

EDITORIAL

Towards defining the full spectrum of important house dust mite allergens

Tsai *et al.* [1] describe the cDNA cloning and sequencing of the group 11 house dust mite allergen, paramyosin. It is almost certainly the high molecular weight allergen of about 100 kDa noted to bind immunoglobulin (Ig) E frequently in several Western blotting studies [2–5]. Paramyosin and a discrete degradation product can account for bands representing allergens estimated to migrate as both 98 and 92 kDa proteins. It is also glycosylated which could produce further heterogeneity [6]. Data previously published for purified Der f 11 showed it bound IgE in the serum of 82% of mite-allergic subjects and gave dot-blot reactivities with the same staining intensity as reactivity to Der p 2 [6]. This and the ongoing studies of the M-177 (Mag 3) allergen [7,8] are important steps in defining the high molecular weight IgE binding specificities which have largely eluded the recent progress in the characterization of mite allergens. It is timely to discuss not only paramyosin but also some emerging features of the spectrum of allergens produced by *Dermatophagoides* mites. Some salient properties of the allergens are summarized in Table 1.

Early indications suggested that although many proteins produced by *Dermatophagoides* mites induced IgE antibody, the bulk of the allergenic activity resided in a small number of dominant allergens. This was particularly indicated by the finding that frequently over 50% of the IgE binding activity of mite extracts could be attributed to the group 1 and 2 allergens [9,10]. The picture has changed now that other allergens have been defined and produced in sufficient amounts to conduct studies with known and adequate quantities of allergen. The group 3 allergens from *D. pteronyssinus* and *D. farinae* bind IgE with a similar frequency and degree to the group 1 and 2 allergens [11,12] and in the studies reported for *D. farinae*, paramyosin [6], tropomyosin [13] and the allergen designated M-177 [7,8] have been found to bind IgE to a high degree and at a high frequency. Studies with *D. pteronyssinus* showed that the IgE binding to the Der p 9 collagenolytic allergen was similar to the binding to the group 1 allergens [11] and was not cross-reactive with the other serine proteases Der p 3 and Der p 6. The allergens Der p 5 and Der p 7 are interesting because although they only bind IgE in the serum of half the mite-allergic subjects, the strength of binding by responders is similar and often greater than the binding to the major allergen, Der p 2 [14,15]. Der p 4 and 8 allergens bind IgE at frequencies of about 40% but the strength of binding has not been compared with the binding to other allergens and the

group 6 chymotrypsins bind IgE at a frequency of 40% and at a lower level than the major allergens [16]. The allergens of *D. pteronyssinus* and *D. farinae* have 80–90% sequence identity so there is little reason to suspect any difference in the allergenicity of the homologous proteins from each species. There has been a discrepancy reported for the IgE binding of the group 10 allergens, where studies with Der p 10 have not shown the high frequency or strength of IgE binding reported for Der f 10 [17]. The study on Der f 10 was conducted in Japan and the study of Der p 10 was conducted in Europe so factors other than the nature of the allergens are likely to be important, particularly since these allergens have 99% amino acid sequence identity.

The group 1 and 2 allergens are found in abundant quantities in mite body extracts [18] while the group 3, 5, 7, 10 and the M-177 allergens are only minor or unstable constituents [7,13,19–21]. It is therefore not known how well the extracts represent the important allergens. The reasons for low concentrations in extracts could be several fold. The group 3 allergens are known to be major constituents of mite faeces despite their paucity in body extracts [19] and degradation products have been shown for the group 7 allergens [21] and M-177 allergens [8]. Additionally, the information available would suggest that mite bodies are not the main source of allergens. The data obtained by Tovey *et al.* for Der p 1 [22] shows that almost all the allergen in the environment is of faecal origin and assuming a steady state concentration, 15% of the Der p 1 in the environment would have been produced fresh each day. In addition to components of bodies and faeces, mite secretions could release allergens into the environment. For example, the group 2 allergens are homologues of proteins produced by the epithelial cells of respiratory and epididymal surfaces [22] and the M-177 allergen is, amongst other tissues, found in the lining of the oesophagus and gut [7]. Likewise, although paramyosin is a muscle protein it has been found as an abundant non-filamentous protein in lipid bodies of the tegument and oesophageal lining of *Schistosomes* [23], a phenomenon which may explain why it can be used as a vaccine. The source of mite paramyosin would therefore be of interest and is discussed by Tsai *et al.* [1]. The concentration in whole mite extracts appears to be substantial as judged by the strong immunostaining with a monoclonal antibody in Western blotting of mite extract compared with the staining of purified protein [6].

