

EAACI/DGAI Symposium Reviews

Recombinant mite allergens

New technologies for the management of patients with asthma

Chapman MD, Smith AM, Vailes LD, Arruda LK. Recombinant mite allergens.

Allergy 1997; 52: 374–379. © Munksgaard 1997.

The introduction of molecular cloning techniques has led to advances in allergen identification and sequencing, production of recombinant allergens, identification of B-cell and T-cell epitopes, and tertiary structural analysis of allergen molecules. Over 10 groups of mite allergens have been cloned from *Dermatophagoides* spp., as well as several homologous allergens from *Euroglyphus maynei*, *Lepidoglyphus destructor*, and *Blomia tropicalis*. The availability of these allergens has made it feasible to consider their use for both diagnostic and therapeutic purposes. Several recombinant *Dermatophagoides* allergens show comparable reactivity on skin testing and in serologic assays to natural allergens, and cocktails of the recombinant proteins could be used as diagnostic reagents. New technologies have been developed for detection of allergen-specific IgE and for environmental allergen detection using rapid diagnostic tests. Novel approaches to immunotherapy are also being investigated, including T-cell-peptide-based vaccines, allergen variants which lack IgE reactivity, and naked DNA vaccines. The application of allergen biotechnology should lead to improvements in the management of mite-allergic patients with asthma and represents a logical step toward reducing asthma mortality and morbidity.

**M. D. Chapman, A. M. Smith,
L. D. Vailes, L. K. Arruda**

Asthma and Allergic Diseases Center,
Departments of Medicine and Microbiology,
University of Virginia Health Sciences Center,
Charlottesville, Virginia, USA

Key words: allergens; asthma; biotechnology;
house-dust mites; immunotherapy.

Martin D. Chapman, PhD
Asthma and Allergic Diseases Center
Box 225
University of Virginia HSC
Charlottesville, VA 22908
USA

Accepted for publication 17 December 1996

The application of molecular biology techniques to allergen identification and analysis has made a major impact over the past 5 years, especially for dust-mite allergens. Over 10 allergens have now been cloned from the genus *Dermatophagoides*, and many have been expressed in bacteria or yeast as immunoreactive recombinant proteins. These allergens include the established major allergens (e.g., Der p 2), as well as new allergens that have been identified and defined as a result of cloning studies (e.g., Der p 5 and Der p 7). Amino acid sequence data have been used to derive the function of mite allergens, through sequence similarity searches of protein databases, and to design experiments to define epitopes recognized by IgE (and IgG) antibodies and by T cells of allergic patients (1–3). Homology searches and molecular modeling have been used to predict three-dimensional allergen structure, and definitive structures are being determined by nuclear magnetic resonance

spectroscopy or X-ray crystallography using recombinant allergens (4). For the clinician, the “bottom line” in these studies is the development of new diagnostic products and new immunotherapeutic approaches to allergy treatment (Table 1).

On a worldwide basis, mites are the most important allergens associated with asthma, and, in spite of the extensive availability of antiasthma drugs over the past 20 years, the mortality and morbidity of asthma have continued to increase in Western populations. Knowledge of the molecular biology and immune response to mite allergens will allow the inflammatory mechanisms involved in bronchial hyperreactivity to be established and will provide a rational basis for developing immunotherapies and allergen-avoidance procedures. This review describes recent progress in the molecular biology of mite allergens and outlines the clinical applications of “allergen biotechnology”.

Table 1. Impact of molecular biology on research and clinical practice in allergic disease

- Identification and cloning of allergens
- Sequence homology searches – biologic function
- Production of recombinant allergens
- Identification of antigenic determinants
 - Site-directed mutagenesis (IgE Ab)
 - Derivation of T-cell clones and epitope mapping
 - Analysis of allergen-specific immune response
- Molecular modeling/three-dimensional structure
- **Development of new diagnostic and therapeutic products**

Molecular biology

Mite allergens that fulfill the criteria for inclusion in the systematic WHO/IUIS allergen nomenclature are listed in Table 2. This list of 10 allergens accounts for most of the IgE-binding bands originally identified by crossed immunoelectrophoresis or immunoblotting techniques (5). Most patients have strong IgE responses to the group 1 and group 2 allergens, but the prevalence and magnitude of IgE antibody responses to the other allergens are usually lower. However, serologic analyses of the prevalence of IgE antibody responses may vary according to the sensitivity of the IgE assay used and on how the patient population is selected. Some studies have described patients who have high levels of IgE to other allergens; e.g., Der p 7 or Der p 10 (2, 6).

