

Comparison of the levels of the major allergens *Der p* I and *Der p* II in standardized extracts of the house dust mite, *Dermatophagoides pteronyssinus*

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Summary

Allergy to the house dust mite *Dermatophagoides pteronyssinus* is mediated by IgE to the major allergens *Der p* I and *Der p* II in the majority of mite-allergic patients. In recent years, standardized preparations of *D. pteronyssinus*, commercially available from several sources, have become widely used for the diagnosis and immunotherapy of mite allergy. As standardization implies uniformity of allergen composition and potency, we directly compared the absolute and relative quantities of *Der p* I and *Der p* II in six different commercial standardized extracts of *D. pteronyssinus*. Our findings reveal variability in levels of both *Der p* I and *Der p* II, producing ratios of *Der p* I/*Der p* II ranging from 1.1/1 to 6/1. Although the content of minor allergens in the extracts was not evaluated here, their contribution to the overall reactivity of mite-allergic patients to the commercial extracts was judged to be minimal. This was demonstrated by showing that plasma depleted of reactivity to both *Der p* I and *Der p* II had virtually no residual IgE directed against extract components. The variation in the proportion of *Der p* I and *Der p* II among different *D. pteronyssinus* extracts is likely to influence their biological effectiveness. Patients with reactivity against only *Der p* I or *Der p* II, who were found to comprise approximately one-third of the mite-allergic population, may not respond optimally to extracts containing relatively low levels of the allergen to which they are sensitive.

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Introduction

Allergy to the house dust mite *Dermatophagoides* species (*D. pteronyssinus* and *D. farinae*) is a common form of allergic disease. Mite sensitivity has been associated with asthma, atopic dermatitis, and perennial rhinitis [1]. The two major allergens of *D. pteronyssinus*, *Der p* I, a 24 kDa component of mite faeces, and *Der p* II, a 14 kDa component of mite bodies, bind IgE in the majority of mite-allergic patients, and account for up to 75% of the total IgE reactivity to mite [2–7]. In 1984, an International Standard for *D. pteronyssinus* was adopted by the World Health Organization (NIBSC 82/518), and standardized skin test extracts are now commonly used to diagnose and to treat allergy to house dust mite. The proteolytic

activity of several mite antigens, including *Der p* I [8–12], however, questions the uniformity of even standardized mite extracts. In this study, we compared six commercially available standardized skin test extracts for their content of *Der p* I and *Der p* II. This direct comparison of allergen levels revealed a wide variability in both the absolute and relative quantities of the major mite allergens among the different extracts tested.

Additional, minor, allergenic components of *D. pteronyssinus* include *Der p* III [9], *Der p* IV [11], *Der p* V [13], and *Der p* VI [8]. A longitudinal study of children in which the onset of IgE responsiveness to *D. pteronyssinus* was documented revealed that IgE present early in the course of mite sensitivity was directed against components distinct from *Der p* I and *Der p* II in most subjects [14]. The effectiveness of mite extracts may therefore be determined not only by their content of *Der p* I and

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Der p II, but also by the presence of any minor allergens that contribute to the overall sensitivity to house dust mite. In order to assess the contribution of the minor allergens to the *D. pteronyssinus* sensitivity of mite-allergic adults, we depleted IgE directed against *Der p* I and/or *Der p* II from a pool of plasma derived from 18 mite-allergic (skin test 4+) subjects. Results not only confirmed the major roles of both *Der p* I or *Der p* II, but indicated that minor allergens did not play a dominant role in the responsiveness of this group of mite-allergic patients. Because of the importance of *Der p* I and *Der p* II in the allergenic response to house dust mite, our findings of variability in the content of these allergens in commercial skin test extracts encourage the adoption of more stringent criteria to establish uniformity of mite extracts used for diagnosis and treatment.

Materials and methods

Patient plasma

Plasma samples were obtained from mite-allergic subjects (*D. pteronyssinus* skin test 4+) that were diagnosed at either the Lahey Clinic (Burlington, MA, USA), or the New England Medical Centre (Boston, MA, USA). Patients were not undergoing immunotherapy at the time of sampling. A pool of equal volumes of plasma samples from each of 18 mite-allergic subjects (*D. pteronyssinus* skin test 4+) selected for the presence of IgE reactive to *Der p* I and/or *Der p* II was used in some experiments.

Antibodies

The MoAbs 4C1 [15] and 5H8 [16,17] specific for the group I allergens of mite, and MoAb 6D6 [6] specific for the group II allergens were purchased from Dr Martin Chapman, University of Virginia, Charlottesville, VA, USA; MoAb 5H8 was biotinylated using NHS-biotin. Polyclonal rabbit antiserum (antiserum #427) was prepared by immunization of a New Zealand white rabbit with native *Der p* II in complete Freund's adjuvant, followed by boosting in incomplete Freund's adjuvant.