The existence of many proteins with a high frequency of

Table 1. House dust mite allergens^a

Allergen	M _w (kDa)	Function	IgE binding %	IgE binding strength ^b	Concentration in extracts (μg/mL)
Group 1	25	Cysteine protease	70–90	High	10–100 ^c
Group 2	14	Epithelial secretion?	70–90	High	5–50 ^c
Group 3	25 ^d	Trypsin	70–90	High	< 1 ^e
Group 4	57	Amylase	45	?	?
Group 5	15	?	50 ^f	High	< 1
Group 6	25	Chymotrypsin	40	Moderate	?
Group 7	31, 29, 26	?	50 ^f	High	< 1
Group 8	26	Glutathione-S-transferase	40 ^f	?	Low ^g
Group 9	30 (PAGE)	Collagenolytic serine protease	90	High	?
Group 10	37	Tropomyosin	50–95	Variable–high	Low ^g
Group 11	92, 98	Paramyosin	80	High	High ^h
Group 12	14	?	50 ^f	?	?
Group 13	15	Fatty acid binding protein	10 ^f	Usually low	?
Mag 1	39 (PAGE)	?	30 ^f	?	?
Mag 3 (M177)	177 (PAGE)	?	70	High	0–30 ⁱ
Mag 29	70	hsp 70	10 ^f	?	?

(a) data reviewed in [37]; (b) binding of responders in comparison with major allergens; (c) approximate range from [18]; (d) migrates at 30 kDa by PAGE; (e) 0.2 μg/mg protein [19]; extracts approximately 3 mg/mL protein [18]; (f) data for recombinant allergen only; (g) only small quantities can be isolated from extracts; (h) see text; (i) estimated from data showing that degradation products detected in some extracts as 0.9% of protein but not in others [8].

IgE binding (13 allergens with 40% or more) agrees with the banding patterns found in Western blotting studies. The advance brought about by the recent molecular analyses has been to show that the binding is often quantitatively large and that the blotting patterns are produced by reactivities to a series of unrelated allergens. The group 7 allergens do, however, show different and variable molecular weights due to secondary modifications [21] and the degradation products of M-177 produce a variety of IgE binding peptides [8]. Now that the different allergens have been defined it will be possible to produce them in sufficient quantities to determine their role in the allergic responses. Already the results with IgE binding show that many of them could be more important than first indicated. The evidence that extracts may be a poor representation of the allergens which cause disease raises the possibility that some investigations have been compromised, because only extracts or the major allergens of extracts were used as a source of allergens. The group 1 and 2 allergens are, however, found in high levels in extracts and are important allergens for 70–90% of subjects so, at the broadest level, they probably act as markers of sensitization. There has usually been good correlation between T-cell responses to the group 1 and 2 allergens [5,24], between the group 2 and M-177 allergens [25] and, as reported in preliminary form, between the group 1 and group 7 allergens [26]. The data, however, are quite limited

and comparative studies of the allergens appear critical. Such studies could also be important for improving immunotherapy. Therapy using allergen preparations containing a balanced and adequate concentration of the important allergens could be more effective and consistent. New strategies of immunotherapy such as the use of peptides or, recombinant and genetically modified recombinant proteins, are critically dependent on determining the important allergens.

Although there is no direct method for determining the role of the different allergens in disease, recombinant or purified natural allergens could be used for surrogate studies. IgE binding activity can be determined either in absolute quantities or as a comparison with the titres to purified markers such as the group 1 or 2 allergens. Note that since it appears that mite extracts do not contain adequate quantities of many allergens, the determination of IgE binding activity as a percentage of binding activity of mite extracts may have limited value. Cytokine release by peripheral blood mononuclear cells stimulated by allergens can be readily determined by ELISA for IFN γ and IL-5 and RT-PCR can be used to estimate IL-4 synthesis. While allergens which induce high IgE responses and sensitize cells for TH2 cytokine production are likely to be active participants in the production of disease, it could be argued that the induction of allergic inflammation has different dose–response or kinetic characteristics to sensitization. The determination of the

importance of the individual allergens in the effector phase of the inflammation is therefore a challenge. Some progress could be made by measuring the amount of allergen inhaled, although information on the bioactivity of the different allergens and the active particle size would be required. At the T-cell level it is possible that advances in cell sorting and cytokine detection technology may be employed. Activation markers such as the IL-12 β_1 chain [27] and the chemokine CCR3 receptor [28] could be used to purify T cells recently activated by allergen, so their cytokine reactivity and other properties, such as the presence of P- and E-selectins [29], can be ascertained. Both circulating cells and cells isolated from inflammatory sites could be examined.

