated differently, perhaps via a chitin pathway.

The structures of the group 5, 7&21 allergens suggest that they have structures and that can bind lipid, glycoprotein and glycolipids to interact with the innate immune system and affect antigen delivery[2,96]

Component resolved diagnosis

The proprietary ImmunoCAP ISAC array contains the natural group 1 and recombinant group 2 allergens of *D. farinae* and *D. pteronyssinus*, and rDer p 10. It also contains potential cross-reacting tropomyosins, glutathione-S- transferase and arginine kinase proteins from other species.

Results with these reagents showed an almost excellent concordance of the sum of the IgE anti-Der p1&2 titres and those to HDM extract in 55 Japanese subjects.[55] The two subjects who failed to bind Der p 1 or 2 had anti-HDM titres of 1–2 IU/mL, levels not usually associated with sensitisation. Similar results, 94%, were found in France[97] and with custom reagents 96% in Australia with titres of non-Der p 1&2 allergens exceeding 3.5 IU/mL in only 0.05%. Results from Sao Paulo Brasil however showed lesser, 83%, binding to a combination of Der p1&2 but this was 95% with a cut-off of 2 IU/mL for the HDM titre.[5] The lesser concordance might result from the high degree of cockroach and *B. tropicalis* sensitisation in Sao Paulo, for example cross-reactivity with Blo t 21.[48] Similarly Singapore where only 78% of subjects had IgE binding to a combination of Der p 1&2[8] has a high prevalent sensitisation to *B. tropicalis*.

Although binding to Der p 10 alone has been described[56] it was not found in subjects that did not bind Der p 1&2 binding in studies in Japan[55] or Australia[7] where, for Australia, Der p 4 with contributions from Der p 5&7 could be implicated. The combined Der p1&2 titres can provide superior precision by using known and repeatable concentrations of the reactants without confounding cross reactivity and moreover they have been shown to correlate best with bronchial responsiveness to HDM.[55] The use of serodominant allergen components is not necessarily a compromise from the current practice since many proprietary extracts often contain undetectable levels of the other important allergens.[98] Additionally since 20–30% of subjects have discordant titres to Der p 1&2,[8,27,55] the use of extracts with 6-fold variations in these components[98] can produce variable measurements. Adding the group 23 allergens would provide a third serodominant allergen and a useful change would be to include the high-IgE binding Der p 2.0104 variant. Consideration of Der f 2 variants might also be important.

Scabies is already a known major confounder because scabies-infected or exposed subjects have high-titre IgE binding to Der p 4 (amylase) and arginine kinase (Der p 20) with the anti-Der p 4 persisting in previously exposed subjects. In the tropics of Australia[40] this has likely resulted in the misclassification in genetic and epidemiological studies of respiratory disease in indigenous subjects.[38] The use of component resolved diagnosis would clearly recognise the lack of Der p1&2 antibodies and the high titres to Der p 4&20. This is likely to impact on the usefulness of HDM allergen extract testing in many countries such as Fiji where the prevalence of scabies infection is 20%.[99] A large impact of scabies will be in developing regions where changes in lifestyle are resulting in increased incidences of atopic disease necessitating monitoring to predict health service needs. However, taking the United Kingdom as an example, where the population prevalence of scabies is 0.27%[100] there will be a significant number of incorrect diagnoses in most regions.

It has been reported that subjects with IgE antibodies to a large spectrum of allergens are the most likely to have nasal and skin as well as respiratory symptoms[8,101,102] but this does not apply to the severity of respiratory disease as observed in Europe,[56,103] and Australia.[7,11] where children recruited from a hospital emergency department had the same pattern of allergen recognition as stable asthmatics included those with persistent and recurrent asthma. The large difference found was the paucity of IgG antibody in the severe groups.[7,11] The recent demonstration that HDM-allergic atopic dermatitis patients have a high prevalence of IgE antibodies to the body components Der p 10, 11&14 not found in subjects with respiratory disease[61] suggests this might be prognostic for the persistence of eczema from childhood.

Conclusions

The probability of developing disease after HDM sensitisation is proportional to the degree of sensitisation measured by IgE antibody titre.[104] It progresses from 30% at a titre of 3 IU/mL to 60% at the highest titres. From another perspective the IgE antibody titres of subjects diagnosed with allergic disease by clinical criteria, by specialist allergists, are above 3.5 IU/mL.[105] It is now apparent that this is mostly binding to a small defined group of HDM components.

From studies of *D. pteronyssinus* 50–70% of IgE binding of extracts is due binding to Der p1, 2 & 23 that collectively induce responses in nearly all HDM allergic subjects and most of the rest is constituted by IgE to one or all of the mid-tier allergens Der p 4, 5, 7&21. The finding with Der p 23 should be corroborated as a priority. Binding to the serodominant and mid-tier HDM components follow a typical pattern with the size of the responses to the mid tier allergens being, when present, proportional to the responses to serodominant allergens. This pattern has also been found in children with severe disease with the difference in their responses being a paucity of IgG.

Given the dominance of Der p 1&2 in HDM extracts, and lack of mid-tier and cross reactive allergens in a great many of them, the use of components would be sufficient to determine unequivocal allergic sensitisation and to indicate potential cross reactions. In line with evidence unravelling in tropical Australia cross-reactive anti-scabies antibodies contraindicate the use HDM extracts in many environments where allergic disease might be expected to increase hand-in-hand with the increased sanitation of living conditions. IgE binding to Der p 4&20 and the absence of binding to Der p 1&2 can identify this. Consideration of using the most reactive group 2 variants should be made and this could be more important for the efficacy of immunotherapy.

The allergenicity of group 14&22 and recently denominated allergens need further investigations that use pure allergens, eliminate carbohydrate binding and ascertain absolute IgE binding titres. Many denominated allergens have not met expectations when examined under such conditions as elaborated elsewhere.[106] The availability of HDM genome will also provide information and it is noted that the group 2-like Der f 22 gene has been identified.

The recent discovery that subjects with atopic dermatitis have a higher prevalence of IgE responses to the body but not the faecal allergens of HDM provides a new avenue of research for this disease.

Conflict of interest

House dust mite patents of Telethon Kids Institute assigned for commercial use with WRT as an inventor.

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