

Recent Progress in Mite Allergen Immunochemistry

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INTRODUCTION

House dust mites of the genus *Dermatophagoides* are a major source of dust allergen and a common cause of symptoms in IgE mediated diseases (1-7). Mite identification in dust samples from houses in many countries has demonstrated the occurrence of three *Dermatophagoides* species (5,8). These species, *D. farinae*, *D. pteronyssinus*, and *D. microceras*, do not interbreed and, although they can coexist within the same household, their geographic distributions vary. In most parts of Europe, *D. pteronyssinus* is frequently identified (3,5), whereas in the US and far eastern countries *D. farinae* is prevalent and may be found in house dust together with *D. pteronyssinus* (9,10). *D. microceras* has also been found in some parts of Europe (11,12); however, on a worldwide basis, its distribution is less well documented.

Although mite allergic individuals can be exposed to different mite species, several studies have suggested that dust mites produce allergens that are antigenically related. In 1969, Voorhorst and Spijksma reported that *D. farinae* and *D. pteronyssinus* produce the "same allergen," based on correlations between skin test reactions to *D. farinae* and *D. pteronyssinus* extracts in mite allergic patients (13). Using skin testing and the radioallergen absorbent test (RAST), others have also shown good correlations between IgE antibodies to both species (14-17).

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Because of the importance of mite allergens in IgE-mediated illnesses, the purification of allergens from *Dermatophagoides* species has been vigorously pursued in several laboratories (18–25). The availability of several purified allergens has permitted investigations of human antibody responses to individual mite allergens with particular interest in crossreactivity at IgE binding sites. Cellular immune responses to mite allergens have also been examined. More recently, monoclonal antibodies (Mabs) have been used extensively in studies of antigen binding sites on purified mite allergens (26–29). These Mabs have been used to develop quantitative assays for mite allergen in house dust samples and commercial allergen extracts (30). This review describes the purification and properties of the major mite allergens, the applications of allergen specific Mab, and their relevance in the management of allergic disease.

PURIFIED MITE ALLERGENS

Although a number of antigens and allergens have been identified in mite extracts using serologic techniques (crossed immunoelectrophoresis [CIE], crossed radioimmunoelectrophoresis [CRIE], immunoblotting, and so on) (31–37), we focus here on allergens that satisfy the criteria of purity and allergenic activity recently recommended by the WHO/International Union of Immunological Societies (IUIS) subcommittee on allergen nomenclature (38). The criteria recommended to judge purity are based on

1. Molecular size (e.g., SDS-polyacrylamide gel electrophoresis [SDS-PAGE], gel filtration);
2. Molecular charge (e.g., isoelectric focusing [IEF], ion-exchange chromatography);
3. Immunochemical properties (e.g., CIE, CRIE);
4. Hydrophobicity (e.g., reverse-phase high pressure liquid chromatography [HPLC]); and
5. Chemical properties (e.g., *N*-terminal amino acid sequencing, amino acid composition).

Two groups of mite allergens fulfill these criteria and have been purified in several laboratories from various starting materials (e.g., whole culture, mite body, and mite excreta extracts)

(18–25). In most cases, their identification has been confirmed by exchanges of reagents among investigators. The systematic allergen nomenclature, endorsed by the WHO, identifies purified allergens by the first 3 letters of the genus, followed by the first letter of the species and a Roman numeral to indicate the order of purification (38). Thus, the first allergen purified from *D. pteronyssinus* is *Der p* I (formerly called Ag P₁, Dp 42, or Dpt 12) (18,20,21).

