

The spectrum of low molecular weight house dust mite (*Dermatophagoides pteronyssinus*) allergens with emphasis on *Der p* II

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Summary

Analysis by protein blotting of sera from 96 different house dust mite-allergic subjects revealed previously unrecognized complexity of low molecular weight (MW) (< 20 kD) IgE-binding proteins in extracts of whole bodies of *Dermatophagoides pteronyssinus*. Of 11 different IgE-binding components of MW < 20 kD identified, two (MW ~ 16 kD and ~ 15 kD), showed both a high frequency (88% and 49% respectively) and a high intensity of IgE-binding. The ~ 16 kD component, identified as allergen *Der p* II, showed the highest frequency of IgE antibody reactivity of any of the major *D. pteronyssinus* allergens including *Der p* I and *Der p* III.

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Introduction

Despite the clinical and research interest shown in the house dust mite *Dermatophagoides pteronyssinus* in recent years [1–3], emphasis has generally been confined to a few 'major' allergens [1] and the 'complete' spectrum of the many allergens in this species has not been defined and confirmed.

With the introduction of protein blotting [4] and its application to the identification of allergens in a diverse range of plant and animal materials [5,6], including *D. pteronyssinus* [2,7–9], definitions of allergens in many sources have been confirmed and extended. Application of protein blotting to *D. pteronyssinus* whole body and culture extracts has revealed the presence of a larger number of allergens than previously reported [2,8] and demonstrated some important aspects of the detection and/or stability of allergens *Der p* I [10] and *Der p* II [9]. In particular, these studies demonstrated several low molecular weight (MW) IgE-binding components that were similar in both culture and whole body extracts.

Blotting of *D. pteronyssinus* whole body proteins followed by probing with a large number of sera from

mite-allergic subjects was effective in delineating a set of allergenic proteins with closely related electrophoretic mobilities and MWs of < 20 kD. Results from electrophoretic studies demonstrating the complexity of this group of rapidly migrating IgE-binding proteins which reinforce the importance of *Der p* II as a mite allergen are presented.

Materials and methods

House dust mite extract and allergens

Dried, visually clean mite (*D. pteronyssinus*) bodies (1 g) (Commonwealth Serum Laboratories (CSL), Parkville, Victoria, Australia) were homogenized in glass using a Teflon blender for 15 min at 0°C in 20 ml of phosphate-buffered saline (PBS) pH 7.3. After sonication for 5 min, the extract was stirred at 4°C for 1 hr, centrifuged at 18 000 g for 30 min, and concentrated by ultrafiltration to 14 mg/ml of protein, determined by the method of Bradford [11]. All preparative steps were carried out in a cold room at 4°C or with cooling by ice. The extract was stored at –20°C.

Purified *Der p* I was provided by Dr T. A. E. Platts-Mills (Charlottesville, Virginia, U.S.A.).

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Sera

Sera from 96 house dust mite-allergic subjects diagnosed on the basis of case history, a positive skin-prick test to *D. pteronyssinus* extract (CSL or Hollister-Stier, Spokane, U.S.A.) and a positive RAST (3+ or 4+) to Pharmacia (Uppsala, Sweden) *D. pteronyssinus* allergen discs were obtained from the Allergy Clinic, Royal North Shore Hospital, Sydney. Cord serum and sera from non-allergic and pollen-allergic subjects were obtained and tested as previously described [7,9].

A mouse monoclonal antibody to *D. pteronyssinus* allergen *Der p* II (DpX) was provided by Dr R. C. Aalberse (Red Cross Blood Transfusion Service, Amsterdam, The Netherlands).

Sodium dodecylsulphate-polyacrylamide gel electrophoresis and electrotransfer to nitrocellulose

Sodium dodecylsulphate (SDS)-polyacrylamide gel electrophoresis (PAGE) [12] was carried out as described previously [8]. Mite proteins (1.5 mg), without prior reduction with 2-mercaptoethanol, in 300 µl of buffer were applied to each gel and subjected to electrophoresis at 200 V for 15 hr. Materials and methods for electrotransfer of separated proteins were as described previously [7,13] with the exception that nitrocellulose (NC) transfers were blocked for 17 hr at room temperature (24°C) with 0.1% Tween 20 in PBS [9].