Whether or not the biological activity of allergens enhances their ability to sensitize or produce inflammation remains to be proven despite the existence of several plausible mechanisms. As discussed by Tsai *et al.* [1] paramyosin is not an enzyme unlike many of the other mite allergens, and this possibly shows that proteins do not require an adjuvant bioactivity to be allergens. However, although paramyosin is a core structural protein of thick filaments of invertebrate muscles, it may have additional functions as a secreted non-filamentous protein [23]. Paramyosin from other species has been shown to block the binding of the C1q complement component [30] and in this manner could interact with immune responses. Additionally, the possibility that paramyosin is presented in lipid bodies at exposed surfaces of the mite (by analogy with studies in helminths) may add adjuvant activity. Perhaps more importantly, it is possible or even likely that the exposure to paramyosin would be accompanied by the exposure to other allergens or mite proteins with enzymatic activity.

People infected with the trematode and nematode helminths, *Schistosoma mansoni* [31] and *Onchocerca volvulus* [32] produce IgE antibodies to paramyosin. Anti-paramyosin antibodies have shown extensive cross-reactivity between such disparate invertebrates as insects, spiders, earthworms and helminths [33,34] so the possibility that the mite antiparamyosin IgE antibodies are cross-reactivities must be considered. The studies of Tsai *et al.* showed that the anti-Der f 11 IgE antibodies were found in mite-allergic subjects with high IgE binding to Der p 2 and that they were not found in skin test-negative subjects [6]. Although this shows that the mite-allergic subjects have high IgE anti-Der f 11 titres, the skin tests used to select allergic patients were performed with mite extracts which contain Der f 11. The presence of cross-reactivity in people not sensitized by mites has therefore not been excluded. A larger study on IgE binding specificities of subjects first selected by reactivity to Der f 11 is indicated.

Although antibodies to paramyosins of many species have shown cross-reactivity, their level of amino acid

sequence identity is modest. The paramyosin of the trematode *Schistosoma japonicum* for example shows 32% identity with paramyosin from *Drosophila melanogaster* and 33% with paramyosin from the nematode *Onchocerca volvulus*. Paramyosin from the nematodes has 50% identity with *Drosophila melanogaster* paramyosin. The Der f 11 has 60% identity with *Drosophila melanogaster* paramyosin, 50% identity with the nematode paramyosin and 34% with the trematode paramyosin. This pattern is consistent with that of an arthropod and the degree of identity is of similar magnitude to that found amongst the species which have been shown to have antigenically cross-reactive paramyosins. The level of identity of mite paramyosin with paramyosins of other species, however, is no greater than that found for the inter-species identities of most other mite allergens, for which there is little indication of cross-reactivity. The cysteine protease group 1 allergens have 30% identity to arthropod and helminth homologues, the trypsins show 45% identity to mammalian and other arthropod trypsins and the chymotrypsins 30%. The paramyosin may therefore have a greater conservation of tertiary structure or perhaps particular regions are both conserved and antigenic. Some evidence for the later has been reported [33]. It is important to emphasize that any broad cross-reactivity with mite paramyosin remains to be demonstrated. The group 10 allergen tropomyosin is, however, known to be cross-reactive amongst a variety of invertebrates [17]. Tropomyosin which regulates calcium flux in muscles is not restricted to invertebrates and has a highly conserved amino acid sequence. Der p 10 has 76% identity to tropomyosin of *Drosophila melanogaster* and 59% identity to mammalian tropomyosin. It may be useful in certain circumstances to use cocktails of defined allergens or to remove potentially cross-reactive antigens to avoid misdiagnosis. Lynch *et al.*, for example, showed that in a tropical area with a high level of helminth infection, while skin test and IgE binding activity to mite extracts correlated poorly with allergic disease, IgE antibodies to recombinant Der p 2 had a strong correlation [35].

The identification of the high molecular weight Der p 11 as a major allergen is an important step in characterizing the complete spectrum of mite IgE binding specificities. This paramyosin allergen can explain the frequent IgE binding detected in Western blotting in the 95–100 kDa region. An important question remaining is whether or not additional frequently recognized high molecular weight bands reported by some investigators [36] are separate entities, modifications of paramyosin or degradation products of the 177 kDa M-177 allergen. It is extremely difficult to compare the details of different Western blotting studies, particularly with high molecular weight allergens which are poorly resolved by the standard polyacrylamide gel electrophoresis conditions employed. Definitive work with purified and cloned

allergens is required. The high molecular weight allergens are recognized at high frequency by IgE from the serum of children [5,36] so despite their current Cinderella status they may be important determinants in the development of allergic sensitization.

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W. R. THOMAS

W. SMITH

TVW Telethon Institute for

Child Health Research

Division of Molecular Biology

PO Box 855

West Perth WA 6872

Australia