Group I Allergens

Der p I was initially purified from culture media containing mite excreta using a variety of physiochemical techniques (salt precipitation, gel filtration, block electrophoresis, and preparative isoelectric focusing) (18). As judged by skin testing, histamine release, and measurements of serum IgE antibodies, *Der p* I is a major allergen—i.e., most mite allergic patients (approx 80%) have IgE ab to *Der p* I (18,20,21). *Der p* I is a heat labile glycoprotein that has closely related physiochemical properties to *Der f* I from *D. farinae* and *Der m* I from *D. microceras* (19,39). Collectively, these three allergens can be referred to as Group I mite allergens (25). They have the same mws (24,000) and exist in multiple isoallergenic forms with comparable pI ranges (pIs 4.7–7.1) (19). In addition, *Der p* I and *Der f* I have similar, but not identical, amino acid compositions and N-terminal sequences. In keeping with these physiochemical similarities, there is a very good correlation between IgE ab to *Der p* I and *Der f* I by quantitative intradermal skin testing, the radioallergosorbent test (RAST), and radioimmunoassay (RIA) (19). However, only partial antigenic identity is evident between *Der f* I and *Der p* I on immunodiffusion against rabbit antisera, and Balb/c mice immunized with either allergen in complete Freund's adjuvant (CFA) develop a predominantly species-specific response (40,41). In humans, the presence of species specific IgE ab to Group I allergens has also been demonstrated in sera from mite allergic patients (42). Furthermore, mite allergic subjects can be exposed to allergens produced by more than one *Dermatophagoides* species. Consequently, concordant sensitivity (dual allergies) has been offered as an explanation for the close correlation in IgE ab responses to *D. pteronyssinus* and *D. farinae* allergens in hu-

mans. However, solid phase immunoabsorption experiments have shown that the bulk of IgE and IgG ab to *Der f* I ($\geq 82\%$) can be removed by a *D. pteronyssinus* immunosorbent even in sera from patients exposed exclusively to *D. farinae* (19). In addition, using the same absorbed sera, extensive crossreactivity between IgE ab to *D. farinae* and *D. pteronyssinus* allergens has been demonstrated by RAST using disks coated with crude extracts containing the Group I and other mite allergens (Fig. 1).

Other parameters of the immune response to *Der p* I have been investigated and emphasize the value of using purified allergens in studying allergic diseases. In antigen binding experiments, an excellent correlation between IgE and IgG ab to *Der p* I was demonstrated in sera from allergic patients, supporting the view that factors that control IgE ab also influence other antibody isotypes (43). Two studies have examined the natural history of IgE and IgG ab specific for inhalant allergens in different age groups. Few children under 2 years had detectable IgE or IgG ab to *Der p* I or *Lol p* I (a major allergen from rye grass); however, both the concentration and prevalence of these antibodies increased from the second to fifth year in skin test positive children (44). A progressive fall in IgE and IgG ab to *Der p* I, as well as IgE ab to crude *D. pteronyssinus* extract by RAST, was demonstrated in a long term study of patients with asthma diagnosed up to 40 y previously (45). No positive correlations were observed, however, between decreasing antibody levels and loss of symptoms. In studies of cellular immune responses, a significant proliferative response to *Der p* I was detected in peripheral blood mononuclear cells (PBM), predominantly of the T-helper phenotype, from *D. pteronyssinus* sensitive patients (46). In addition, IL-2 has been used to maintain and expand cultures of PBM stimulated with partially purified mite allergen (47). This method has made it possible to obtain allergen specific T-cell lines that can be used in studies on the regulation of IgE ab production in vitro. Patch testing with purified *Der p* I for 48 h resulted in the recruitment of basophils and eosinophils in the skin of atopic dermatitis patients having immediate skin test reactions to *Der p* I (48). This study demonstrated that an acute eczematous response characteristic of cutaneous basophil hypersensitivity could be induced by application of inhalant allergen to the skin.