Probing of nitrocellulose blots and autoradiography

Blocked 5-mm wide NC strip transfers were incubated overnight with sera (individual or pooled samples) diluted 1–10 in PBS containing 0.005% Tween 20, 0.01% sodium azide and 0.5% bovine serum albumin (BSA), washed extensively for 30 min with 0.05% Tween 20 in PBS and incubated overnight with [¹²⁵I]anti-IgE (Pharmacia) 80 000 c.p.m./strip in 0.05% Tween 20–BSA buffer. After washing with 0.005% Tween 20 in PBS, strips were air-dried and loaded into a Kodak X-omatic cassette, KP68375 (Eastman Kodak Co., Rochester, U.S.A.) together with photographic film (Fuji Rx-safety, Tokyo, Japan). The cassette was stored at –70°C for 3 days. Incubation buffer, cord serum, sera from non-allergic healthy adults and sera from subjects allergic to pollens but not house dust mites were used as controls.

Results

Identification of IgE-binding mite components by protein blotting

Protein blotting studies employing mite whole body extract and sera from 96 different subjects allergic to

D. pteronyssinus revealed a total of 32 different IgE-binding components, 11 of which had MW < 20 kD (Fig. 1). Three of the proteins identified (numbers 9, 10 and 11) (strips m and n) were found to have MW ≤ 10 kD. The MW of the smallest IgE-binding component (number 11) was only ~6 kD. Employing a monoclonal antibody specific for *Der p* II (strip r), it was possible to demonstrate that component number 5 (MW ~16 kD) was *Der p* II. Under these electrophoretic conditions *Der p* II runs with an apparent MW of ~16 kD. Because of the frequent recognition and heavy staining of *Der p* II, component 6 was often difficult to detect. Close examination of strip h, however, revealed the presence of components 6 and 7 close together while strip g clearly showed components 5 and 7 and suggested that the heavy staining of component 5 may be obscuring the presence of component 6.

Some sera reacted strongly with components of MW < 20 kD (particularly *Der p* II) but did not bind to *Der p* I (located at MW ~25 kD on our gels) (Fig. 1, strips c, e, f, i, j, k, l and o). Five of the sera studied (RAST radioactive uptakes of [¹²⁵I]anti-IgE of 6.1, 12.9, 21.4, 23.9 and 41.1%) showed monospecific recognition of *Der p* II (for example see strips f and k, Fig. 1).

No bands were detected on the autoradiographs when cord serum, or sera from subjects allergic to ryegrass pollen or subjects who were not clinically allergic to mites, were used to probe mite protein transfers prior to the addition of [¹²⁵I]anti-human IgE (Fig. 1, strips t and u). Likewise, no reaction was detected between the transferred mite proteins and the labelled anti-IgE alone (strip v).

Frequency of recognition of the 11 low MW mite IgE-binding components

By far the most commonly recognized component was *Der p* II (component 5) which reacted with IgE antibodies in the sera of 88% of subjects. Only two of the other 10 components, numbers 4 and 7, reacted with more than 20% of the sera tested (Table 1). Component 7 (MW ~15 kD) was recognized by IgE antibodies in almost half of the sera examined.

Recognition of allergen *Der p* II relative to other important mite allergens

The importance of allergen number 5 (*Der p* II) and its pre-eminent position among the 32 IgE-binding components of *D. pteronyssinus* identified so far [2], can readily be seen from the frequency values listed in Table 2. A comparison of the three major allergens *Der p* I, *Der p* II and *Der p* III revealed that the recognition frequency