Mite allergenic extracts and purified standards

Standardized *D. pteronyssinus* extracts were obtained from the following sources: ALK (ALK America Inc., Milford, CN, USA; lot #1452/13255901), Meridian (Meridian Bio-Medical, Inc., Round Rock, Texas, USA; lot #1U5), USA Hollister-Stier (Miles Inc., West Haven, CN, USA; lot #B42K8623), Greer (Greer Labs,

Inc., Lenoir, NC, USA; lot #GB70-13B-5UR7), Antigen Laboratories (Liberty, MO, USA; lot #D28051391), and Nelco (Nelco Labs Inc., Deer Park, NY, USA; lot #9216090G). All extracts were used at least 12 months prior to the expiration date provided by the manufacturer. The allergens *Der p* I and *Der p* II were purified as follows and quantitated for use as reference standards. Lyophilized mite bodies and feces were purchased from Dr L. G. Arlian, Wright State University, Dayton, OH, USA. *Der p* I and *Der p* II were isolated by affinity purification using MoAbs 4C1 [15] and 6D6 [6], respectively, as described [6], and quantitated using the BCA assay kit (Pierce Chemical Co., Rockford, IL, USA). Purity was determined by HPLC analysis, and purity and quantitation checked by amino acid analysis. The CBER reference standard for *D. pteronyssinus* (lots E4-Dp and E5-Dp) were obtained from the U.S. Food and Drug Administration (Bethesda, MD, USA).

Direct ELISA

ELISA plates (Costar Corp., Cambridge, MA, USA) were coated overnight at 4°C with 2.5 µg/ml *Der p* I or *Der p* II, isolated by affinity purification using MoAbs 4C1 [15] or 6D6 [6], respectively. The coated plates were washed three times with phosphate buffered saline (PBS) containing 0.05% Tween-20 (PBS-Tween), blocked for 1 h with PBS containing 0.5% gelatin and 1 mM polyvinylpyrrolidone (gelatin-PVP), then incubated with human plasma diluted from two to 2000-fold with PBS-Tween, overnight at 4°C. The plates were washed, incubated for 1 h at room temperature with 1 µg/ml biotinylated goat-anti-human IgE (Kirkegaard and Perry Labs, Inc., Gaithersburg, MD, USA), followed by streptavidin conjugated to horseradish peroxidase (1:10 000 dilution in PBS-Tween) (Southern Biotechnology Associates, Inc., Birmingham, AL, USA). Binding was detected using the peroxidase substrate TMB (3,3',5,5'-tetramethylbenzidine) (Kirkgaard and Perry Labs). The TMB reaction was stopped by acidification with phosphoric acid, and the products were read in a plate reader at 450 nm.

Capture ELISA

ELISA plates (Costar) were coated overnight at 4°C with approximately 3 µg/ml of the specific anti-group I MoAb 4C1 [15], or the specific anti-group II MoAb 6D6 [6], in PBS. The coated plates were washed three times with PBS-Tween, blocked with gelatin-PVP, then incubated with purified preparations of *Der p* I, *Der p* II, or the commercially available standardized *Der p* extracts. Binding to MoAb 4C1-coated plates was

detected with biotinylated MoAb 5H8 [16,17] specific for *Der p I*, and binding to MoAb 6D6-coated plates was detected using polyclonal rabbit anti-*Der p II* serum #427 at a dilution of 1:1000 in PBS-Tween, followed by biotinylated goat-anti-rabbit Ig at 1:2000 dilution (Kirkegaard and Perry Labs). Binding of biotinylated antibodies was detected using horseradish peroxidase-linked streptavidin (Southern Biotechnology Associates) and TMB substrate (Kirkegaard and Perry Labs), as described. All incubations were performed at room temperature for 1 h. *Der p I* and *Der p II* levels in the allergenic extracts were determined by comparison with a standard curve run with purified allergens. Quantitative results were reproducible within a factor of 2.

Western Blot

Purified *Der p I* (5 µg/lane), purified *Der p II* (5 µg/lane), or the *Der p* allergenic extracts (50 µl/lane of a 1:50 dilution) were reduced by boiling in the presence of dithiothreitol (DTT), and were run on 15% reducing SDS-polyacrylamide gels. Western blots were performed as described by Bond *et al.* [18]. Briefly, gels were transferred to nitrocellulose membranes using a Transphor apparatus (Hoefer Instruments, San Francisco, CA, USA). The membranes were blocked with 1% defatted milk in a solution of 25 mM Tris-HCl, pH 7.5, 0.17 M NaCl, and 0.05% Tween-20 (blot solution), then incubated with the following antibodies: a 1:2000 dilution of a polyclonal mouse antiserum directed against *Der p I* (anti-23.1), a 1:5000 dilution of a polyclonal rabbit antiserum directed against *Der p II* (rabbit 427), or a 1:10 dilution of a pool of plasma from 18 mite-allergic (skin test 4+) patients (pooled human plasma).