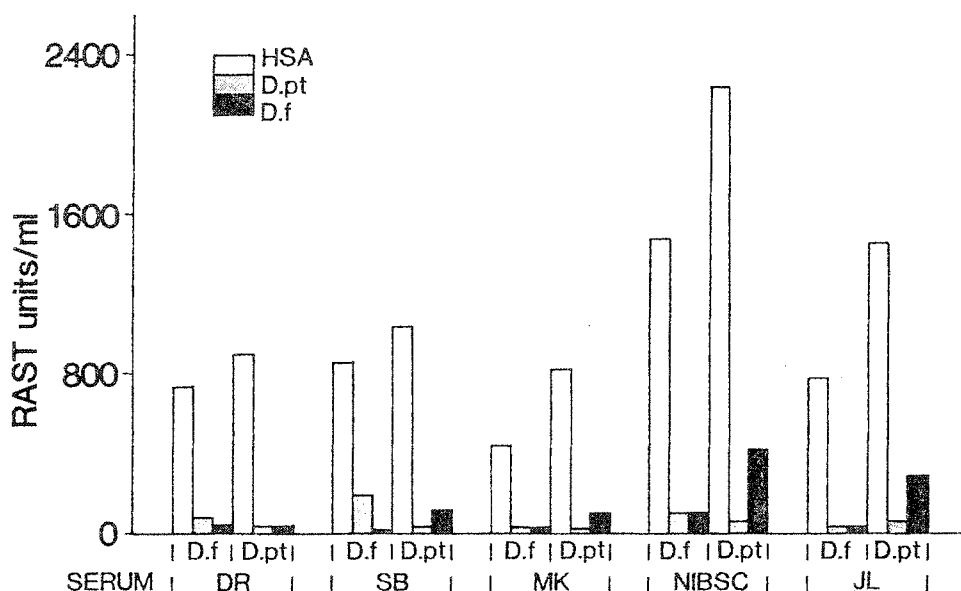


Fig. 1. Immunoabsorption showing crossreactivity of human IgE ab to *D. pteronyssinus* and *D. farinae* allergens. Sera were obtained from five mite allergic patients. Immunosorbents were human serum albumin control $\times$ HSA (open bars); *D. pteronyssinus* extract (hatched bars); and *D. farinae* extract (black bars). Results show concentration of IgE ab (RAST U/mL) to mite allergen coated RAST disks (D. f = *D. farinae* disks; D. pt = *D. pteronyssinus* disks) following absorption. In all five patients $\geq 77\%$ of IgE ab to *D. farinae* were removed by the *D. pteronyssinus* immunoabsorbent and $\geq 83\%$ of IgE ab to *D. pteronyssinus* were removed by the *D. farinae* immunoabsorbent.

Group II Allergens

The physiochemical methods used to purify Group II allergens suggested that they had different biochemical properties from the Group I allergens. Both *Der f* II and *Der p* II precipitate at higher ammonium sulfate concentrations (50–80%) than *Der f* I and *Der p* I ($\leq 50\%$). Thus, partial separation of the two groups can be achieved by establishing 50% saturated ammonium sulfate (SAS) precipitates of crude mite extract rich in Group I allergens followed by a 50–80% SAS cut rich in Group II allergens. Multiple pIs in the range of 5.5–8.2 and 5–7 have been reported

for *Der f* II and *Der p* II, respectively (20,24). The heterogeneity of these allergens on isoelectric focusing overlaps with the Group I allergens, thus hampering their separation. *Der p* II (DpX) was partially purified by Lind using gel filtration, ammonium sulfate precipitation, and hydrophobic interaction chromatography on phenyl Sepharose (20). A basic component of *Der f* II (pI = 8.0) was isolated from crushed *D. farinae* mites by Holck et al. using repeated preparative isoelectric focusing (23). Yasueda et al. isolated *Der f* II from a 60 to 95% SAS cut of *D. farinae* by anion and cation exchange chromatography and gel filtration (24). In contrast to Group I allergens, Group II allergens appear to be relatively heat stable.

Recently, we have purified both *Der f* II and *Der p* II by Mab affinity chromatography using a crossreacting Mab produced by Robert Aalberse (25). This Mab was raised in a Balb/b mouse immunized with *D. pteronyssinus* extract. It also recognizes, however, *Der f* II and a *D. microceras* protein (presumably *Der m* II). *Der p* II and *Der f* II have the same mw (15,000), similar amino acid compositions, and differ in only 4 of the first 35 *N*-terminal amino acid residues (comprising approximately 1/3 of the full sequence).

