

EDITORIAL

Mite allergens groups I-VII. A catalogue of enzymes

Yasueda *et al.* have now described how chymotrypsin is one of the relatively small number of house dust mite allergens which show IgE binding with 40% or more of allergic sera [1]. It is timely to look at why this and other allergens produced by the house dust mite are enzymes and to summarize the very significant recent progress made in characterizing mite allergens. The answer to the enzyme question which is relevant to allergens from all sources will be complex but it should not be dismissed. Only a small number of proteins regularly induce allergic responses and some proteins such as the group I and II mite allergens are clearly more allergenic than others. This can be seen by comparing protein profiles of electrophoresed mite extracts with corresponding IgE immunoblots [2,3]. Regular exposure of experimental animals to antigen leads to transient IgE responses and tolerance, not a persistent allergic state [4], so it is probable that other influences participate in allergic sensitization such as genetic and environmental factors, age of exposure and biological properties of the allergen. The suggestion that an enzymatic or other biological activity may enhance or be required for an antigen to induce a persistent allergic response is supported by the finding that many environmental allergens have such activity and by the high degree of sensitization produced by enzymes in commercial or domestic and pharmaceutical use. There is also a myriad of plausible mechanisms which an enzyme could use to augment allergic sensitization.

The group I mite allergens which react with IgE in most mite-allergic patients and often account for 50% or more of the anti-mite specificities [5,6] are cysteine proteases [7] and the group III allergens [8] which have been reported to react at a similar frequency but at a lower level are trypsins [9]. Chymotrypsin can now be added to the list along with *Der p* IV which is amylase [10] and evidence is accumulating that the group II allergens are lysozymes [11]. One of the intriguing aspects which arose when *Der p* I was recognized as a cysteine protease was that its homologue from the papaya, papain, had previously been documented to induce sensitization in factory workers who inhaled papain dust during its production for a meat tenderizer [12] or in consumers [13]. Similar sensitivities have been reported to develop in the production or use of other enzymes such as subtilisin in detergents [14], chymopapain used for treatment of slipped discs [15] and amylase in bakers allergy [16]. Environmental allergens with well-defined enzyme activities other than mite allergens are alcohol dehydrogenase from mould [17] and

Asp f I [18] whose cytotoxic activity is mediated by catalytic cleavage of ribosomal RNA [19]. Most of the venom allergens are also enzymes [20]. Other allergens whose possible molecular functions could be hypothesized to assist sensitization are *Mus m* I and *Fel d* I. The major urinary proteins, α 2 μ globulins, which comprise the mouse allergen *Mus m* I have extensive sequence homology with a family of lipid binding proteins appear to be transporters with ligand activity for a variety of lipid molecules possibly including pheromones [21]. One of the chains of cat allergen *Fel d* I has sequence homology with uteroglobulin an anti-inflammatory which both inhibits phospholipase A2 and has steroid binding functions [22].

The function or mechanism of action of most of the aeroallergens from grass and weeds is not known so it is difficult to give many examples of allergens which would lack an adjuvant biological activity. *Chi* I from the non-biting midge *Chironomus thummi thummi* [23] is, however, a potent aeroallergen and since it is a complex of haemoglobin molecules is unlikely to have any such activity. Another probable example is the actin-binding profilin, *Bet v* II, from birch pollen [24]. Some food allergens without enzyme activity are the ion transport proteins α -lactalbumin from milk, *Gad c* I from cod and the allergens parvalbumin, ovalbumin and conalbumin from eggs [20]. Although the chironomid haemoglobin shows that not all allergens are enzymes it is possible that it could be presented in a special way including in association with an enzyme. Certainly attempts to reproduce prolonged allergic responses in experimental animals have been a problem. The outcome of such experiments has been transient IgE production followed by tolerance unless the exposure was coupled with some treatment to upset the homeostasis of the immune system. Such co-stimuli included the adjuvant aluminium hydroxide, pertussigen, oestradiol and nitrogen dioxide [4]. The mechanisms by which biological activity could influence the immune system are diverse which is in keeping with the range of enzymes which become allergens. They may act by altering epithelial permeability or the cellular or matrix ligands affecting cell migration or directly on cells. Signal transduction could be caused for example by allosteric changes or cross-linking produced by hydrophobic interactions between or within modified receptors. The interaction of the enzymes with substrate or ligands such as protease inhibitors could also influence the presentation and processing of antigen. In this respect the administration of antigen coupled to the protease inhibitor α_2 macroglobulin has already been shown to increase the