Incubations were performed in blot solution overnight at room temperature. The blots were then incubated with 1:2000 dilutions of biotinylated goat anti-mouse, anti-rabbit, or anti-human Ig (Kirkegaard and Perry Labs), as appropriate, followed by a 1:10 000 dilution of streptavidin-labelled peroxidase (Southern Biotechnology Associates). Binding was detected using the enhanced chemiluminescence (ECL) system (Amersham Corp., Arlington Heights, IL, USA). The results were qualitatively reproducible.

Depletion assays

Cyanogen bromine-linked Sepharose (75 mg) (Pharmacia, Uppsala, Sweden) was acidified, then equilibrated with 0.1 M sodium bicarbonate, pH 8.5, and incubated with 250 µg of either purified *Der p I* or *Der p II* overnight at 4°C. The conjugated Sepharose was washed five times in 0.1 M sodium bicarbonate, pH 8.5, blocked with 0.5 M

glycine buffer, pH 8.5 for 3 h at room temperature, then resuspended and stored in PBS containing 0.3% bovine serum albumin, 10 mM EDTA, 0.1% Tween-20, and 0.05% sodium azide. Pooled plasma from 18 mite-allergic subjects was diluted 1:10 in PBS-Tween and incubated overnight at room temperature with 10 µl of *Der p I*-conjugated and/or *Der p II*-conjugated Sepharose, in a final volume of 200 µl. The Sepharose was washed three times with PBS-Tween and the amount of bound plasma IgE was determined by incubation of the Sepharose with biotinylated anti-human IgE followed by ¹²⁵I-streptavidin (Amersham). The efficiency of depletion of *Der p I*- and/or *Der p II*-specific IgE from the plasma was monitored by direct ELISA of the unbound material. The ability of IgE in plasma depleted of *Der p I* and/or *Der p II* reactivity to bind to mite extracts was determined by direct ELISA using ELISA plates (Costar) coated with the extracts (1:50 dilution in PBS). An efficiency of 90% depletion of IgE reactive to *Der p I* or *Der p II* was consistently observed.

Results

Der p I and *Der p II* levels in mite allergenic extracts

The levels of *Der p I* and *Der p II* in six different commercially available *Der p* allergenic extracts were determined by quantitative capture ELISA using purified *Der p I* and *Der p II* as standards. The allergens were captured using MoAbs directed against the group I (*Der p I*) or group II (*Der p II*) mite allergens, then detected using specific second antibodies. This assay method was highly specific for *Der p I* or *Der p II*, and, although it was not meant to provide an absolute quantitation of allergen levels in each extract, it did allow a relative comparison of allergen levels in extracts from different sources. Results were reproducible to within a factor of 2. Standard curves for *Der p I* and *Der p II* are shown in Fig. 1a and b, respectively, and the *Der p I* and *Der p II* ELISA results for the extracts are shown in Fig. 1c and d, respectively. The quantitation of allergen levels in the six extracts is shown in Table 1. *Der p I* levels in the extracts ranged from 12 µg/ml (extract F) to 30 µg/ml (extracts D and E). *Der p II* levels ranged from 3 µg/ml (extract B) to 15 µg/ml (extract D). The ratio of *Der p I*/*Der p II* on a weight basis ranged from 1.1/1 (extract F) to 6/1 (extract B) and, on a molar basis, from 0.64/1 (extract F) to 3.6/1 (extract B) (Table 1). For comparison, two different lots of the U.S. FDA CBER reference standard for *D. pteronyssinus* (E4-Dp and E5-Dp) were assayed and found to contain a *Der p I*/*Der p II* weight ratio of 1.2/1 and 1.1/1, respectively (Table 1).

