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PURIFICATION AND CHARACTERIZATION OF THE MAJOR ALLERGEN FROM *DERMATOPHAGOIDES PTERONYSSINUS*-ANTIGEN P_1[1]

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A purified allergen was obtained from *D. pteronyssinus* culture by gel filtration, block electrophoresis, and preparative isoelectricfocusing (IEF). The antigenic activity on IEF was predominantly distributed in two peaks (pI 4.7 to 5.4 and pI 6.6 to 7.1), which were completely cross-reactive on immunodiffusion and by cross-inhibition of radioimmunoassay. These fractions had the same m.w. (24,000) and a very similar amino acid composition. The common antigen in each fraction was designated *Dermatophagoides pteronyssinus* antigen P_1. In seven, symptomatic mite allergic subjects skin test reactivity to antigen P_1 and crude mite extract was closely correlated. There was also a very good correlation between IgE-binding activity (BA) for P_1 measured by antigen-binding radioimmunoassay and IgE antibody (ab) to *D. pteronyssinus* measured by using the radioallergosorbent test (RAST) ($r = 0.82$, $p < 0.001$). RAST experiments showed that up to three-quarters of the IgE ab to *D. pteronyssinus* was directed against P_1, and that IgE ab to P_1 accounted for on average 12% of the total serum IgE. These data strongly suggest that P_1 is the major allergen of *D. pteronyssinus*. By immunodiffusion, SDS-PAGE, and radioimmunoassay antigen P_1 appeared to be present as a large proportion (10 to 20%) of the protein in crude mite extract. Our results support the view that the importance of an inhalant allergen is largely determined by its physical properties, and by the quantities inhaled during natural exposure.

Positive immediate wheal and flare responses to prick tests with extracts of house dust were first reported over 50 years ago (1, 2). In the United Kingdom 5 to 10% of the population develop symptoms from dust, and in many parts of the world up to 85% of patients with extrinsic asthma show positive skin tests with dust extracts (3–5). The identification of a pyroglyphid mite, *Dermatophagoides pteronyssinus*, as the most important source of allergens in house dust by Voorhorst *et al.* (6) was a major breakthrough in house dust allergy. The occurrence of this mite in dust samples from many countries appeared to explain the "universal" nature of house dust allergens (6). An analysis of over 100 dust samples showed that mite numbers correlated well with the allergenic potency of dust extracts (7). Moreover, reactivity to house dust and to mites of the genus *Dermatophagoides* has been closely correlated by using skin testing, the radioallergosorbent test (RAST)[3] and provocation tests (3–9). There is good evidence that allergy to *Dermatophagoides* sp accounts for a large proportion of allergic reactions to dust in Europe (8, 10, 11), Asia (4), and the west coast of the United States (12). On the other hand house dust may also contain other important allergens, e.g., animal danders and fungal spores (13). Mites are probably a less common source of dust allergens in the eastern United States and in mountainous areas of Switzerland (6, 14).

Many different pollen allergens have been obtained in a highly purified form. These allergens have been widely used for studies on antibody (ab) responses and cellular responses, and also as standards for the allergen content of pollen extracts (15–17). The characterization of mite allergens by isoelectricfocusing (IEF) (18, 19), gel filtration (10, 20), ion-exchange (10, 19), or affinity chromatography (21) has not resulted in the isolation of a purified allergen. Recently, we reported the partial purification of a mite allergen, F_4P_1, which could be used in an antigen-binding radioimmunoassay (RIA) to quantitate IgG-, IgA-, and IgE-binding activity (BA) for *D. pteronyssinus* (22). The F_4P_1 preparation (m.w. 15 to 25,000) had very good skin test reactivity and up to 68% of the ^{125}I-labeled material could be bound by hyperimmune sera in RIA. We report here the purification and characterization of the major allergen to *D. pteronyssinus*-antigen P_1. The antigen is present on a heat-sensitive glycoprotein that has a m.w. of 24,000 and exhibits a wide range of isoelectric points. Antigen P_1 appears to comprise a substantial proportion of the protein in crude mite extract.