In keeping with their striking structural similarities, there appears to be considerable crossreactivity between *Der p* II and *Der f* II in binding human IgE ab (25). *Der f* II has clearly been shown to be a major allergen (approximately 90% of mite allergic patients have IgE ab to *Der f* II) (24,25). In addition, both *Der f* II and *Der p* II have been shown to have IgE ab binding activities distinct from *Der f* I and *Der p* I, respectively, in RAST inhibition experiments and CRIE (20,24). In contrast to the fecal associated Group I allergens, Group II allergens appear to be relatively more concentrated in extracts made from isolated mites (49).

A third *D. farinae* allergen, *Der f* III (mw 29,000) has recently been purified from spent growth media by repeated gel filtration (25). Isoelectric focusing reveals isoallergens at a low pH (pI 4.2–4.8) that differs from the behavior of *Der f* I and II on IEF. In addition, comparisons of the *N*-terminal amino acid compositions demonstrate that they are structurally distinct proteins (25). In antigen binding RIAs, *Der f* III appears to be a minor allergen,

and, although homology and crossreactivity with *D. pteronyssinus* allergen has yet to be established, patients exposed exclusively to *D. pteronyssinus* have IgE ab that bind *Der f* III. IgE ab to these *D. farinae* allergens (*Der f* I, II, and III) varies among mite allergic individuals and patients commonly have IgE ab to more than one allergen. Thus, the clinical response of patients receiving house dust or mite allergen immunotherapy may be difficult to judge without knowing whether an extract used contains allergens that have stimulated IgE ab in the patient being treated.

MITE ALLERGEN SPECIFIC MONOCLONAL ANTIBODIES

The development of allergen specific monoclonal antibodies (Mabs) has progressed dramatically over the last 3–5 yr. Currently, Mabs with different specificities for the Group I and Group II allergens and for *Der f* III are available. These monoclonals have been used to purify mite allergens by affinity chromatography; to develop immunoassays for measuring allergen concentrations in dust samples and commercial extracts; and to investigate the repertoire of antigenic sites on purified mite allergens.

Mab Affinity Purification of Mite Allergens

Conventional biochemical methods of purification require large quantities of culture material, are time consuming (a single purification can take several weeks) and give a relatively low yield (a few mg). In contrast, Mabs can be used to affinity purify milligram quantities of allergen within a few days from considerably less starting material. These purifications are single step procedures resulting in approximately 90% pure allergen. When followed by either size exclusion or reverse phase HPLC, homologous allergen preparations can be obtained. Once established, columns containing Mab on solid phase can be used repeatedly without loss of activity. Table 1 outlines protocols currently being used to affinity purify 6 different mite allergens. Crossreacting Mabs 4C1 and CLB-DpX show binding specificities for Group I and Group II allergens, respectively, whereas 5A12 is species specific for *Der f* III.

Table 1
Outline of Mab Chromatography for Affinity Purifying Mite Allergens

	Group I	Group II	
Allergens	<i>Der p I</i> <i>Der f I</i> <i>Der m I</i>	<i>Der p II</i> <i>Der f II</i>	<i>Der f III</i>
Source material	Excrement fraction of mite culture	50–80% ammonium sulfate fraction of "spent" mite culture	
Mab	4C1 ^a	CLB-CpX ^a	5A12 ^b
Elution conditions	0.005 M glycine/ 50% ethylene glycol pH = 10.0	0.1 M glycine/0.5 M NaCl, pH = 11.5	

^aCrossreacting Mabs 4C1 and CLB-DpX are group specific and bind either Group I or Group II allergens, respectively.

^bMab 5A12 is specific for *Der f III*.