Within the allergenic extracts, which contain solubilizing

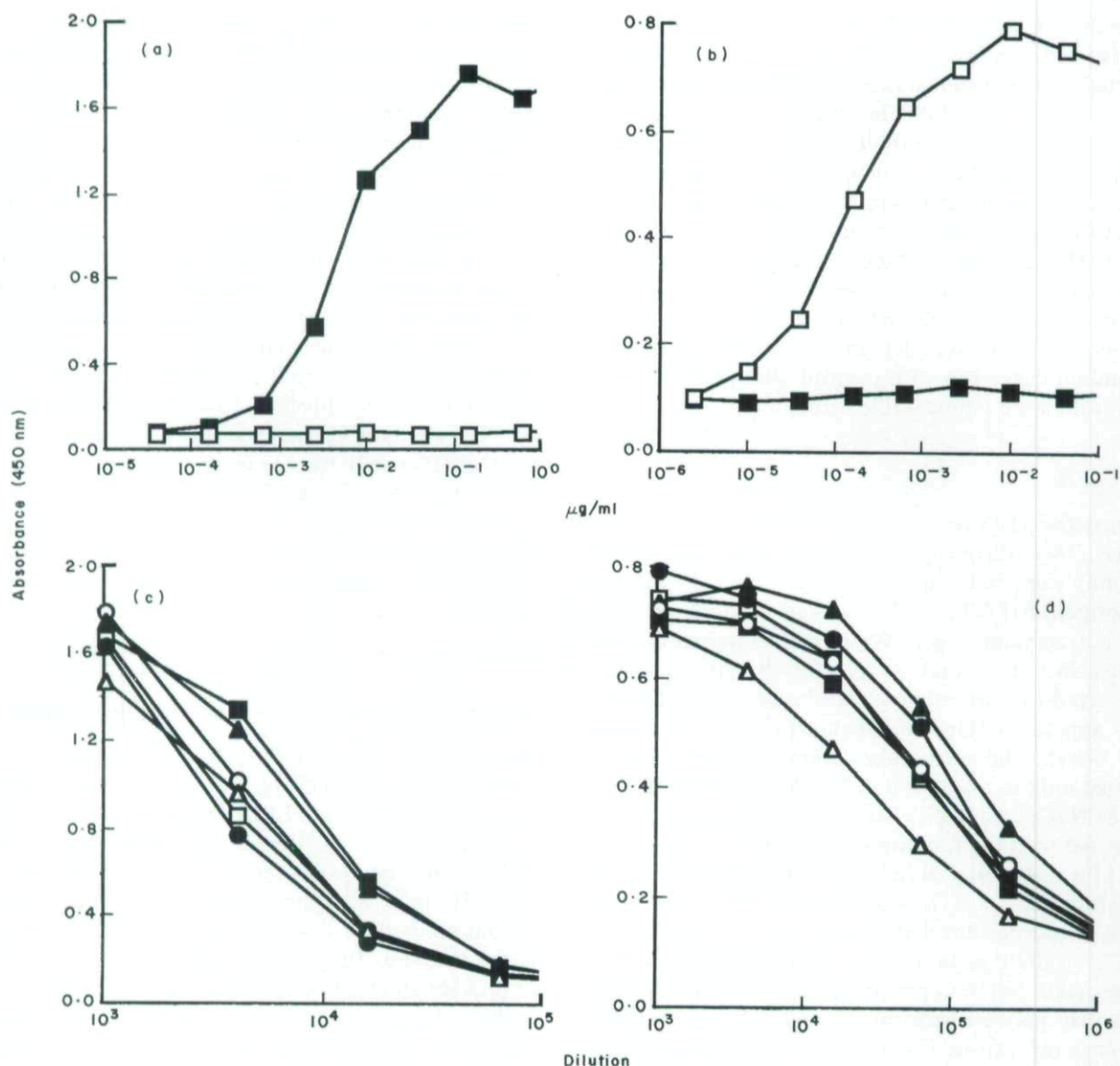


Fig. 1. Capture ELISA for determination of *Der p I* and *Der p II* levels in mite allergenic extracts. Six commercial *Der p* allergenic extracts (Extracts A through F) were assayed for content of *Der p I* or *Der p II* using specific ELISAs. Reactivity was quantitated as absorption at 450 nm (A 450) of the peroxidase reaction product (a) *Der p I* capture ELISA: standard curve and specificity for *Der p I* (■) over *Der p II* (□). (b) *Der p II* capture ELISA: standard curve and specificity for *Der p II* (□) over *Der p I* (■). Binding of mite allergenic extracts in: (c) *Der p I* capture ELISA; and (d) *Der p II* capture ELISA. (□). Extract A. (△). Extract B. (○). Extract C. (▲). Extract D. (■). Extract E. (●). Extract F.

materials such as phenol, *Der p I* and *Der p II* may not be in totally native form. This would affect our ability to detect these allergens by the capture ELISA assays utilized above, as these assays are specific for native allergens and do not detect denatured forms (data not shown). In order to assay both native and non-native forms of the allergens, Western blots were performed in which the extracts were run on denaturing SDS-polyacrylamide gels and transferred to nitrocellulose membranes, which were probed with mouse

polyclonal anti-*Der p I* sera or rabbit polyclonal anti-*Der p II* sera. These antisera detect both native and denatured forms of *Der p I* and *Der p II*, respectively (data not shown), and should therefore reflect the total allergen content of the extracts. The results are qualitatively consistent with those of the capture ELISA, showing that, of the six extracts tested, extract F contains the lowest levels of *Der p I* (Fig. 2a) and extract B contained the lowest levels of *Der p II* (Fig. 2b).

Table 1. *Der p*I and *Der p*II levels in standardized mite extracts*

Extract	<i>Der p</i> I ($\mu\text{g/ml}$)	<i>Der p</i> II ($\mu\text{g/ml}$)	Ratio of <i>Der p</i> I/ <i>Der p</i> II	
			(Weight) $\dagger$	(Molar) $\ddagger$
A	15	7	2.1/1	1.2/1
B	18	3	6/1	3.6/1
C	21	7	3/1	1.8/1
D	30	15	2/1	1.1/1
E	30	7	4.3/1	2.5/1
F	12	11	1.1/1	0.64/1
E4-Dp§	12	10	1.2/1	0.70/1
E5-Dp§	24	22	1.1/1	0.64/1

* *Der p*I and *Der p*II concentrations were calculated by comparison to standards of known concentration using a specific capture ELISA (see Materials and methods).