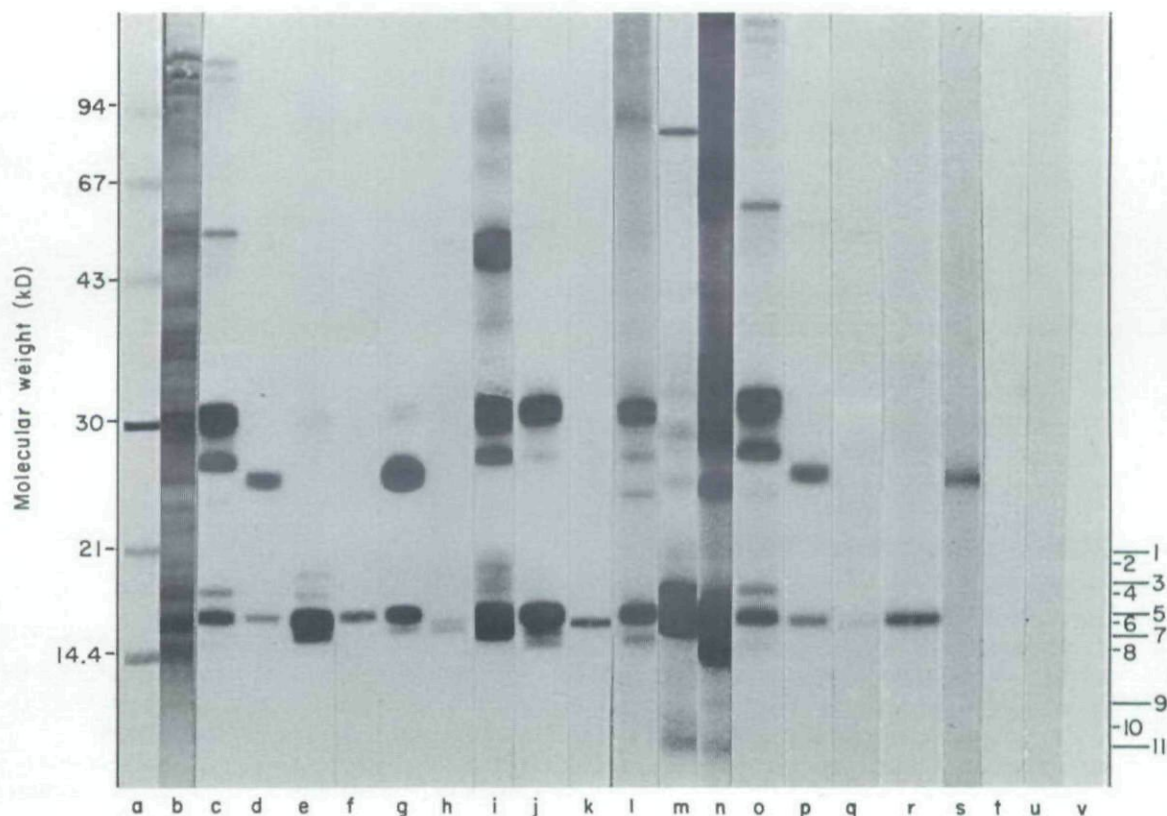


Fig. 1. Identification by autoradiography of the IgE-binding *D. pteronyssinus* body components present in the MW region ~6–20 kD. Mite proteins were immobilized on nitrocellulose strips (b–v) following their separation by SDS-PAGE. Strips c–v are autoradiographs. Strip a, low MW markers, b, amido black protein stain of separated mite body components. Strips c–q with transferred mite proteins incubated first with individual human serum samples from house dust mite-allergic subjects and then with [^{125} I]anti-human IgE. Strip r, incubated first with anti-*Der p* II monoclonal antibody and then with [^{125} I]goat anti-mouse IgG heavy and light chains (Cappel, West Chester, U.S.A.). Strip s, allergen *Der p* I, immobilized on nitrocellulose following SDS-PAGE and then detected with a serum pool from mite-allergic subjects followed by [^{125} I]anti-human IgE; t, incubated with cord serum followed by [^{125} I]anti-human IgE; u, incubated first with serum from a subject non-allergic to house dust mites but highly allergic to grass pollens, and then with [^{125} I]anti-human IgE; v, incubated with [^{125} I]anti-human IgE alone. Component number 5, strips j, p and q represent examples of high (3+), medium (2+) and low (1+) levels respectively of IgE-antibody binding (see Table 3).

for *Der p* II alone was 9%. For *Der p* I and *Der p* III, however, the recognition frequencies of these allergens alone were 0 and 1% respectively.

If one looks at the frequencies of recognition of only one of these three allergens (*Der p* I, *Der p* II or *Der p* III) together with any one, or more of the other 29 IgE-binding components identified, the recognition frequency of *Der p* II (19%) again predominates. Recognition of *Der p* II, *Der p* I or *Der p* III, regardless of the combination of other allergens recognized, occurred in 88, 43 and 68% of subjects respectively (Table 2).

Estimation of the quantity of specific IgE bound to each of the low molecular weight allergens identified

By assessing, on an arbitrary scale, the intensity of the different bands on autoradiographs (Fig. 1, see IgE-

binding component 5, strips j, p and q), an attempt was made to determine which of the low MW mite components bound the greatest quantity of [^{125} I]anti-IgE (and hence IgE) antibodies (Table 3). Of the 11 components identified with MW < 20 kD, only two (components 5 and 7) showed strong (3+) binding of IgE/[^{125} I]anti-IgE with a high proportion (74% and 47% respectively) of subjects' sera. Strong binding (as shown by the density of a band on the autoradiograph) of components 1, 2, 3, 4 and 8 occurred with at least one serum but, for all components other than 5 and 7, the majority of positive sera showed only weak binding (Table 3).