MATERIALS AND METHODS

Purification of antigen P_1. The preparation of a partially purified *D. pteronyssinus* allergen, F_4P_1, from an aqueous extract of *D. pteronyssinus* culture, Extract I (ExI), has been described in detail previously (22). Briefly, gel filtration of ExI on Sephadex G-100 yielded six fractions that were tested by skin testing and direct RAST. The most allergenic fraction, F_4 (m.w. 15 to 25,000), gave two protein peaks after pevikon block electrophoresis one of which migrated with β-mobility and reacted strongly with rabbit antiserum to ExI on immunodif-

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[3] Abbreviations used in this paper: RAST, radioallergosorbent test; BA, binding activity; ExI, an aqueous extract of whole *D. pteronyssinus* culture; IEF, isoelectricfocusing; BBS, borate-buffered saline, pH 8.0; SDS-PAGE; polyacrylamide gel electrophoresis in sodium dodecyl sulfate; RIA, radioimmunoassay; HSA, human serum albumin; Rye I, the group I protein from rye grass pollen; CM, culture medium.

fusion. This fraction, F_4P_1, was rechromatographed over Sephadex G-100 and further purified by preparative IEF using the LKB 2117 Multiphor (23). The reagents and apparatus for IEF were obtained from LKB, Bromma, Sweden. A gel containing 4.0% (w/v) Ultrodex, 3.5% (v/v) ampholines, pH 3.5 to 10, 0.5% (v/v) ampholines, pH 9 to 11, and 12 mg F_4P_1 in 100 ml distilled water was poured into a 24.5 x 11.0 x 0.5 cm acrylic frame containing electrode strips soaked in either 1 M NaOH (−) or 1 M H_3PO_4 (+). The gel was air dried for 4 to 5 hr and focused at a constant 8 watts for 15 to 16 hr at 10°C. Gel sections (0.5 cm) were then eluted twice with 2.5 ml borate-buffered saline, pH 8.0 (BBS), and centrifuged at 2000 × G for 15 min. The pH, protein content, and F_4P_1 content of the first eluate were measured. Two peaks designated Fr.5 and Fr.14 were pooled on the basis of their F_4P_1 content (see *Results*, Fig. 1). Carrier ampholines were removed from these samples by gel filtration through a 35.0 x 1.0 cm column of Sephadex G-50. The final yield of Fr. 5 and Fr. 14 was 4 mg and 2 mg, respectively. For RIA 10 to 25 μg of allergen solutions were labeled with 1.0 mCi of ^{125}I by using a modified chloramine-T technique (24). The specific activity of the labeled allergens was 20 to 40,000 cpm/ng and up to 85% of the radioactivity was precipitable with trichloroacetic acid.

Gel electrophoresis. Analytical sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed in a slab gel apparatus by using a discontinuous buffer system (25). The resolving gel contained 15% acrylamide in 0.38 M Tris-HCl, pH 8.8. It was overlaid with a spacer gel containing 5% acrylamide in 0.13 M Tris-HCl, pH 6.8. Both gels contained 0.1% SDS. Allergen solutions containing 5 to 10 μg of protein were boiled for 2 min in 4% SDS, 0.1 M dithiothreitol, and 8 M urea and applied to sample wells. Electrophoresis was carried out for 3 to 4 hr at 15 mA with electrode buffer containing 0.025 M Tris, 0.19 M glycine, and 0.1% SDS, pH 8.3.

Preparation of purified human antibodies to D. pteronyssinus. Serum was obtained from two mite allergic individuals. The ab from each donor was prepared by passing 220 ml serum in 0.1 M ethylenediaminetetraacetic acid over a 2.5-ml column of Sepharose 2B linked to ExI by using the cyanogen bromide technique (26). The flow rate was 10 to 15 ml/hr. The column was washed with 500 ml BBS and then eluted with 40 ml 0.5 M glycine, pH 2.8. Fractions of 2 ml were collected in tubes containing 0.5 ml 2 M Tris-HCl, pH 8.5. The fractions was assayed for IgE ab by antigen-binding RIA (22), pooled and concentrated down to 1 ml. In each case, approximately 20% of the applied ab was recovered.