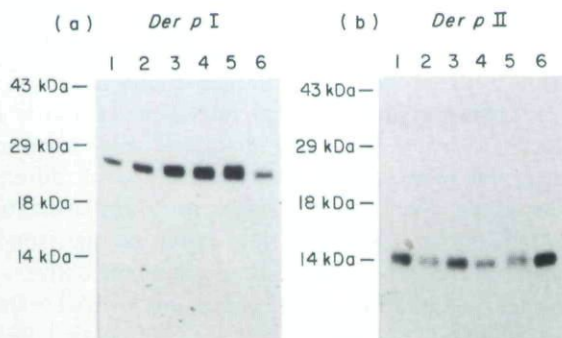
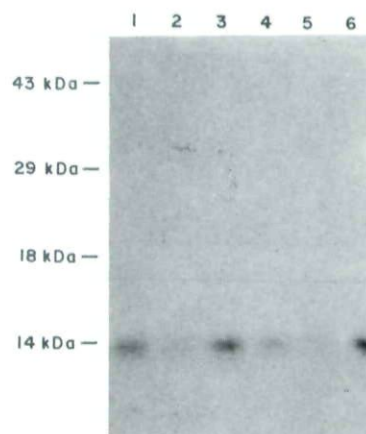
$\dagger$ Ratio based on $\mu\text{g/ml}$ concentrations of *Der p*I and *Der p*II.

$\ddagger$ Calculated using mol. wt. values of 24 kDa for *Der p*I and 14 kDa for *Der p*II.

§ E4-Dp and E5-Dp are two lots of CBER/FDA reference standard for *D. pteronyssinus*.

Human IgE binding to standardized mite extracts

The assays reported above compared the total *Der p*I and *Der p*II levels in the mite allergenic extracts. Because all of the allergen may not be in a form reactive with human IgE, we went on to compare plasma IgE reactivity to the *Der p*II contained in the six extracts by Western blot. Proteins in the six extracts were separated on sodium dodecyl sulphate—polyacrylamide gel electro-

**Fig. 2.** Western blot of mite allergenic extracts probed with: (a) Mouse antisera specific for *Der p*I; (b) Rabbit antisera specific for *Der p*II. Lane 1, extract A; lane 2, extract B; lane 3, extract C; lane 4, extract D; lane 5, extract E; lane 6, extract F.**Fig. 3.** Western blot of mite allergenic extracts probed with a pool of plasma from 18 mite-allergic (skin test 4+) patients. Lane 1, extract A; lane 2, extract B; lane 3, extract C; lane 4, extract D; lane 5, extract E; lane 6, extract F.

phoresis (SDS—PAGE), transferred to nitrocellulose, and the membrane probed with the pooled plasma from 18 mite-allergic subjects. This assay was unable to evaluate *Der p*I reactivity because, in contrast to the specific mouse antisera utilized above, human plasma IgE is unable to detect *Der p*I by Western blot. This is most likely a result of the antigen denaturation produced by boiling the sample in SDS-containing buffer in preparation for electrophoresis. ELISA results confirm that human plasma IgE binds to native *Der p*I, but not to *Der p*I that has been denatured by boiling or by treatment with SDS (data not shown). *Der p*II, however, can bind human IgE on a Western blot, and results of this assay show that all six extracts contained *Der p*II that bound to human IgE, with extracts B and E displaying the weakest binding (Fig. 3).

Depletion of *Der p*I and/or *Der p*II reactivity from plasma of mite-allergic subjects

The only IgE-binding component detectable in the six *Der p* allergenic extracts by Western blot had the electrophoretic mobility of *Der p*II (mol. wt. 14 kDa) (Fig. 3). This does not rule out the possibility, however, that additional allergenic components are present but, like *Der p*I, are not detectable by Western blot. To address this possibility, assays were performed in which the pool of plasma from 18 mite-allergic patients was depleted of IgE reactive to native *Der p*I and/or *Der p*II by incubation with allergen-conjugated Sepharose, then assayed for residual IgE binding to extract components. The plasma depleted of *Der p*I reactivity still contained complete *Der p*II reactivity, and that depleted of