Discussion

Although dialysable fractions of house dust mite extracts have been reported to contain potent allergenic activity

Table 1. Frequencies of recognition by IgE antibodies of low molecular weight *Dermatophagoides pteronyssinus* allergens

Allergen number	Approximate MW (kD)	Frequency of recognition* of allergen
1	19	9.6
2	18	19.1
3	17.5	17.0
4	17	21.3
5†	16	88.3
6	15.5	3.2
7‡	15	49.0
8	14	12.8
9	10	4.3
10	8	2.1
11	6	2.1

* Frequencies of recognition by IgE antibodies in sera of 96 house dust mite-allergic subjects.

† *Der p* II.

‡ Recently cloned as a recombinant protein and shown to bind IgE antibodies [14,15].

Table 2. Frequencies of recognition by IgE antibodies of *D. pteronyssinus* allergen *Der p* II relative to allergens *Der p* I and *Der p* III

Recognition of allergen(s)				
<i>Der p</i> II	<i>Der p</i> I	<i>Der p</i> III	One or more of the other 29 allergens	Frequencies of recognition* of allergens(s)
+	-	-	-	9
-	+	-	-	0
-	-	+	-	1
+	-	-	+	19
-	+	-	+	0
-	-	+	+	4
+†				88
	+‡			43
		+§		68

* Frequencies of recognition by IgE antibodies in sera of 96 house dust mite-allergic subjects.

† Recognition of *Der p* II alone or with any one, or more, of the other 31 allergens including *Der p* I and *Der p* III.

‡ Recognition of *Der p* I alone or with any one, or more, of the other 31 allergens including *Der p* II and *Der p* III.

§ Recognition of *Der p* III alone or with any one, or more, of the other 31 allergens including *Der p* I and *Der p* II.

Table 3. Classification of binding of IgE antibodies in subjects' sera according to the estimated quantity of IgE bound* to each of the low molecular weight mite allergens

Allergen		Number of sera showing IgE binding			
No.	Approximate MW (kD)	Total	Strong†	Medium†	Weak†
1	19	9	1	1	7
2	18	18	2	3	13
3	17.5	16	2	2	12
4	17	20	5	2	13
5‡	16	84	62	9	13
6	15.5	3	0	1	2
7	15	47	22	9	16
8	14	12	4	3	5
9	10	4	0	0	4
10	8	2	0	0	2
11	6	2	0	1	1

* Directly proportional to the amount of [¹²⁵I]anti-IgE retained by each mite component transferred to nitrocellulose and reacted with IgE antibodies in subjects' serum.

† Estimate on a scale of 1+ (weak) (see component 5, strip q), 2+ (medium) (components 5, strip p) or 3+ (strong) (component 5, strip j), of intensity of staining on autoradiographs following reaction of nitrocellulose transfers with subjects' sera and then [¹²⁵I]anti-human IgE.

‡ Allergen *Der p* II.

[16], and protein blotting studies have shown a number of IgE-binding *D. pteronyssinus* components of MW < 20 kD [8,9], the experiments described here have revealed a previously unrecognized complexity of allergenic proteins in the 'low' MW region (i.e. < 20 kD) of *D. pteronyssinus* components in whole body extract. This complexity was shown by examining a far larger number of sera from house dust mite-allergic subjects than appears to have been examined in the past and by the employment of optimized blocking conditions that lead to increased immunodetection of IgE-binding components [9].

Der p II has been established as a major low MW allergenic component of *D. pteronyssinus* [2,17]. Our results reinforce this conclusion and suggest that this allergen is the most important of the 32 IgE-binding components identified so far in *D. pteronyssinus* whole body extract [2]. Eighty-eight per cent of the 96-mite-allergic subjects examined had serum IgE antibodies that reacted with *Der p* II while only 43% and 68% of subjects had antibodies complementary to *Der p* I and *Der p* III, respectively. IgE antibody reactivities of *Der p* I, II and

III, with or without recognition of other allergens, also emphasized the dominant position of *Der p* II.

The experiments described here also clearly showed that the low MW mite components that demonstrated the highest frequencies of IgE-binding (components 5 (*Der p* II) and 7) also showed the greatest intensity of binding as shown by the depth of staining on the autoradiographs. Conversely, components showing low binding frequencies with subjects' sera also demonstrated a low intensity of antibody binding. This may prove to be a common feature of major allergens in *D. pteronyssinus* and other allergenic extracts.

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