Table 1. *D. pteronyssinus* allergens

	MW (kD or Mr)	Function	IgE binding frequency (% sera)	Sequence
<i>Der p I</i>	25	Cysteine protease	80-100	cDNA [7]
<i>Der p II</i>	14	Probably lysozyme	80-100	cDNA [32]
<i>Der p III</i>	28/30	Trypsin	70-100	N-term [9]
<i>Der p IV</i>	56-60	Amylase	40	N-term [10]
<i>Der p V</i>	15	?	40	cDNA [30]
<i>Der p VI</i>	25	Chymotrypsin	40	N-term [1]
<i>Der p VII</i>	22,26,28	?	40	cDNA [31] (provisional)

* Values vary with different reports depending on methods, extracts and types of patients.

proliferative response of T cells to antigen [25]. The production of proteases and protease inhibitors by T cells, NK cells and mast cells indicates that they may play a fundamental part in immunoregulation. Finally, Finkelmann has shown that immunizing mice with active papain induces 10-30 times more IL-4 and other TH₂ lymphokines than the injection of non-activated papain [26].

An alternative view is however that since many proteins are enzymes then many enzymes will be allergens. The fact that the allergens which are enzymes have a wide variety of activities supports this contention by showing there is no special pathway to allergic sensitization. For the mite the special property of the enzymes may simply be that they are secreted for digestion and become incorporated in the dung ball which facilitates their dissemination and delivery to the mucosa at high concentration. *Der p I* has for example been documented to be present in dung balls at the very high concentration of 10 mg/ml [27]. The probability that the digestive enzymes of the mite would be allergens has been used very effectively, particularly by Stewart, to separate a number of hydrolytic enzymes by substrate chromatography and demonstrate IgE binding [28]. In the case of amylase and trypsin this has led to the purification and N-terminal sequencing of significant allergens [9,10]. Yasueda *et al.* have also taken full advantage of substrate affinity purification by using this to incontrovertibly distinguish chymotrypsin from other allergens including the group III trypsins [1].

The publication by Yasueda *et al.* [1] describes one of a number of significant mite allergens characterized recently so it is perhaps time to take stock of the progress in this area. It is especially useful that the IgE binding activity of chymotrypsin was described in comparison with *Der p I* and *Der p II*. The group I and II bound IgE in

almost 100% of the sera and their strong reactivity was confirmed. The reactivity to chymotrypsin was less frequent, 40%, and considerably weaker. Studies with Western blotting [2] and those of Stewart *et al.* [9] with purified mite trypsin have shown that nearly all patients recognize the group III trypsin allergens but like chymotrypsin the degree of reactivity was considerably less than to *Der p I* [9]. It is possibly this lower reactivity which resulted in the conclusion of Heymann [8] that only 17% of patients had reactivity to *Der f III* since subsequent dot blot immunoassays have shown that at least 70% of allergic sera react with this allergen [29]. Other characterized allergens with binding frequencies similar to chymotrypsin are *Der p IV* and the '15 K' allergen described from the analysis of a cDNA clone [30] and which is to be designated *Der p V* by the allergen nomenclature committee. A further allergen with the same IgE binding frequency has similarly been described from a cDNA clone [31]. The clone encoded a 22 kD protein but interestingly antibodies to the cloned allergen reacted with Mr 28, 26 and 22 K components in mite extracts as determined by Western blotting. The chymotrypsin would now be designated *Der p VI* and when published the allergen described by Shen will be *Der p VII*. Table 1 summarizes these developments.

Studies with Western blotting and other electrophoretic techniques are difficult to interpret because of variations in the allergen concentrations of extracts, the influence of the denaturing conditions on the allergenicity and the lack of quantitation. There are also variations in the migration of allergens due to different conditions used in different laboratories and run to run variations. They are nevertheless interesting to compare with the results obtained with purified molecules. The recent study of Ford *et al.* [3] showed that in a series of 14 patients eight

showed reactivity to one to two bands, six with three to six bands and three with a larger number including one with reactivity to at least 13 components. Tovey and Baldo [2] identified fractions with high frequency reactivity. Fractions of Mr 95, 54–56, 32–30, 26, 16 and 15 K had over 40% or greater reactivity while most other fractions showing less than 10%. The Mr 16 K band in their conditions corresponds to *Der p* II (MW 14131) and the 15 K to *Der p* V defined by molecular cloning [30]. The 32/30 K fractions are *Der p* III and it is tempting to speculate that the 54–56 K bands correspond to *Der p* IV. Chymotrypsin would now be responsible for reactivities in the 25–27 K regions along with *Der p* I which does not react well by Western blotting and the proposed *Der p* VII.