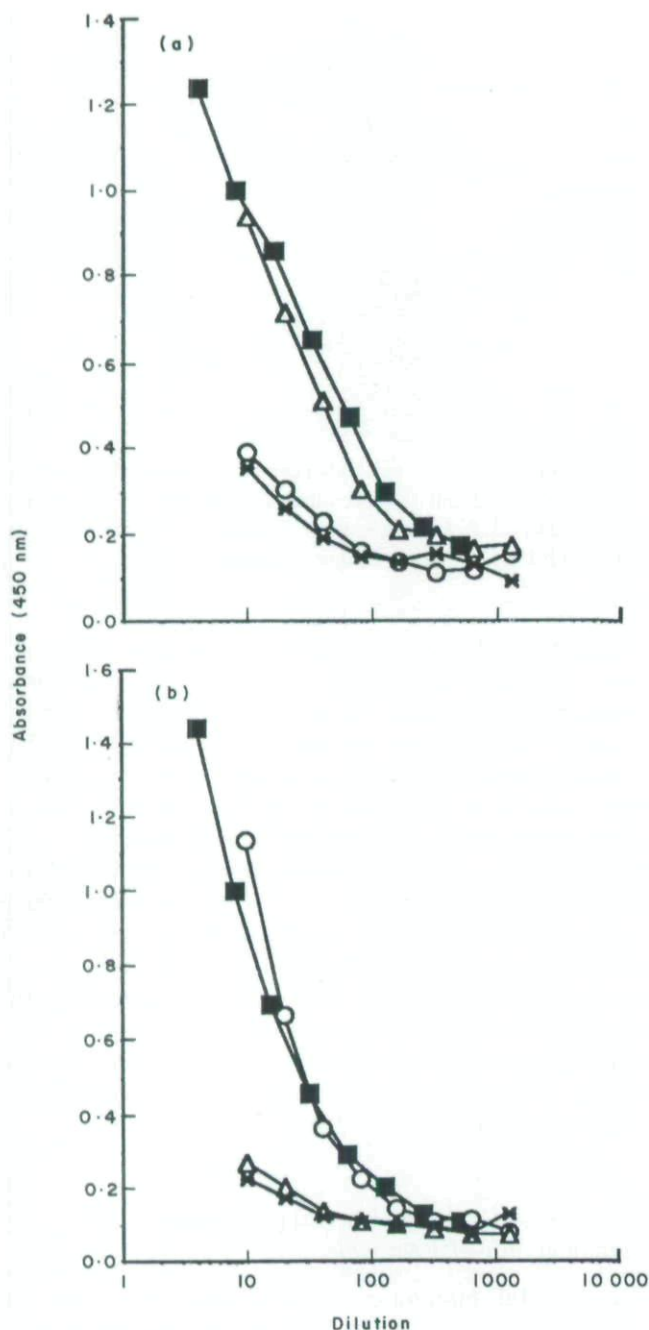


Fig. 4. *Der p*I- or *Der p*II-reactivity of human plasma depleted of mite-specific antibodies. A pool of plasma from 18 mite-allergic patients was incubated with Sepharose conjugated to: (○) *Der p*I; (△) *Der p*II; or (×) *Der p*I and *Der p*II. After incubation, the plasma was tested for residual binding to *Der p*I (a) or *Der p*II (b) by direct ELISA. The binding of untreated plasma is shown for comparison (■).

*Der p*II reactivity retained complete *Der p*I reactivity, as assayed by direct ELISA on plates coated with *Der p*I or *Der p*II (Fig. 4a and b). When plasma samples were

incubated with both *Der p*I- and *Der p*II-conjugated Sepharose, however, the reactivity to both of these purified allergens was effectively depleted (Fig. 4a and b). An approximately 10 times higher plasma concentration was required to achieve a given level of binding to *Der p*I (an absorbance at 450 nm of 0.3) (Fig. 4a) or *Der p*II (an absorbance at 450 nm of 0.2) (Fig. 4b) in the depleted plasma as compared with control (untreated) plasma, indicating an efficiency of depletion of approximately 90% in each case. The plasma depleted of both *Der p*I and *Der p*II reactivity no longer displayed IgE reactivity to the skin test extract (Meridian) by direct ELISA (Fig. 5), suggesting that, within the sensitivity limits of the ELISA, there are no substantial levels of IgE directed against components of the extract other than *Der p*I or *Der p*II. Because the subjects whose plasma comprised the pool used in these studies had been selected for the presence of IgE to *Der p*I and/or *Der p*II, however, it is possible that some mite-allergic subjects with low levels of IgE to these allergens may have a more significant response to minor allergens.

Reactivity of a mite-allergic patient population to *Der p*I and/or *Der p*II

To estimate the prevalence of *Der p*I-exclusive or *Der p*II-exclusive mite responders, plasma from 49-allergic patients (*D. pteronyssinus* skin test 4+) having IgE reactive with mite allergens was tested for IgE to *Der p*I and *Der p*II by direct ELISA. Of these 49 subjects, 31 (63%) had IgE to both major allergens, 12 (24%) had detectable IgE to *Der p*I only, and six (12%) had detectable IgE to *Der p*II only. Thus, the number of *Der p*I-exclusive responders was twice that of *Der p*II-exclusive responders.