Mab-Based Assays for Quantitative Measures of Mite Allergen

The effective management of patients with allergic asthma and rhinitis owing to mite allergen exposure would be greatly enhanced by the availability of rapid, simple, and quantitative measurements of mite allergen in house dust samples and extracts. Initially, inhibition RIAs using rabbit polyclonal antibodies to *Der p I* (18) or to crossreacting epitopes on *Der p I* and *Der f I* (P_1 Equivalent assay) (40) were developed to quantify Group I allergens. Those assays established that *Der p I* and *Der f I* are concentrated primarily in mite fecal particles, rather than in mite bodies, cuticles, or eggs (40,50). Mite fecal particles are 10–40 μ m in diameter (similar to pollen grains) and remain airborne for 15–30 min following disturbance of household dust (51). Analysis of dust samples from bedding, carpets, and sofas in homes of mite allergic asthmatics showed that P_1 Eq antigen levels ≥ 10 μ g/g of dust were associated with increased symptoms (40). Sea-

sonal variations were also clearly demonstrated (52). Results showed that mite allergic patients may experience attacks of asthma and rhinitis more frequently during fall and early winter months (August–December), when $\geq 10 \mu\text{g}$ of AgP_1 Eq/g dust are more likely to occur.

There are problems, however, using immunoassays that depend on polyclonal antibodies. First, the antisera used are heterogeneous in composition and often require immunoabsorption if they are to be made monospecific. Second, polyclonal inhibition assays are technically involved and require the availability of highly purified radiolabeled allergen. RAST inhibition assays involve pooled human antisera that vary in their composition of specific IgE ab and that understandably are limited in supply.

Monoclonal antibody based immunoassays have several advantages over existing techniques. First, once clones are established, the supplies of Mab required to carry out immunoassays are essentially unlimited. Second, the specificity of Mabs remains constant with time and purified allergen is no longer a required reagent. Third, these assays are direct binding assays by design and are rapid, technically straightforward, sensitive, and reproducible. Figure 2 shows a two-site Mab assay for measuring either *Der f* I or *Der p* I. In this assay, allergen samples (e.g., mite or house dust extract) are incubated in microtiter wells coated with either anti-*Der p* I or *Der f* I Mab. Bound allergen is then detected using a second radiolabeled (or enzyme labeled) Mab of different epitope specificity to the same allergen. Effective radiolabeling of detecting Mab (e.g., 4C1, the crossreacting Mab for Group I allergens) is accomplished by using Mab purified from crude ascites by preparative IEF (Fig. 3). Fractions showing maximum binding to ^{125}I -allergen demonstrate the monoclonality of the antibody and are pooled and concentrated. These assays are quantitated using a reference preparation (*D. pteronyssinus* NIBSC International Reference 82/518) containing a known amount of the allergen to be measured to form a control curve. Figure 4 shows binding curves obtained for dilutions of purified *Der p* I, the NIBSC International Reference preparation 82/518, and a whole *D. pteronyssinus* culture extract using the Mab assay specific for *Der p* I. Using a 40% SAS cut of *D. farinae* extract, no significant binding was detected. Similarly, the Mab