Although unproven, the allergens characterized to date could account for most of the high frequency reactivities found by Western blotting. The most obvious task ahead is to document the importance of these allergens compared to each other and to whole mite extracts both in terms of IgE binding and T cell activation. It will be possible to make a comparison of immune responses to the major and less significant allergens and the structural knowledge of the allergens will provide a base for developing recombinant and peptide-based allergens or modified allergens for immunotherapy to what are probably all the important allergens. The use of a trivalent preparation of purified allergens from timothy grass for immunotherapy has already been examined by Osterballe [33] and it was not effective but recent data on the grass allergens suggest the preparation would be deficient in the group I grass allergen and should be re-evaluated. To conclude on a different note, the sequence for most of the *Eur m* I allergen from *Euroglyphus maynei* has been obtained from genomic DNA [34]. The sequence is about 80% homologous to both *Der f* I and *Der p* I as they are to themselves. Residues conserved in the *Dermatophagoides* allergens tend to be conserved in *Eur m* I and non-conserved residues were most often the same as one on either *Der f* I or *Der p* I. Since it has been reported that this species can reach at-risk levels in 25% of homes in some regions [35] there is a need to develop *E. maynei* specific allergen constructs, for example species-specific peptide epitopes to determine the importance of primary sensitization to this mite.

References

- 1 Yaseuda H, Mita H, Shida T, Ando T, Sugiyama S, Yamakawa H. Allergens from *Dermatophagoides* mites with chymotrypsin activity. *Clin Exp Allergy* 1993; 23:384–90.
- 2 Tovey ER, Baldo BA. Comparison by electroblotting of IgE-binding components in extracts of house dust mite bodies and spent mite culture. *J Allergy Clin Immunol* 1987; 79:93–102.
- 3 Ford SA, Tovey ER, Baldo BA. The spectrum of low molecular weight house dust mite (*Dermatophagoides pteronyssinus*) allergens with emphasis on *Der p* II. *Clin Exp Allergy* 1989; 20:27–31.
- 4 Holt PG, McMenamin C. Defence against allergic sensitization in the healthy lung: the role of inhalation tolerance. *Clin Exp Allergy* 1989; 19:255–62.
- 5 Chapman MD, Platts-Mills TAE. Purification and characterization of the major allergen from *Dermatophagoides pteronyssinus*-antigen P1. *J Immunol* 1980; 125:587–92.
- 6 Van der Zee JS, Van Swieten P, Jansen HM, Aalberse RC. Skin tests and histamine release with P1-depleted *Dermatophagoides pteronyssinus* body extracts and purified P1. *J Allergy Clin Immunol* 1988; 81:884–95.
- 7 Chua KY, Stewart GA, Thomas WR *et al.* Sequence analysis of cDNA coding for a major house dust mite allergen, *Der p* I. Homology with cysteine proteases. *J Exp Med* 1988; 167:175–82.
- 8 Heymann PW, Chapman MD, Aalberse RC, Fox JW, Platts-Mills TAE. Antigenic and structural analysis of group II allergens (*Der f* II and *Der p* II) from house dust mites (*Dermatophagoides* spp). *J Allergy Clin Immunol* 1989; 83:1055–67.
- 9 Stewart GA, Ward LD, Simpson RJ, Thompson PJ. The group III allergen from the house dust mite *Dermatophagoides pteronyssinus* is a trypsin-like enzyme. *Immunology* 1992; 75:29–35.
- 10 Lake FR, Ward LD, Simpson RJ, Thompson PJ, Stewart GA. House dust mite-derived amylase: allergenicity and physicochemical characterization. *J Allergy Clin Immunol* 1991; 87:1035–42.
- 11 Stewart GA, Bird CH, Thompson PJ. Do the group II allergens correspond to lysozyme? *J Allergy Clin Immunol* 1992; 90:141–2.
- 12 Baur X, Konig G, Benize K, Fruhmman G. Clinical symptoms and results of skin test, RAST and bronchial provocative tests in thirty three papain workers. *Clin Allergy* 1982; 12:9–16.
- 13 Mansfield LE, Ting S, Haverly RW, Yoo TJ. The incidence and clinical implications of hypersensitivity to papain in an allergic population, confirmed by blinded oral challenge. *Ann Allergy* 1985; 55:541–43.
- 14 Belin LG, Norman PS. Diagnostic tests in the skin and serum of workers sensitised to *Bacillus subtilis* enzymes. *Clin Allergy* 1977; 7:55.
- 15 Grammar LC, Patterson R. Proteins: chymopapain and insulin. *J Allergy Clin Immunol* 1984; 74:635–40.
- 16 Baur X, Fruhmman G, Haug B, Rasche B, Reihner W, Weiss W. Role of *Aspergillus* amylase in baker's asthma. *Lancet* 1986; i:43.
- 17 Shen HD, Choo KB, Lee NH *et al.* The 40-kilodalton allergen of *Candida albicans* is an alcohol dehydrogenase: molecular cloning and immunological analysis using monoclonal antibodies. *Clin Exp Allergy* 1991; 21:675–81.
- 18 Arruda LK, Platt-Mills TA, Fox JW, Chapman MD. *Aspergillus fumigatus* allergen I, a major IgE-binding protein