As a result of sequence homology searches, the biologic function of several mite allergens has been established. The allergens include several enzymes, such as cysteine and serine proteases (Der p 1, Der p 3, Der p 9), amylase (Der p 4), and glutathione transferase (Der p 8), as well as allergens of unknown function (Der p 5 and Der p 7) (7–10). The proteases are excreted at high concentration in mite feces, and protease activity has been demonstrated in mite extracts and in house-dust extracts (11). The group 2 allergens have homology with a human epididymal protein, and it has been

suggested that these allergens are produced by male mites as part of the reproductive process (12). The observation that several mite allergens are proteases led Stewart and colleagues to propose that the protease activity plays an intrinsic role in enhancing or initiating IgE antibody responses. Support for this hypothesis comes from recent data showing that purified Der p 1 can cleave CD23 on B-cell lines, and that both Der p 1 and Der p 9 proteases cause detachment of epithelial cell monolayers *in vitro* and release of proinflammatory cytokines from human bronchial epithelial cells (11–13). Thus, these proteases could enhance passage of allergen molecules through the respiratory mucosa and upregulate IgE production through their action on CD23.

In addition to *Dermatophagoides* species, major allergens have also been cloned from *Euroglyphus maynei*, *Lepidoglyphus destructor*, and *Blomia tropicalis*. Extracts of these species are not commercially available, and recombinant allergens may provide the best approach to producing their allergens. Homologs of the *Dermatophagoides* group 1 and group 2 allergens have been amplified from *E. maynei* cDNA and the major *L. destructor* allergen, Lep d 2, shows ~40% sequence identity to *Dermatophagoides* group 2 (15, 16). Allergens produced by *Dermatophagoides* spp. were not detected by immunoassay in *B. tropicalis* extracts, and immunoabsorption and RAST inhibition studies have suggested that *Blomia* allergens are largely species specific (17). Molecular cloning has identified a series of *B. tropicalis* allergens, one of which shows 40% amino acid sequence homology to Der p 5 (18). The prevalence of IgE antibody to this allergen, Blo t 5, is 60–70% in patients from tropical countries (e.g., Brazil, Colombia, Singapore), who may be exposed to high levels of *B. tropicalis* in their homes. Other *B. tropicalis* allergens include a troponin and several allergens of 15–25 kDa which have been partially characterized (18–20). Because *B. tropicalis* is not found in temperate climatic regions, analysis of IgE responses to the group 5 allergens in exposed and nonexposed patients provides a useful model for comparing antigenic cross-reactivity between *Blomia* and *Dermatophagoides* spp. A surprisingly large number of patients (~60%) exposed primarily to *Dermatophagoides* allergens (e.g., from the UK) give positive skin tests to *B. tropicalis* extracts, suggesting allergenic cross-reactivity. However, most of these patients have no detectable IgE antibody to Blo t 5, in contrast to patients from South America, who show positive IgE antibodies to both group 5 allergens (21, 22). This suggests that there are other cross-reacting allergens in *Blomia* and *Dermatophagoides* spp. that remain to be identified.