Discussion

Allergenic extracts are often crude preparations containing, in addition to allergens, an extensive array of components that do not bind IgE (data not shown). The adoption of international reference standards for allergenic extracts used for diagnosis and treatment ensures the effectiveness and uniformity of different commercially available preparations. Standardization also provides a basis for quantitative comparison of commercial extracts. Standardized allergenic extracts of the house dust mite, *D. pteronyssinus*, are available from a number of sources. Because of the proteolytic activity of the major allergen *Der p*I and even of the minor allergens *Der p*III (trypsin), *Der p*IV (amylase), and *Der p*VI (chymotrypsin) [8–12], however, extracts enriched for these allergens may be susceptible to

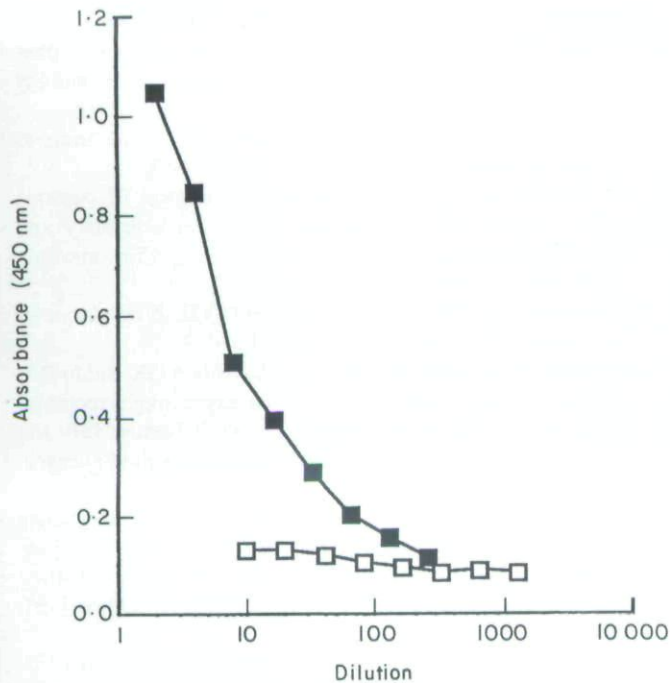


Fig. 5. Binding of human plasma depleted of *Derp*I and *Derp*II reactivity to mite allergenic extract. A pool of plasma from 18 mite-allergic patients was incubated with Sepharose conjugated to *Derp*I and *Derp*II. The depleted plasma was then tested for binding to standardized mite extract (Meridian) by direct ELISA (□). The binding of untreated plasma is shown for comparison (■).

degradation, perhaps resulting in allergen loss and leading to variability in composition. Furthermore, because *Derp*I is a component of mite faeces and *Derp*II is a component of mite bodies, the conditions used for mite culture may influence the relative levels of *Derp*I and *Derp*II in the extracts. Heymann *et al.* [6] have confirmed that mite extracts rich in faecal material contain higher ratios of group I/group II allergens than those rich in mite bodies. Heterogeneity in the composition of commercial *D. pteronyssinus* extracts has previously been demonstrated by Bousquet *et al.* as variability in the nature and degree of enzymatic activities detected in the extracts [19], and by Chapman *et al.* [20] as a greater than 10-fold variation in *Derp*I concentration among different commercial house dust extracts. In this report, we directly compare the content of the major allergens *Derp*I and *Derp*II in a random sampling of six commercially available standardized extracts of *D. pteronyssinus*. Because there may be lot to lot variation in the extracts provided by each manufacturer, this study is not intended as a specific comparison of different commercial sources of extract, but rather as an indicator of the range

of allergen content to be found in standardized preparations.

Among the six commercial extracts tested, there was variability in levels of both *Derp*I (12–30 µg/ml), and *Derp*II (3–15 µg/ml). The ratio of *Derp*I/*Derp*II ranged from 1.1/1 (extract F) to 6/1 (extract B). The variation that we observed in *Derp*I levels is less than that previously reported in a sampling of mite extracts from four different manufacturers, reported by Chapman *et al.* in 1987 [20]. Although this decreased variability may be due to our sampling of extracts, it is conceivable that standardization methods have improved in the past few years. Nevertheless, only one of the commercial extracts that we tested, extract F, contained the same ratio of *Derp*I/*Derp*II as the CBER *D. pteronyssinus* standards that were tested. This ratio, 1.1/1, is the same as that reported for an OBRR/FDA *D. pteronyssinus* standard by Heymann *et al.* in 1989 [6], indicating that extract consistency is achievable. Although the impact of variations in allergen composition on the biological effectiveness of the extracts was not evaluated here, we may speculate that the different extracts would have variable potency, especially in allergic subjects with exclusive sensitivity to either *Derp*I or *Derp*II. Our analysis of plasma isolated from 49 allergic patients (skin test 4+) with mite-specific IgE estimated exclusive responders to comprise approximately one-third of the mite-allergic population.

The biological effectiveness of the extracts may also be influenced by the presence of minor allergens of *D. pteronyssinus*, such as enzymatically active *Derp*III and *Derp*VI. To evaluate the contribution of minor allergens to the IgE reactivity of mite extracts, we depleted mite-reactive plasma samples of IgE directed against *Derp*I and/or *Derp*II. This depletion abolished IgE reactivity to *D. pteronyssinus* extract, a finding which, although it does not rule out the possibility that low levels of minor allergens are present in extracts, is not consistent with a role for allergens other than *Derp*I or *Derp*II in the IgE reactivity of mite-allergic subjects to standardized extracts. It is important to note, however, that subjects were selected for this study based not only on 4+ skin test reactivity to *D. pteronyssinus*, but also on the presence of IgE to *Derp*I and/or *Derp*II. Our analysis of 70 mite-allergic subjects (4+ skin test reactivity) indicates that 75–80% have IgE to these allergens that is detectable by the assay methods utilized here (unpublished observations). The possibility exists, therefore, that mite-allergic subjects that lack IgE to *Derp*I and/or *Derp*II may have a more dominant response to minor allergens.