Double antibody inhibition RIA for F_4P_1 or P_1. One-tenth milliliter of 1/20,000 rabbit antibody to ExI in 1/50 normal rabbit serum was incubated with 0.1 ml of diluted sample for 2 hr in plastic tubes. Seven nanograms of ^{125}I-labeled F_4P_1 in a volume of 0.1 ml were added for a further 2 hr. The complex was then precipitated with 0.1 ml of goat anti-rabbit IgG diluted 1:6, sufficient to precipitate all the rabbit IgG, for 16 hr at 4°C. The precipitates were washed three times in BBS and counted in a gamma-counter (NE 1600, Nuclear Enterprises Ltd., Edinburgh, United Kingdom). Samples were diluted in 0.3% BSA BBS. Serial 2-fold dilutions of a preparation of F_4P_1 (22) were made from 1 μg/ml to 1 ng/ml and used to construct a control curve from which values for unknown samples were obtained. This assay was modified for the determination of antigen P_1 by using 2 ng ^{125}I-labeled Fr. 14 and dilutions of cold Fr. 14 for the control curve. In cross-inhibition studies cold Fr. 5 or Fr. 14 at 4.0, 0.5, 0.125, or 0.032 μg/ml was used to inhibit the binding of 2 ng of ^{125}I-labeled Fr. 5 or Fr. 14 by either IgG or IgE ab in a preparation of specifically purified ab from an allergic subject. The ab was diluted 1/160 in 1/50 normal human serum. Monospecific goat antiserum to the Fc fragment of either human IgG or IgE was used as a second antibody to precipitate.

In the stability experiments 20 μg ExI or F_4P_1 in 0.1 ml BBS (pH 8.0) was incubated for 1 hr at room temperature, 37, 56, or 80°C. The same quantities of allergen were incubated in 0.1 M buffers at pH 2.0, 4.0, 7.0, 9.4, and 12.0 for 16 hr at 37°C. The pH was neutralized with 0.1 ml 0.3 M phosphate buffer, pH 7.7. All the experiments were performed in duplicate. Dilutions of the heat- or pH-treated samples were assayed for P_1 content as described above.

RAST, RAST absorption. The RAST was performed with paper discs coated with ExI as described previously (22). RAST absorption was carried out as described by Gleich and Jacob (27). Dilutions of serum (1:2 to 1:20) or specific ab (1:200 to 1:400) were absorbed overnight with 0.5 ml 2% Sepharose beads coupled to ExI, antigen P_1 (IEF Fr. 5) or human serum albumin (HSA). The beads were removed by centrifugation and the supernatant was assayed for total IgE.

Skin testing. Intradermal skin tests were carried out by injecting 0.05 ml of serial 10-fold dilutions of each allergen preparation in 0.05% HSA in phenol saline. The allergen solutions were sterilized through an 0.22-μm filter (Millipore Corporation, Bedford, Mass.) before use. The skin response was measured after 15 min and a wheal of >6 x 6 mm was regarded as a positive (++) response.

RESULTS

Purified allergens from IEF. The partially purified allergen, F_4P_1, showed only a single major band on Laurell electrophoresis but multiple bands on IEF over a wide pI range. This suggested two possibilities; either that the preparation contained other proteins that could be separated by IEF, or that the allergen was present in multiple isoelectric forms. Preparative IEF was used to determine the distribution of allergen at different isoelectric points. The antigenic activity detected by inhibition RIA eluted almost in parallel with protein and was predominantly distributed in two peaks, fraction 5 (Fr. 5) and Fr. 14 with pI of 4.7 to 5.4 and 6.6 to 7.1, respectively (Fig. 1). On immunodiffusion with rabbit antiserum a single line was seen against Fr. 5 and Fr. 14, which showed complete identity. This line was identical with the major line against ExI (Fig. 2). Highly concentrated extract of the *D. pteronyssinus* culture

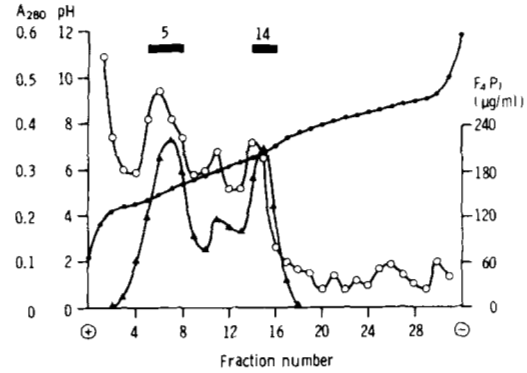

Figure 1. Preparative isoelectricfocusing of partially purified *D. pteronyssinus* extract, F_4P_1. The pH (●——●), protein content (absorbance at 280 nm) (○——○), and F_4P_1 content (▲——▲) of each fraction is shown. The quantity of F_4P_1 was determined by inhibition RIA, by using immunosorbent purified rabbit antibody to ExI. Fr. 5 and Fr. 14 were pooled as indicated (▬).