Table 2. Purified allergens from *D. pteronyssinus* and *D. farinae*

Allergen	Prevalence of IgE Ab	MW (kDa)	Biologic function
Group 1	80–90%	25	Cysteine protease
Group 2	> 90%	14	Epididymal protein
Group 3	~60%	28–30	Serine protease (trypsin)
Der p 4	40%	60	Amylase
Group 5	40%	14	Unknown
Der p 6	40%	25	Chymotrypsin
Group 7	50%	22	Unknown
Der p 8	40%	26	Glutathione-S transferase
Der p 9	> 60%	30	Collagenolytic serine protease
Group 10	> 80%	36	Tropomyosin

Emerging diagnostic technologies

Recombinant allergen diagnostics

With the exception of the group 1 allergens, most of the recombinant mite allergens that have been produced show strong reactivity to IgE antibodies, as judged by serology and by immediate skin testing. Group 1 allergens produced in bacteria or yeast show partial reactivity to IgE antibodies as compared to the natural allergen, whereas there is an excellent correlation between IgE binding to natural or recombinant group 2 allergens. Similarly, recombinant group 5 and group 7 allergens show strong reactivity to IgE antibody (the natural molecules have not been isolated as yet, and the recombinants are the sole source of the allergens). These results suggest that it would be possible to use a "cocktail" of three or four recombinant mite allergens for diagnostic purposes, either for skin testing or *in vitro* assays. We are carrying out a large, multicenter skin test study in several parts of the world and have currently tested over 100 patients with recombinant group 5 allergens. Patients from Brazil and Singapore give positive skin tests to both allergens, correlating with serum IgE antibody responses (22, 23). Overall, the prospects for using recombinant mite allergens as diagnostics look promising, especially for allergens such as *Blomia*, where commercial extracts are not available. Another advantage of recombinant allergens is that they could be readily standardized in mass units, and the content of recombinant cocktails could be accurately defined.

Chimeric antibodies

We have recently collaborated with Drs Rob Aalberse and Janine Schuurman (University of Amsterdam, The Netherlands) to produce chimeric allergen-specific antibodies, using the F(ab)'2 domains of monoclonal mouse anti-Der p 2 coupled to the Fc portion of human IgE, IgG1, or IgG4 (24). These molecules behave essentially as human allergen-specific probes and can be used to quantitate antibody responses. The chimeric IgE anti-Der p 2 can be used to quantitate IgE antibody assays in terms of absolute units of IgE. Moreover, the binding curves for IgE binding to a panel of indoor allergens (e.g., Der p 1, Fel d 1, Can f 1) are parallel to those of the anti-Der p 2 IgE chimeric antibody; therefore, this antibody can be used to measure IgE (e.g., by ELISA) potentially to any single allergen (25). These assays are designed for use in epidemiologic or immunogenetic studies to compare IgE responses to specific allergens and could also be used for serodiagnosis, in conjunction with recombinant allergens. In addition, allergen-specific

chimeric antibodies will be useful tools for investigating the biologic function of different antibody isotypes.

Rapid diagnostic tests for allergen analysis

Advances in molecular techniques can also be applied to environmental allergen testing. The "gold standard" for indoor allergen analysis has been measurement of allergen in reservoir dust samples or air samples by ELISA with monoclonal antibodies. However, it has been clear for some time that we need more rapid tests, which may be used in outpatient clinics, doctors' offices, pharmacies, or patients' homes, in order to encourage patient compliance with avoidance procedures and to make environmental allergen monitoring more widely available. Over the past year, more rapid semiquantitative colorimetric tests have been developed to use antibodies or allergens on nitrocellulose membranes. Some of these tests, e.g., DUSTSCREEN™, can detect multiple allergens within 3–4 h and can be used to assess a patient's environmental allergen exposure on dust samples assayed during the course of an office visit. A further refinement has been the development of rapid diagnostic tests using gold-labeled monoclonal antibodies. These tests are extremely sensitive (to 100 pg allergen/test) and can be fabricated on dipsticks to detect allergens within 10 min (26). Immunogold tests have been developed for mite group 2 and cat allergen Fel d 1. They are suitable for home testing and use technology similar to that of pregnancy tests. The development of rapid allergen detection tests will greatly expand the use of environmental allergen monitoring, and this in turn should lead to better design of allergen-avoidance devices and procedures.

