The Allergenic Specificities of the House Dust Mite

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The most important house dust mites are *Dermatophagoides pteronyssinus* and in drier areas *D. farinae*. In subtropical and tropical regions the glycyphagid mite *Blomia tropicalis* is a major source of allergen, which co-exists with *D. pteronyssinus*. The group 1 and 2 allergens of *Dermatophagoides* mites are clearly major specificities and it is likely that these allergens could be the basis of new strategies of immunotherapy for many mite-allergic subjects. About 20% of patients, however, do not have IgE antibody to the group 1 and 2 allergens, and even though this is a minority, it constitutes a large population. There are also many other house dust mite allergens which have high IgE binding activity but these are present in low and variable concentrations in mite extracts, usually at less than 1% of the group 1 and 2 allergens. It must be appreciated that mite extracts are arbitrary preparations that do not accurately represent the relative concentrations of allergens in inhaled air. There is now the opportunity to produce more representative and more balanced formulations of allergens, possibly by mixtures of recombinant allergens. It is likely that the group 3, 5, 7 and 9 allergens will be important along with the high molecular weight group 11, 14, 15 and 18. The tropomyosin group 10 may be an important cross-reacting allergen. *B. tropicalis* is, because of its distribution in highly populated regions with increasing affluence, a very important allergen. It has low-grade cross-reactivity with *Dermatophagoides* but most allergens only have 30-40% sequence identity between the different families so they require different allergens for immunotherapy and new diagnostic measures are required to distinguish the sensitivity between the mite families. Studies on *B. tropicalis* allergens are required to identify the major allergens that do not appear to be the group 1and 2 specificities. Component resolved diagnosis is a newly developing procedure that uses allergen arrays to provide a diagnostic format to differentiate between cross-reacting allergens and to identify the optimal formulation of allergens for different patients. *(Chang Gung Med J 2004;27:563-9)*

Key words: **house dust mite, *Dermatophagoides pteronyssinus*, *Dermatophgoides farinae*, *Blomia tropicalis*, allergens, mite extracts, diagnosis, immunotherapy.**

Allergenic House Dust Mites

Allergens have been described from the families Acaridae, Glycophagidae and Pyroglyphidae.[1,2] The acarid mites Acarus siro and *Tyrophagus putrescentiae* are minor sources of house dust allergens. For Glycyphagidae, *Lepidoglyphus destructor* is primarily a storage mite while *Blomia tropicalis* has emerged as a prominent house dust mite in tropical and subtropical regions.[3]

The pyroglyphid mite *Dermatophagoides pteronyssinus* is distributed from temperate to tropical regions. Other important pyroglyphid mites are *D. farinae* found in drier regions. *Euroglyphus maynei* is found in temperate regions but in lesser abundance.

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Mite Extracts and Allergens

Extracts of *D. pteronyssinus* contain high concentrations of the group 1 and 2 allergens, usually between and 20 and 100 µg/ml.[4] Their high allergenicity can account for the allergenicity of the extracts. The concentrations of most other allergens are unknown but probably poor. Der p 7 is present at under 1 µg/ml[5] and recent unpublished data has shown that Der p 3 is present at less than 1 µg/ml. Experiments measuring the trypsin enzymatic activity of this allergen demonstrated a 200 fold higher concentration in spent mite media.[6] Recent direct measurements confirm this (authors, unpublished). Based primarily on purification yields Der p 5, 10, 11 and 14 are present in low quantity.[7-10] There is evidence that Der p 3, 7 and 14 are unstable in the extracts.[11,12,5] The group 14 is homologous to lipid transport molecules and shows abundant immunostaining in mite bodies.[13]

Allergens present in low amount in extracts can induce high titres of IgE so the questions are whether or not the allergens are present in high concentration in inhaled air or if they are potent at low concentration. It is also possible that the amount of allergen required to induce allergic responses in the airways is more than that required to induce IgE.

Calculations based on the allergen content of bodies and faeces[14] show that allergen extracted from bodies can only contribute about 1 % of the group 1 allergen in dust. Moreover since 100 mites/g of dust corresponds to about 2 µg of Der p 1[15] it can be further calculated that this sensitising level will be produced each week. Extracts can be produced by adding faecal material but preparations made with a high faecal content have been shown to contain low quantities allergen.[16] The age of the faeces could be important or there could be a threshold where proteolytic activity becomes dominant.

Several reasons can account for the low concentrations of allergens. Firstly allergens could be secreted proteins. Secondly some proteins are unstable in aqueous extracts such as Der p 14 which could be stabilised in lipid particles. Other proteins might be unable to withstand exposure the hydrolytic enzymes. Allergens in the mite are compartmentalised so, for example, haemolymph proteins are not mixed with digestive enzymes. Additionally, some proteins may be labile but are mites produce sufficient quantities of fresh allergen for sensitisation.