medium (CM) also showed a single line, but this line showed no cross-reaction with either Fr. 5 or Fr. 14 (Fig. 2). The fractions were also compared by using cross-inhibition of RIA. Dilutions of cold Fr. 5 or Fr. 14 were used to inhibit the binding of ^{125}I-labeled Fr. 5 or Fr. 14 by either IgG or IgE ab from an allergic person (see *Methods*). The inhibition curves were very similar when using either the homologous or the heterologous antigen as inhibitor (Fig. 3). In both cases binding could be inhibited by up to 94% with the opposite fraction. Furthermore, absorption of allergic sera with Fr. 5 linked to Sepharose beads completely removed (>95%) the BA for radiolabeled Fr. 14 (data not shown). In keeping with the view that Fr. 5 and Fr. 14 showed antigenic identity both fractions gave very similar skin test responses in allergic individuals. Quantitative intradermal skin tests on 11 skin test-positive individuals and four nonallergic controls showed a good correlation between sensitivity to each purified fraction and sensitivity to ExI (Table I). With Fr. 5 two individuals gave positive (++) reactions using 10^{-5} μg/ml of purified allergen (i.e., ~5 pg). One subject (N.J.) who was only weakly sensitive to mites gave a ++ skin response to a 100-fold higher dilution of ExI than of the purified fractions, suggesting that he was probably reacting to different allergens in the crude mite extract. On the basis of all these criteria Fr. 5 and Fr. 14 appeared to be antigenically identical.

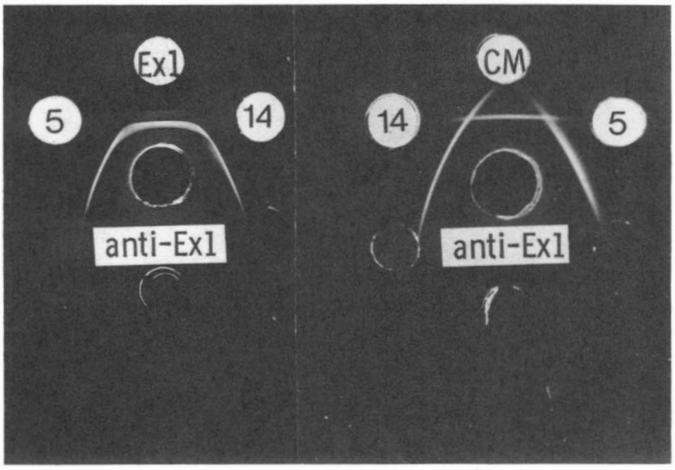

Figure 2. Immunodiffusion analysis of purified fractions. The center well contained hyperimmune rabbit antiserum to extract of whole *D. pteronyssinus* culture (anti-Ex I) (22). Immunodiffusion against ExI, IEF fractions 5 and 14, and extract of the culture medium (CM) is shown.

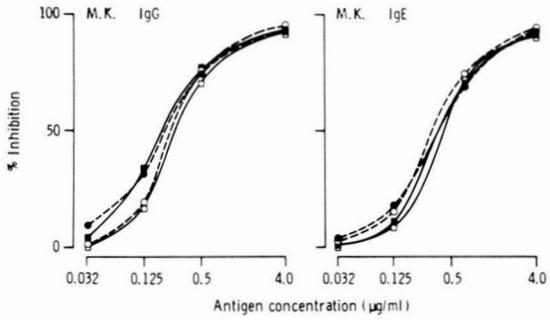

Figure 3. Cross-inhibition by using IEF purified fractions. Binding by human IgG or IgE antibody to ^{125}I-labeled Fr. 5 (*circles, dashed line*) or ^{125}I-labeled Fr. 14 (*squares, solid line*) was inhibited by using either the homologous (*closed symbols*) or heterologous antigen (*open symbols*). Human antibodies were precipitated by using a second antibody; goat anti-IgG or goat anti-IgE, respectively.