New therapeutic strategies

Recombinant allergens and the amino acid sequence information derived from cDNA cloning are being used to develop new approaches to the allergen-specific treatment of allergic disease. Clearly, cocktails of recombinant allergens could be used instead of allergen extracts in conventional immunotherapy protocols. Several studies have documented the quantity of major mite, cat, or pollen allergen required to achieve effective immunotherapy (27). Using recombinant allergens would enable more consistent allergen doses to be administered with, perhaps, a lower frequency of adverse reactions. However, even with more refined allergen dosing, the possibility remains that immunotherapies using IgE-reactive allergens will continue to have side-effects similar to those of natural

allergen extracts currently in use. For solution of these problems, attention is being focused on new forms of immunotherapy designed to downregulate or inactivate allergen-specific T cells, or to alter the T-cell phenotype from Th2 to Th1. These therapies would involve allergen peptides or variants that no longer bind IgE, or specialized delivery systems that would not result in interactions with IgE antibodies. Epitope mapping studies using synthetic peptides have identified a series of T-cell epitopes on the group 1 and group 2 mite allergens (reviewed by van Neerven et al. [3]). An immunodominant Der p 1 peptide has been identified, inhalation of which in experimental animals can downregulate antibody responses and protect against challenge with natural allergen administered in Freund's adjuvant (28). However, human subjects appear to recognize a broad repertoire of T-cell epitopes on the group 1 and 2 allergens, and it is not clear that immunotherapy using individual peptides would be sufficient to abrogate the T-cell response to natural allergens (3). Another approach which we have suggested is to use full-length mite allergen cDNAs which have been modified by site-directed mutagenesis so that they have reduced binding to IgE antibodies (29, 30).

Substitution of cysteine residues (Cys 21 and 73) on Der p 2 can reduce IgE reactivity on skin testing by up to 100-fold, and these cysteine variants appear to retain T-cell reactivity. Thus, it would be possible to carry out site-specific mutations and produce recombinant allergen variants that retain T-cell epitopes but no longer bind IgE. This is somewhat analogous to attempts in the past with polymerized allergen extracts or allergen digests, but the advantage of recombinant variants is that the mutations can be clearly defined (an important point for regulatory purposes).

A key question that has not been considered in previous studies of immunotherapy is whether allergen immunization early in life would be more effective at preventing the development of IgE responses and subsequent allergic disease. Attention has recently focused on the importance of allergen exposure early in life and whether or not it would be possible to induce allergen-specific Th1 responses in the neonatal period which would not progress to Th2 responses after subsequent natural allergen exposure. It is well known that children born to one or two atopic parents are at high risk of developing allergic disease; thus, such children could be a target for early intervention, either by immunotherapy or allergen avoidance. Holt has proposed that immunization with allergens together with a Th1 adjuvant in the first 1–2 years of life could result in the development of "nonpathogenic" IgG responses without IgE antibody production

(31). Such an adjuvant could include interleukin (IL)-12 and recent experiments have shown that IgE antibody responses were suppressed in mice immunized with ovalbumin-IL-12 constructs (Dr Dale Umetsu, Stanford University, USA, personal communication).

Another recent approach has been the development of plasmid or "naked" DNA vaccines which preferentially induce Th1 responses. In mice, immunization with β -galactosidase in a cytomegalovirus expression vector (pCMV) induced IgG2a, but not IgE responses, and also inhibited ongoing secondary IgE responses (32). This type of vaccine has been shown to suppress IgE antibody responses, airway hyperreactivity, and histamine levels in bronchoalveolar lavage fluids in rats immunized with pCMV coupled to mite allergen Der p 5 (33). Naked DNA vaccines can be administered intradermally with standard scratch test applicators (as used in delayed hypersensitivity testing). They are thought to induce Th1 responses either through a presenting allergen on class I HLA molecules or by inducing production of IL-12 from antigen-presenting cells (32).