The relative importance of Der p 1 and 2 has been investigated by bronchial challenge.[17] The allergens elicited similar immediate responses to extracts but late reactions were smaller. This implies that other allergens are important. Additionally about 20% of mite-allergic patients do not produce IgE to the group 1 and 2 allergens[18,19] and, given the high frequency of mite allergy, this constitutes a major population. Thus of the 6% of Australasian children with asthma attributed to mite allergy, 20% (280,000) will have a non-Der p 1 and 2 allergy.[20]

Der p 1 and 2 constitute 1-2% of the protein found in extracts[4]. The immunoreactivity of other proteins must therefore be considered. One non-allergenic polypeptide, ferritin heavy chain, has been shown to be highly immunogenic[21] and to induce a balanced Th1/Th2 cytokine response.

Rank Order of Allergenicity (Table 1)

1. Pyroglyphideae

The group 1 and 2 allergens of *Dermatophagoides sp*. induce high titres of IgE[22][18] and Th2 cytokines in 80% of allergic patients [23,24]. The allergens Der p 3, 5, 6, 7 and 8 induce IgE in about 50% of subjects usually at lower titres.[25] Recent work has found less IgE binding to Der p 8[26] than the original estimate. The IgE binding to Der p 4 is frequent but usually with low titres.[27] More recently the Der f 16 gelsolin allergen with 47% IgE binding has been described.[28]

Der p 9 is a serine protease different to the trypsin and chymotrypsin group 3 and 6 allergens. It was shown to have very high IgE binding activity[29] but the same study also showed unusually high titres against the group 3. Further study is indicated.

The group 10 tropomyosin allergens are evolutionary conserved and cross-react amongst species such shell fish and parasites.[30] Probably for this reason the frequency of reactivity has varied from extremely high in Japan (80%)[8] and Zimbabwe (55%) to low in Europe.[31]

High molecular mass allergens are high IgE binding specificities. The 92/98 kDa paramyosin (group 11) allergens binds IgE in 80% of allergic subjects[9] and the 98 and 60 kDa chitinase enzymes (Der f 15 and 18) bind IgE from about 70% and 54% of allergic subjects.[32,33] The chitinases are important allergens for allergic dogs[32][33] and several studies

Table 1. House Dust Mite Allergens

Group	Biochemical function	MW cDNA * (SDS-PAGE)	Species**	IgE binding ***
1	cysteine protease	25 000	Dp, Df, Dm, Ds Em, Bt	80
2	(Niemann Pick C2 homologue)	14 000	Dp, Df, Ds, Em, Ld, Tp, Gd, As	80
3	trypsin	25 000 (30 000)	Dp, Df, Ds, Em, Bt	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, 29, 26 000)	Dp, Df, Ld	50
8	glutathione-S-transferase	26 000	Dp	20-40
9	collagenolytic serine protease	no cDNA (30 000)	Dp	90
10	tropomyosin	37 000	Dp, Df, Bt, Ld	50-95
11	paramyosin	96 000 (92, 98 000)	Df, Bt	80
12	unknown	14 000	Bt	50
13	fatty acid binding protein	15 000	Bt, Ld, As	10-20
14	vitellogenin/apolipophorin-like	177 000 (variable)	Df, Dp, Em	90
15	98K chitinase	62 500 (98, 105 000)	Df	70
16	gelsolin	55	Df	50
17	Ca binding EF protein	30	Df	35
18	chitinase	60 000	Df	55
19	anti-microbial peptide	7 000	Bt	10

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

** Species designated by initials: *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Euroglyphus maynei*. *Dermatophagoides siboney*, *Dermatophagoides microceaus*, *Lepidoglyphus destructor*, *Blomia tropicals*, *Tyrophagus putrecentiae*, *Glycophagus domesticus*, *Ascaris siro*

*** Binding frequency (% patients, variation due to different studies)

have shown that the group 1 and 2 allergens are not as allergenic[34,35]. The group 14 homologue of apolipophorin and vitellogenin, binds IgE in 80% of subjects allergic to *D. farinae*[10,13] and *D pteronyssinus* (authors unpublished). The allergen, first characterised as the 177 kDa M-17 protein, degrades in extracts or is processed to smaller peptides[10].