- is a member of the mitogillin family of cytotoxins. *J Exp Med* 1990; 172:1529-32.
- 19 Fando JL, Alaba I, Escarmis C, Fernandez-Luna JL, Mendez E, Salinas M. The mode of action of restrictocin and mitogillin on eukaryotic ribosomes. Inhibition of brain protein synthesis, cleavage and sequence of the ribosomal RNA fragment. *Eur J Biochem* 1985; 149:29-34.
 - 20 Marsh DG, Goodfriend L, King TP, Lowenstein H, Platts-Mills TA. Allergen nomenclature. *J Allergy Clin Immunol* 1987; 80:639-45.
 - 21 Mancini MA, Majumdar D, Chatterjee B, Roy AK. $\alpha 2\mu$ -globulin in modified sebaceous glands with pheromonal function: localization of the protein and its mRNA in preputial, meibomian, and perianal glands. *J Histochem Cytochem* 1989; 37:149-57.
 - 22 Morgenstern JP, Griffith IJ, Brauer AW *et al.* Amino acid sequence of *Fel d* I, the major allergen of the domestic cat: protein sequence analysis and cDNA cloning. *Proc Natl Acad Sci USA* 1991; 88:9690-4.
 - 23 Baur X, Aschauer H, Mazur G, Dewair M, Prelicz H, Steigemann W. Structure, antigenic determinants of some clinically important insect allergens: chironomid hemoglobins. *Science* 1986; 233:351-4.
 - 24 Valenta R, Duchene M, Pettenburger K *et al.* Identification of profilin as a novel pollen allergen: IgE-autoreactivity in sensitized individuals. *Science* 1991; 253:557-8.
 - 25 Osada T, Noro N, Kuroda Y, Ikai A. Murine T cell proliferation can be specifically augmented by macrophages fed with specific antigen: alpha 2-macroglobulin conjugates. *Biochem Biophys Res Commun* 1987; 146:26-31.
 - 26 Finkelman FD, Urban JF Jr. Cytokines: making the right choice. *Parasitology Today* 1992; 8:311-14.
 - 27 Tovey ER, Chapman MD, Platts-Mills TAE. Mite faeces are a major source of house dust allergens. *Nature* 1981; 289:592-3.
 - 28 Stewart GA, Lake FR, Thompson PJ. Faecally derived hydrolytic enzymes from *Dermatophagoides pteronyssinus*: physicochemical characterisation of potential allergens. *Int Arch Allergy Appl Immunol* 1991; 95:248-56.
 - 29 Thomas WR, Chua KY, Smith WA. Molecular polymorphisms of house dust mite allergens. *Exp Appl Acarology* 1992; 16:153-64.
 - 30 Tovey ER, Johnson MC, Roche AL, Cobon GS, Baldo BA. Cloning and sequencing of a cDNA expressing a recombinant house dust mite protein that binds human IgE and corresponds to an important low molecular weight allergen. *J Exp Med* 1989; 170:1457-62.
 - 31 Shen HD, Chua KY, Lin KL, Hsieh KH, Thomas WR. Molecular cloning of a house dust mite allergen with common antibody binding specificities with multiple components in mite extracts. *Clin Exp Allergy* 1993; in press.
 - 32 Chua KY, Doyle CR, Simpson RJ, Turner KJ, Stewart GA, Thomas WR. Isolation of cDNA coding for the major mite allergen *Der p* II by IgE plaque immunoassay. *Int Arch Allergy Appl Immunol* 1990; 91:118-23.
 - 33 Osterballe O. Immunotherapy with grass pollen major allergens. Clinical results from a prospective 3-year double blind study. *Allergy* 1982; 37:379-88.
 - 34 Kent NA, Hill MR, Keen JN, Holland PWH, Hart BJ. Molecular characterisation of group I allergen *Eur m* I from house dust mite *Euroglyphus maynei*. *Int Arch Allergy Appl Immunol* 1992; 99:150-2.
 - 35 Colloff MJ, Stewart GA, Thompson PJ. House dust mite acarofauna and *Der p* I equivalent in Australia: the relative importance of *Dermatophagoides pteronyssinus* and *Euroglyphus maynei*. *Clin Exp Allergy* 1991; 21:225-30.

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