Conclusions

The mite allergen Der p 1 was the first inhaled allergen to be cloned, and advances in the molecular biology of mite and other allergens over the past 10 years have spawned a burgeoning of allergen biotechnology. Recombinant mite allergens have already been used extensively in research studies, and, inevitably, these molecules will be introduced into clinical practice over the next few years. Current indications are that recombinant allergens will be used to develop new diagnostic reagents and procedures which will allow the sensitivity of allergic patients to be more clearly defined. The efficacy of new forms of treatment will have to be determined by clinical trials of allergen peptides or other vaccines. Trials of the efficacy of the cat allergen Fel d 1 peptides are being carried out, and preliminary results indicate that these peptides can cause reductions in symptom scores (34). Formulation of peptide vaccines for mite allergens will require further studies of T-cell responsiveness in large populations of mite-allergic patients (and nonatopic controls), as well as quantitative comparisons of T-cell responses to the "group of 10" mite allergens that have been cloned. In view of the importance of mite allergens in asthma, these studies will form an integral part in developing new treatments aimed at reducing asthma mortality and morbidity.

Acknowledgment

This work was supported by National Institutes of Health grants AI 32557 and AI 34607.

References

1. Thomas WR. Molecular analysis of house dust mite allergens. In: Walker MR, Roberts AM, editors. Molecular analysis of mediated hypersensitivity and strategies for immunologic intervention. Chichester: Wiley, 1997; in press.
2. Platts-Mills TAE, Thomas WR, Aalberse RC, Vervloet D, Chapman MD. Report of the Third International Workshop on Indoor Allergens and Asthma. J Allergy Clin Immunol 1997; in press.
3. van Neerven RJ, Ebner C, Yssel H, Kapsenburg ML, Lamb JR. T cell responses to allergens: epitope-specificity and clinical relevance. Immunol Today 1996;17:526-32.
4. Topham C, Srinivasan N, Thorpe C, Overington J, Kalsheker N. Comparative modelling of major house dust mite allergen *Der p* 1: structure validation using an extended environmental amino acid propensity table. Protein Eng 1994;7:869-94.
5. 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.
6. 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;23:934-40.
7. King C, Simpson RJ, Moritz RT, Thompson PJ, Stewart GA. Isolation and characterization of a novel collagenolytic serine protease, *Der p* 9, from the house dust mite, *Dermatophagoides pteronyssinus*. J Allergy Clin Immunol 1996;98:739-47.
8. O'Neill GM, Donovan GR, Baldo BA. Cloning and characterization of a major allergen of the house dust mite, *Dermatophagoides pteronyssinus*, homologous with glutathione S-transferase. Biocimica Biophys 1994;1219:521-8.
9. 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 [published erratum appears in J Exp Med 1990;171(4):1387]. J Exp Med 1989;170:1457-62.
10. Lin KL, Hsieh KS, Thomas WR, Chiang BL, Chua KY. Characterization of *Der p* V allergen, cDNA analysis, IgE mediated reactivity to the recombinant protein. J Allergy Clin Immunol 1994;94:989-96.
11. Robinson C, Kalsheker NA, Srinivasan N, et al. On the potential significance of the enzymatic activity of mite allergens to immunogenicity. Clues to structure and function revealed by molecular characterization. Clin Exp Allergy 1997;27:10-21.
12. Thomas WR, Chua K-Y. The major mite allergen *Der p* 2 – a secretion of the male mite reproductive tract? Clin Exp Allergy 1995;25:666-9.
13. Schulz O, Laing P, Sewell HE, Shakib F. *Der p* 1, a major allergen of the house dust mite, proteolytically cleaves the low-affinity receptor for human IgE (CD23). Eur J Immunol 1995;25:3191-4.
14. Hewitt CRA, Brown AP, Hart BJ, Pritchard DI. A major house dust mite allergen disrupts the immunoglobulin E network by selectively cleaving CD23: innate protection by antiproteases. J Exp Med 1995;182:1537-44.