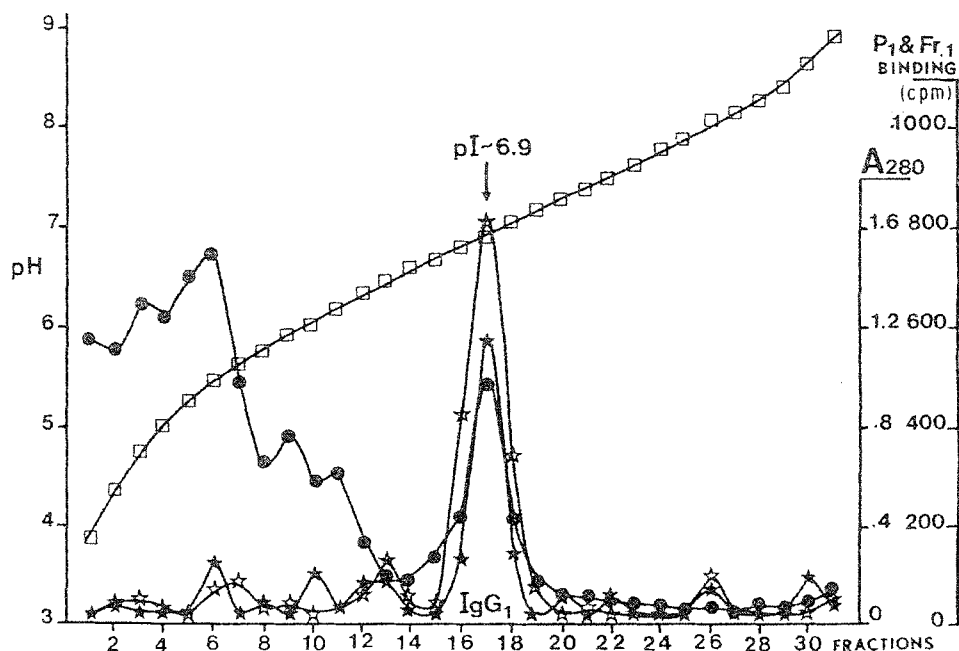


Fig. 3. Preparative IEF of crude ascites containing crossreacting Mab 4C1 specific for Group I mite allergens. The pH ($\square$), protein content (absorbance at 280 nm) ($\bullet$), antiDer p I ($\star$), and antiDer f I ($\star$) concentration of each fraction are shown. Fractions 16–18 were pooled and concentrated for radiolabeling.

lack consistency because of different sensitivities among individual allergic patients and because in RAST inhibition the allergen content of extracts used to coat the solid phase (e.g., disks) may vary (53). These problems are not encountered using Mabs to assess the potency of extracts, particularly with respect to their concentration of major allergens. Using the Mab two-site assays for Group I allergens, Table 2a shows measurements of Der f I and Der p I in three *D. pteronyssinus* and four *D. farinae* commercial mite extracts. Microgram quantities of either Der p I or Der f I were measured and results emphasize the species specificity of these assays. Similar studies using commercial house dust preparations have been carried out (Table 2b) (30). Nanogram, rather than microgram, amounts of Der f I and Der p I were detected. Greater variability in the total concentration of both allergens was observed, and different proportions of Der p I and Der f I in