TABLE I

Intradermal skin tests with D. pteronyssinus allergens

Subject	Minimum Concentration of Allergen Giving a Positive Skin Test (μg/ml)[a]		
	Extract I	Fr. 5	Fr. 14
W.S.	10^{-1}	10^{0}	10^{0}
T.S.	10^{-4}	10^{-4}	$10^{-3.5}$
N.D.	10^{-5}	10^{-5}	10^{-4}
A.J.	10^{-4}	$10^{-4.5}$	10^{-4}
M.C.	10^{1}	10^{0}	10^{1}
N.J.	10^{-2}	10^{0}	10^{0}
M.K.	10^{-5}	10^{-5}	$10^{-4.5}$
J.S.	$10^{-2.5}$	$10^{-2.5}$	10^{-2}
E.T.	10^{-3}	$10^{-3.5}$	10^{-3}
S.J.	$10^{-3.5}$	10^{-4}	10^{-3}
R.B.	$10^{-3.5}$	10^{-4}	$10^{-3.5}$
Controls × 4	$>10^{1}$	$>10^{1}$	$>10^{1}$

[a] Intradermal skin tests were carried out by using 0.05 ml of serial 10-fold dilutions of allergens. A wheal of >6 × 6 mm was regarded as a positive (++) response. Values were extrapolated to the nearest ½ log when the lower dilution gave a strongly positive reaction. 10^{0} = 1 μg/ml. Negative skin tests at 10 μg/ml are recorded as $>10^{1}$.

TABLE II

Amino acid analysis of Fr. 5 and Fr. 14[a]

	Residues/100 Residues of Amino-Acids		Residues/Molecule[b]
	Fr. 5	Fr. 14	
Asx	14.3	13.9	24
Thr	4.6	4.4	8
Ser	7.6	7.8	13
Glx	12.2	12.9	21
Pro	4.9	5.2	9
Gly	11.6	10.3	20
Ala	9.5	9.3	17
½ Cys	2.0	1.0	4
Val	4.2	5.2	7
Met	1.2	1.2	2
Ileu	6.3	6.4	11
Leu	4.3	4.4	8
Tyr	5.2	5.4	9
Phe	2.3	2.2	4
His	2.4	2.6	4
Lys	2.1	2.1	4
Arg	5.4	5.6	10

[a] Twenty micrograms of Fr. 5 and Fr. 14 were hydrolysed *in vacuo* with 1 ml 6 N HCl for 24 hr at 110°C and applied to a Beckman Model 120C amino acid analyser.

[b] Preliminary calculations of the numbers of amino acids/molecule were made for Fr. 5, assuming a m.w. of 24,000, a carbohydrate content of 8.4% (Dr. E. F. Hounsell—personal communication), and that no other components were present. Values are given to the nearest integer.

The amino acid composition of each fraction was very similar apart from slight differences in the numbers of glycine and valine residues (Table II). With the SΔQ system (30) for relatedness among proteins, Fr. 5 and Fr. 14 differed by 4 units, indicating that they were "identical proteins". In contrast, when Fr. 5 was compared with the published amino acid analysis of the purified pollen allergen Rye I (16) it differed by 140 units. On SDS electrophoresis each fraction migrated as a single homogeneous band with an apparent m.w. of 24,000 (Fig. 4). It was also clear that the fractions migrated to the same position as the most heavily staining band in ExI. Since Fr. 5 and Fr. 14 could not be distinguished by their reaction with antibodies, by amino acid analysis or SDS-PAGE it seemed likely that they

represented minor structural modifications of a single protein or glycoprotein.

The common antigen in each fraction has been designated *Dermatophagoides pteronyssinus* antigen P_1. The stability of the antigen was assessed by using a double antibody inhibition RIA (see *Methods*). The antigen is stable at temperatures up to 37°C, loses 50% of its antigenicity after 1 hr at 56°C, and is rapidly denatured at 80°C. Antigen P_1 is stable at neutral pH, but after 16 hr loses more than 50% of its antigenicity at pH 4.0 or pH 9.4, and is completely denatured at pH 2.0 or pH 12.0 (data not shown).