The effectiveness of standardization depends on the attainment of comparable allergen composition and

potency in standardized extracts. The results reported here argue for more stringent criteria to be adopted for the preparation and monitoring of standardized extracts of house dust mite, such that the absolute and relative levels of the major allergens, *Der p*I and *Der p*II, are comparable among the products of different manufacturers. Such measures would enable the maximum effectiveness of immunotherapy with extracts of *D. pteronyssinus* for the mite-allergic population.

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References

- Platts-Mills TAE, Chapman MD. Dust mites: immunology, allergic disease, and environmental control. *J Allergy Clin Immunol* 1987; 80:755–75.
- Chapman MD, Platts-Mills TAE. Purification and characterization of the major allergen from *Dermatophagoides pteronyssinus*—antigen P₁. *J Immunol* 1980; 125:587–92.
- Lind P. Purification and partial characterization of two major allergens from the house dust mite *Dermatophagoides pteronyssinus*. *J Allergy Clin Immunol* 1985; 76:753–61.
- Yasueda H, Mita H, Yui Y, Shida T. Comparative analysis of the physicochemical and immunochemical properties of the two major allergens from *Dermatophagoides pteronyssinus* and the corresponding allergens from *Dermatophagoides farinae*. *Int Arch Allergy Appl Immunol* 1989; 88:402–7.
- Heymann PW, Chapman MD. Recent progress in mite allergen immunochemistry. *Clin Rev Allergy* 1990; 8:51–68.
- 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 mite (*Dermatophagoides* spp.). *J Allergy Clin Immunol* 1989; 83:1055–67.
- Baldo BA, Donovan GR. House dust mite (*Dermatophagoides* spp.) Allergens: Research directions and priorities in the 1990s. In: Baldo BA, ed, *Molecular approaches to the study of allergens*. Basel: Karger, 1990: 28, 138–58.
- Yasueda H, Mita H, Akiyama K et al. Allergens from *Dermatophagoides* mites with chymotryptic activity. *Clin Exp Allergy* 1993; 23:384–90.
- Stewart GA, Ward LD, Simpson RT, Thompson PJ. The group III allergen from the house dust mite *Dermatophagoides pteronyssinus* is a trypsin-like enzyme. *Immunology* 1992; 75:29–35.
- Stewart GA, Thompson PJ, Simpson RJ. Protease antigens from the house dust mite. *Lancet* 1989; ii:462.
- 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.
- Thomas WR. Mite allergens groups I–VII. A catalogue of enzymes. *Clin Exp Allergy* 1993; 23:350–3.
- 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.
- Calkhoven PG, Aalbers M, Schilte PPM et al. Newly generated IgE antibodies to *Dermatophagoides pteronyssinus* in children are directed against components distinct from *Der p*I and *Der p*II. *J Allergy Clin Immunol* 1991; 88:265–71.
- Chapman MD, Heymann P, Sutherland WM, Platts-Mills TAE. Recognition of species-specific and cross-reacting antigenic determinants on house dust mite (*Dermatophagoides*) allergens using monoclonal antibodies. *Int Archs Allergy appl Immunol* 1985; 77:166–8.
- Luczynska CM, Arruda LK, Platts-Mills TAE, Miller JD, Lopez M, Chapman MD. A two-site monoclonal antibody ELISA for the quantification of the major *Dermatophagoides* spp. allergens, *Der p*I and *Der f*I. *J Immunol Methods* 1989; 118:227–35.
- Chapman MD, Sutherland WM, Platts-Mills TAE. Recognition of two *Dermatophagoides pteronyssinus*-specific epitopes on antigen P₁ by using monoclonal antibodies: binding to each epitope can be inhibited by sera from dust-mite allergic patients. *J Immunol* 1984; 133:2488–5.
- Bond JF, Garman RD, Keating KM et al. Multiple Amb a I allergens demonstrate specific reactivity with IgE and T cells from ragweed-allergic patients. *J Immunol* 1991; 146:3380–5.
- Bousquet J, Hale R, Guerin B, Michel F-B. Enzymatic activities of house dust extracts. *Ann Allergy* 1980; 45:316–21.
- Chapman MD, Heymann PW, Wilkins SR, Brown MJ, Platts-Mills TAE. Monoclonal immunoassays for major dust mite (*Dermatophagoides*) allergens, *Der p*I and *Der f*I, and quantitative analysis of the allergen content of mite and house dust extracts. *J Allergy Clin Immunol* 1987; 80:184–94.

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