2. Glycyphagidae

The major allergen of *L. destructor* is Lep d 2.[36] Studies on other allergens are limited although homologues of the group 5, 7 and 10 have been described. Lep d 7 bound IgE at high frequency (62%) while the others were low.[37] Allergens corresponding to tropomyosin and α-troponin have been described but their IgE activity was low.[38] No suggestion of a group 1 homologue has been reported although these allergens react poorly in Western blotting and recombinant polypeptides must be correctly folded.

The major allergen reported for *B. tropicalis* is Blo t 5, which binds IgE in 70% of allergic subjects[39] with little cross-reactivity to Der p 5.[39,40] They do not however appear to be in high titre. No group 2 allergen has been described although Johannsen et al. reported that *B. tropicalis* extracts could block the binding of antibodies to Lep d 2.[41] Group 2 allergens of other species have been easy to detect so the absence is surprising. Recent studies have identified the binding of IgE to the group 11 paramyosin allergen (57% binding frequency).[42,43] Group 3 allergens have been described[44] and shown to bind IgE binding at a frequency of 57%.[45,46] Other IgE binding proteins which have been described are tropomyosin Blo t 10 (30% binding frequency),[47] Blo t 13 a fatty acid binding (10%)[48] and Blo t 12 (50%).[49]

Recently cDNA encoding a polypeptide with 35% homology to Der p 1 was described. The polypeptide bound IgE from 62% of sera from patients allergic to *B. topicalis* but did not bind IgE from patients allergic to *D. pteronyssinus*. The aller-

gen appears to be Blo t 1 but other cysteine proteases related to Der p 1 exist. It is nevertheless an important advance.

Component Resolved Diagnosis

Array technology can be used to measure IgE binding to large panels of recombinant and purified allergens.[50,51] It can identify the major specificities and eliminate cross reactivities such mite tropomyosin. For pyroglyphid mites it can identify non-group 1 and 2 allergic subjects and multisensitive patients. Pittner et al. have shown that patients allergic to Der p 1 and 2 plus other allergens have higher IgE immunoglobulin levels and multisensitivity compared to patients with responses largely restricted to the group 1 and 2.[26] Although this has not been reduced to utility, immunotherapy induces a less favourable response of patients with multiallergen specificities.[52] Lynch et al. have also reported more clinical symptoms in patients with IgE to multiple mite specificities.[53]

Polymorphisms

The group 1 and 2 allergens are both products of single genes[54,55] but are highly polymorphic.

Thirteen of the 20 sequences reported for Der p 1 have been unique.[56] Most of substitutions were sporadic with variations of 1-4 amino acids. Attention should be given to at least two details. Firstly the original sequence of Der p 1 contains a histidine at position 50 while environmental isolates have tyrosine. Secondly position 124 is either a valine or an alanine in an equal ratio. T-cell responses frequently recognise this region and responses to peptides with alanine and valine differ. A combination of the variants Der p 1. 0102 and Der p 1. 0105 could provide representative sequence.

Der p 2 has an evolutionary pattern of variation based on position 40, 47, 111 and 117. They are valine, threonine, methionine and glutamate in Der p 2.0101 and leucine, serine, leucine and glutamine in the most divergent sequence of Der p 2.0104.[56] T-cell cytokine responses induced by these variants are similar but variants with other substitutions could induce lower responses.[24] The 0101 variant bound less IgE although it was well correlated. As well as the aforementioned data from Australia and Taiwan, variants from Korea have been described and show the same pattern except that the 0104-like sequences were predominant.[57] The Der p 2.0104 variants also bound more IgE.

Polymorphisms have been described for Der f 2[55] and Lep d 2[58] and for the latter an isoallergen with a 13 amino acid variation. The IgE binding of Lep d 2.0101 was superior to Lep d 2.0201.

The group 3 allergens of Dermatophagoides sp. also show polymorphism with multiple amino acid changes.[59,60]

Summary

Mite allergy is not caused by exposure to mite extracts. The extracts contain non-allergenic antigens and lack effective concentrations of important allergens. The group 1 and 2 allergens of the Pyroglyphideae present in the extracts are important specificities for 80% of the allergic population but other allergens are important. The 20% of subjects without IgE antibodies to the group 1 and 2 allergens constitute a large population of allergic patients. Several high molecular weight allergens appear important and need further investigation. The polymorphisms of allergens in different regions must also be considered. Biochemical properties of allergens could have adjuvant activities but it should be borne in mind that animals respond to different major specificities than humans. *B. tropicalis* is abundant in highly populated tropical and subtropical regions. Recent studies have provided timely information on its major allergenic specificities but the group 1 and 2 specificities remains uncertain. Pyroglyphid and glycyphid mites often co-exist so reagents to identify the sensitising species are required.

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