IgE antibodies to P_1 in allergic individuals. Skin test reactivity to purified preparations of P_1 correlated with reactivity to crude mite extract in seven symptomatic, mite-allergic subjects who were highly sensitive to ExI (++ reaction with 10^{-3} μg/ml) (Table I). In serum there was an excellent quantitative correlation between IgE ab to P_1 measured by antigen binding assay and IgE ab to *D. pteronyssinus* measured by RAST ($r = 0.82$, $p < 0.001$, $n = 30$). For six sera from allergic persons the quantities of IgE ab to ExI and to P_1 were compared by RAST absorption (Table III). In these samples IgE ab to ExI and to P_1 formed from 15 to 36% and 9 to 21% of the total IgE, respectively. A much higher percentage of the total IgE was directed against each allergen in the two preparations of specific antibody. In all cases IgE ab to P_1 accounted for a large proportion (up to 72%) of the IgE ab to ExI. The quantities of IgE BA and total IgE absorbed by RAST beads coated with P_1 were used to make an estimate of the amount of IgE represented by 1 unit of IgE BA (Table III). These calculations gave a mean value of 1.13 ± 0.44 ng IgE ab/unit IgE BA. When this value was applied to the previous measurements of IgE BA on sera from three groups of mite allergic individuals, IgE ab to P_1 accounted for 8.4 ± 11.5% (n = 54), (31), 10.8 ± 7.4% (n = 60) (32), and 17.4 ± 12.1% (n = 44) (22) of the total IgE. These values (mean 12%) are very similar to those reported by using purified major allergens from pollens (33–35).

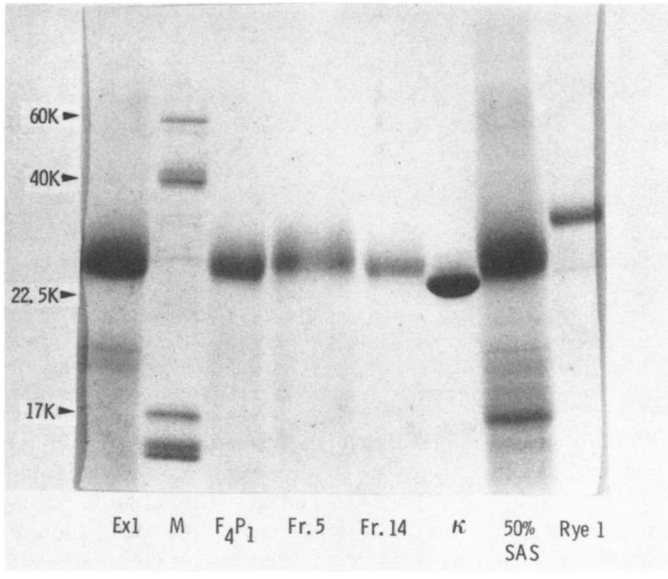

Figure 4. Analysis of *D. pteronyssinus* antigens by SDS-PAGE. A variety of markers were compared with Fr. 5, Fr. 14, F_4P_1, and ExI. *M*, marker solution containing catalase (m.w. 60,000), peroxidase (m.w. 40,000), myoglobin (m.w. 17,000), hemoglobin (m.w. 15,000), and cytochrome C (m.w. 13,000). κ, purified kappa light chain (human Bence-Jones protein) (m.w. 22,500). 50% SAS, an enriched preparation of ExI that precipitated in the presence of 50% saturated ammonium sulfate.

TABLE III

Estimation of the absolute quantities of IgE ab to mite allergens

Patient	Sample	IgE BA for P_1[a]	Total IgE[b]	% Total IgE Directed against[c]		P_1/Ex 1	Estimation of 1 Unit of IgE BA[d]
				Ex 1	P_1		
		units/ml	*units/ml*			%	*ng*
S.B.	Serum	204	708	36	16	44	1.33
P.P.	Serum	576	1,116	30	15	50	0.70
T.W.	Serum	99	180	29	21	72	0.92
D.J.	Serum	180	816	18	9	50	0.98
M.K.	Serum	234	606	15	10	67	0.62
	Sp. Ab.[e]	18,000	19,900	61	40	65	1.06
D.B.	Serum	852	12,480	12	6	50	2.11
	Sp. Ab.[e]	28,000	51,840	50	30	60	1.33

[a] Measured on unabsorbed samples by antigen binding radioimmunoassay (21) by using 2 ng ^{125}I-labeled Fr. 14, serum from a myeloma patient (P.S.) as carrier and monospecific goat antiserum to Fc fragment of IgE to precipitate. Results were obtained from a control curve and expressed in arbitrary units of binding activity (BA)/ml.

[b] Measured on unabsorbed samples by double antibody inhibition radioimmunoassay (28). 1 unit of IgE was assumed to be equivalent to 2.4 ng (29).

[c] The results, expressed as a percentage of the total IgE, are the quantity of IgE absorbed by ExI or P_1 immunosorbent minus the IgE absorbed by an HSA immunosorbent.