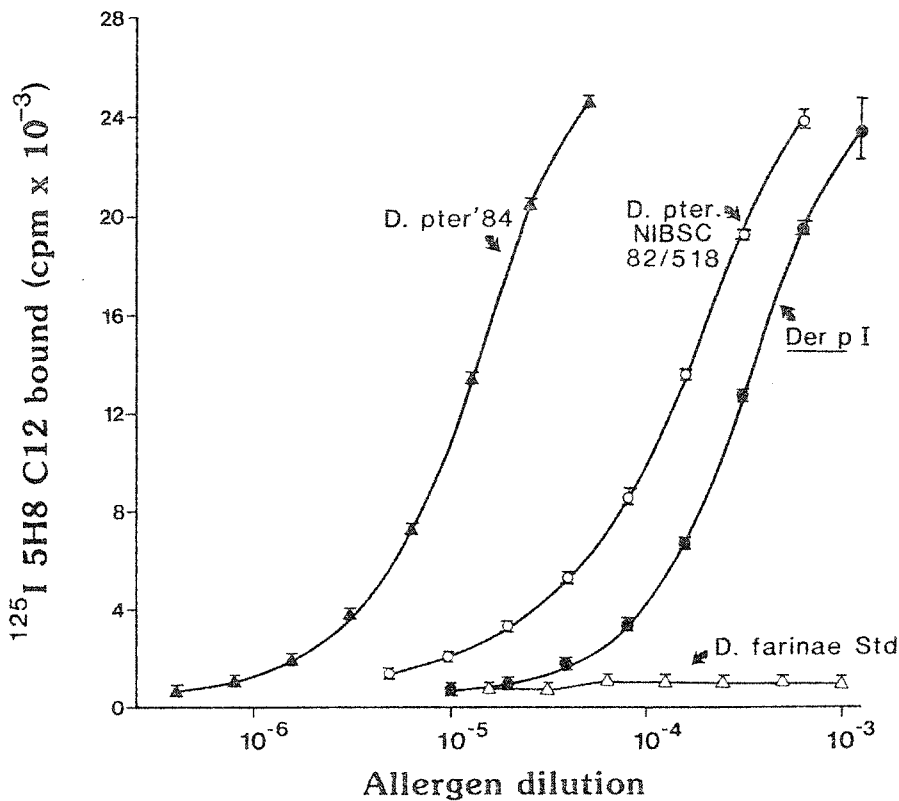


Fig. 4. Binding curves comparing measurements of *Der p I* in mite extracts using the Mab Two-site RIA specific for *Der p I*. Serial twofold dilutions of each extract were tested. Extracts were: *D. pter'84* = whole *D. pteronyssinus* culture (Bencard Ltd.); NIBSC (82/518) preparation of *D. pteronyssinus* approved as an international reference by the WHO; Mab 4C1 affinity purified *Der p I*; and *D. farinae Std* = 40% ammonium sulfate cut of *D. farinae* extract.

each extract were measured, reflecting different numbers of the two mite species present in these preparations. Thus, mite and house dust extracts may differ significantly when used for the diagnosis and treatment of mite allergy.

The extent to which individual allergens (e.g., Group I allergens) reflect the total potency of mite extracts remains to be determined. In contrast to the Group I fecal associated allergens, *Der f II* and *Der p II* may be relatively more concentrated in mite bodies (49). Currently, Mabs to *Der f II*, *Der p II*, and *Der f III* bind

Table 2a
Quantities of *Der p* I and *Der f* I
in Commercial Mite Extracts Determined by Mab RIA

	<i>Der p</i> I μg/mL	<i>Der f</i> I μg/mL
<i>D. pteronyssinus</i>		
A	64.0	0.08
B	58.4	0.02
C	20.5	0.02
<i>D. farinae</i>		
A	<0.01	79.0
B	<0.01	32.0
C	<0.01	32.0
D	<0.01	42.0
E	<0.01	60.8

Table 2b
Quantities of *Der p* I and *Der f* I
in Commercial House Dust Extracts Determined by Mab RIA

Manufacturer	<i>Der p</i> I ng/mL	<i>Der f</i> I ng/mL
A	281	85
B	775	1825
C	738	500
D	16	28
E	96	1063
F	1050	<5
G	179	215

to only a single epitope. Therefore, two site assays for measuring these molecules have not yet been developed. As more monoclonal reagents become available, it will become possible to make direct comparisons of specific allergen levels in mite extracts and, thus, assess the potency of extracts prepared from different source materials more precisely.

Studies of Epitope Specificity Using Mabs to Der p I and Der f I

Significant differences between antibody responses to *Der p* I and *Der f* I in experimental animals (e.g., mice and rabbits) and in mite allergic humans have been demonstrated (19,27,40). Whereas human IgE and IgG antibodies to Group I allergens crossreact extensively, both polyclonal and monoclonal IgG antibodies from mice immunized with *Der f* I or *Der p* I appear to be directed primarily against species-specific epitopes on either allergen.

Mabs to *Der p* I have been successfully produced by several investigators (Lind, Aalberse, Delespesse) including ourselves (26–28). The epitope specificity of these Mabs (35 from 5 different fusions) has been examined, revealing 4 distinct species specific epitopes (27). Seventy percent of these Mabs bound the same epitope, suggesting that they recognize an immunodominant antigenic site. Similarly, the majority of (16 of 18) Mabs raised against *Der f* I bound to the same species specific epitope (19). Only two crossreacting Mabs bound to both *Der p* I and *Der f* I (and *Der m* I) and both were directed against the same epitope. Competitive inhibition experiments showed that most of the epitopes recognized by the Group I Mabs were distinct from human IgE binding sites (19,27).