15. Kent NA, Hill MR, Holland PWH, Han BJ. Molecular characterization of group I allergen *Eur m* I from house dust mite *Euroglyphus maynei*. Int Arch Allergy Appl Immunol 1992;99:150-2.
16. Varela J, Ventas P, Carreira JA, Barbas G, Gimenez-Gallego, Polo F. Primary structure of *Lep d* 1, the main *Lepidoglyphus destructor* allergen. Eur J Biochem 1994; 225:93-7.
17. Arruda LK, Chapman MD. A review of recent immunochemical studies of *Blomia tropicalis* and *Euroglyphus maynei* allergens. Exp Appl Acarol 1992;16:129-40.
18. Arruda LK, Vailes LD, Platts-Mills TAE, et al. Sensitization to *Blomia tropicalis* in patients with asthma and identification of allergen Blo t 5. Am J Respir Crit Care Med 1997;155:343-50.
19. Caraballo L, Avjioglu A, Marrugo J, Puerta L, Marsh D. Cloning and expression of complementary DNA coding for an allergen with common antibody-binding specificities with three allergens of the house dust mite *Blomia tropicalis*. J Allergy Clin Immunol 1996;98:573-9.
20. Puerta L, Caraballo L, Fernandez-Caldas E, et al. Nucleotide sequence of a cDNA coding for a *Blomia tropicalis* allergen. J Allergy Clin Immunol 1997; in press.
21. Smith A, Green R, Custovic A, Woodcock A, Arruda K, Chapman MD. *In vivo* cross-reactivity between IgG Ab to *Blomia* and *Dermatophagoides* spp. and to the recombinant group 5 allergens among mite allergic patients living in the UK [Abstract]. J Allergy Clin Immunol 1996;97: 420.
22. Arruda LK, Rizzo MC, Nasipitz CK, Vailes LD, Chapman MD. Skin test reactivity to recombinant group 5 allergens in mite allergic children living in São Paulo, Brazil. J Allergy Clin Immunol 1997; in press.
23. Chapman MD, Vailes L, Bavbek S, et al. Multi-center study of the biologic activity of recombinant group 5 mite allergens. J Allergy Clin Immunol 1997; in press.
24. Schuurman J, Perdok GJ, Lourens TE, Parren PWHI, Chapman MD, Aalberse RC. The production of a mouse/human chimeric IgE monoclonal antibody to the house dust mite allergen *Der p* 2 and its use for the absolute quantification of allergen specific IgE. J Allergy Clin Immunol 1997; in press.
25. Elderfield A, Schuurman J, Aalberse RC, Smith A, Woodcock A, Chapman MD. Absolute quantitation of mite specific IgE antibody levels using a chimeric mouse Fab/human Fc epsilon antibody to *Der p* 2 [Abstract]. J Allergy Clin Immunol 1996;97:301.
26. Chapman MD, Williams L, Chandler J. "Drawing the line" – rapid diagnostic tests (RDT) for the detection of dust mite, cat and cockroach allergens [Abstract]. J Allergy Clin Immunol 1996;97:302.
27. Use of standardized allergen extracts. Position Statement. American Academy of Allergy, Asthma, and Immunology. J Allergy Clin Immunol 1997; in press.
28. Hoyne GF, O'Hehir RE, Wraith DC, Thomas WR, Lamb JR. Inhibition of T-cell and antibody responses to house dust mite allergen by inhalation of the dominant epitope in naive and sensitized mice. J Exp Med 1993;178: 1783-8.
29. Smith AM, Chapman MD. Reduction in IgE binding to allergen variants generated by site-directed mutagenesis: contribution of disulfide bonds to the antigenic structure of the major house dust mite allergen, *Der p* 2. Mol Immunol 1996;33:399-405.
30. Smith AM, Chapman MD. Localization of antigenic sites on *Der p* 2 using oligonucleotide directed mutagenesis targeted to predicted surface residues. Clin Exp Allergy 1997; in press.

31. Holt PG. A potential vaccine strategy for asthma and allied atopic diseases during early childhood. *Lancet* 1994;344:456-8.
32. Raz E, Tighe H, Sato Y, et al. Preferential induction of a Th₁ immune response and inhibition of specific IgE antibody formation by plasmid DNA immunization. *Proc Nat Acad Sci U S A* 1996;93:5141-5.
33. Hsu C-H, Chua K-Y, Tao M-H, et al. Immunoprophylaxis of allergen induced immunoglobulin E synthesis and airway hyperresponsiveness *in vivo* by genetic immunization. *Nature Med* 1996;2:540-4.
34. Norman PS, Ohman JL, Long AA, et al. Treatment of cat allergy with T cell reactive peptides. *Am J Respir Crit Care Med* 1996;154:1623-8.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.