[d] The quantity of IgE represented by 1 unit of IgE BA was calculated as follows:

$$1 \text{ unit} = \frac{\text{Total IgE (units/ml)} \times 2.4 \times \%\text{ total IgE directed against } P_1}{\text{IgE BA for } P_1 \text{ (units/ml)}} \text{ ng}$$

[e] Immunosorbent purified specific antibody to ExI.

DISCUSSION

Several lines of evidence suggested that antigen P_1 was the major allergen of *D. pteronyssinus*. Reactivity to the purified allergen and crude mite extract was closely correlated by skin testing and showed an excellent correlation in serum. In addition, IgE ab to P_1 accounted for up to three-quarters of the IgE ab to ExI. We have also found that more than 90% of individuals who give positive prick tests to *D. pteronyssinus* extract have detectable serum IgE ab to antigen P_1. In keeping with our previous studies (22) responses to intradermal skin tests with antigen P_1 and ExI were very similar when tested at the same protein concentration. These results correlate surprisingly well with other data which suggest that antigen P_1 represents 10 to 20% of the protein in ExI. However, in agreement with other workers (21), we believe that crude mite extract also contains minor allergens as indicated by one subject (N.J.) who was 100-fold more sensitive to ExI than to antigen P_1 (Table I). The distribution of ab against P_1 is very similar to that for ab to purified pollen allergens (34, 36, 37) in both serum (22, 32) and nasal secretions (M. D. Chapman and T. A. E. Platts-Mills, unpublished data) and includes IgG, IgA, and IgE ab. The evidence suggests that this response, like that to pollen allergens, occurs locally in the nasal mucosa (38).

The apparent m.w. of antigen P_1 on SDS-PAGE (24,000) is very similar to that suggested by Stewart and Turner (21) but a little higher than that suggested by others using gel filtration (7,500 to 20,000) (19, 39). Comparison of the gel filtration data and that derived under reducing conditions on SDS-PAGE confirms that antigen P_1 is a single amino acid chain. It remains possible that the m.w. of antigen P_1 may be slightly overesti-

mated on SDS-PAGE. The presence of a common antigen on proteins with different isoelectric points is not unusual, and has been reported previously for several allergens including Rye I (16), codfish allergen M (40), and *Cladosporium herbarum* allergen 32 (41). However, in almost all cases the pI values of these "isoallergens" differed by less than one pI unit. Our results demonstrate that antigen P_1 occurs in at least two isoallergenic forms that exhibit markedly different isoelectric points. On analytical IEF the two preparations of purified P_1 (Fr. 5 and Fr. 14) each showed more than one band. The heterogeneity on IEF could be explained by differences in carbohydrate content, although several other factors such as conformational changes or differences in amide content may also be involved (42). Recent work has shown that both Fr. 5 and Fr. 14 are glycoproteins, containing galactose, mannose, *N*-acetylglucosamine and *N*-acetylgalactosamine (Dr. E. F. Hounsell, personal communication). The presence of multiple isoallergens over a wide pI range suggests that analytical IEF would not be a useful technique for standardizing mite extracts.

It has previously been thought that allergens required a particular molecular structure (43) or chemically defined determinants (44) in order to elicit IgE ab formation. Highly purified allergens from pollens, codfish, and vespid venoms do not appear to have any chemical properties in common. The major allergens are generally the "most abundant" soluble proteins or glycoproteins with m.w. of between 10,000 and 40,000 (45, 46). Antigens with these properties would readily penetrate the nasal mucosa (47) whereas larger molecules are excluded, and smaller molecules (having fewer determinants) would be expected to be less immunogenic (48). Antigen P_1 has very similar physical properties to the other major allergens. In addition, RIA results suggest that P_1 represents about 10 to 20% of the protein in the crude extract. It also forms the strongest line on immunodiffusion (Fig. 2) and is coincident with the major band on SDS-PAGE (Fig. 4). Recent work has shown that house dust samples contain up to 50 μg P_1/g dust, and that more than 90% of the P_1 can be eluted within 2 min (E. R. Tovey, M. D. Chapman and T. A. E. Platts-Mills, manuscript in preparation). Thus, antigen P_1 is a rapidly eluting glycoprotein, m.w. 24,000, which is "abundant" in extracts of mite culture and house dust. These results strongly support the view that the importance of a protein as an allergen for immediate hypersensitivity is determined by its physical properties. The availability of a purified allergen from *D. pteronyssinus* should greatly facilitate studies on cellular and humoral immunity in patients with perennial rhinitis and extrinsic asthma.

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