The reasons for the striking differences between murine and human antibody responses to Group I allergens are not clear. Repeated low dose immunization (nanogram quantities of antigen in alum) has previously been shown to stimulate an IgE rather than IgG isotype response in mice (54). In this regard, mite allergic individuals are also sensitized with low doses of allergen chronically, albeit without adjuvant and without parenteral inoculation. Mice immunized with *Der p* I or *Der f* I, on the other hand, were hyperimmunized with microgram quantities of allergen (5–10 µg) ip or im in CFA. The different immune responses observed thus form the basis of experiments to examine whether the dose of allergen, routes of administration, and adjuvants used are of importance in influencing not only isotype expression, but the epitope specificity of antibody responses.

Results of such experiments focus attention on differences in antigen processing and presentation that may have important implications in understanding allergen sensitization as well as mechanisms involved in immunotherapy.

SUMMARY

The successful purification of several mite allergens within the last few years has considerably enhanced our understanding of mite allergen immunochemistry. The role of these glycoproteins in stimulating human IgE ab and their role as immunogens in mice and rabbits has been studied extensively in a number of laboratories worldwide. In particular, purified allergens have facilitated the production of murine IgG Mabs that have been used to purify mite allergens by affinity chromatography; to investigate the diversity of antigenic sites on purified allergens; and to develop Mab based immunoassays for measuring allergen concentrations in dust samples and extracts. Full amino acid sequencing of several mite allergens is now in progress together with efforts to identify antigenically important peptide fragments. Such investigations are aimed to further increase our knowledge of humoral and cellular immune responses at the molecular level.

For years, pollen counts have been used to judge airborne pollen allergen levels and to predict, in turn, the severity of symptom days for patients with hay fever. In contrast, simple methods for measuring dust allergens (e.g., mite allergen) have not been available. The development of Mab immunoassays, which can be converted from radiolabeled to enzyme labeled or fluorescence labeled assays, should provide rapid and quantitative measurements of specific mite allergen levels in house dust. Not only can such measurements provide useful clinical information in judging the exposure of patients to mite allergen, but the effectiveness of allergen avoidance regimes can be monitored objectively. By measuring the concentration of specific allergens in extracts, these assays could significantly improve efforts to standardize extracts used for diagnosis and treatment.

ACKNOWLEDGMENT

This work was supported by National Institutes of Health Grants AI-24687 and AI-24261.

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Since this review was written there have been several important advances in the analysis of mite allergens:

1. Chua et al. (1988), Molecular cloning (cDNA) and sequencing of *Der p I* and *Der p II* and sequence similarity searches indicating that *Der p I* is a thiol protease. *J. Exp. Med.* **167**, 175-182.
2. Heymann et al. (1989), Identification and partial sequencing of the 29 kd Group III allergens. These allergens show sequence homology to serine proteases (chymotrypsin and trypsin) *J. Allergy Clin. Immunol.* (Stewart et al., (1989), *Lancet*).
3. O'Hehir et al. (1987), Isolation of human T cell clones directed against the Group I and Group II mite allergens and analysis of their MHC restriction pattern. O'Hehir et al., (1987), *Immunology* **62**, 635-640, and (1988), *Immunology* **64**, 627-632.
4. Luczynska et al. (1989), Development of mAb ELISA assays for the Group I allergens. *J. Imm. Meths.*, **118**, 227-235.
5. Platts-Mills, T. A. E. and de Weck, J. (1989), Analysis of mite allergen levels in house dust which should be considered as risk factors for the development of allergic disease. *J. Allergy Clin. Immunol.* **83**, 416-427.

These new developments in mite allergen immunochemistry have recently been reviewed (Chapman, M. E., ed.). *Curr. Opin. Immunol.* **1**, 647-653, 1989, and *Advances in the Biosciences*, **74**, 281-295, 1989.