

PURIFICATION OF A MURINE IgE-INDUCING
ANTIGEN EXTRACTED FROM THE HOUSE DUST
MITE, *DERMATOPHAGOIDES PTERONYSSINUS*

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Abstract—A major antigen inducing IgE in mice (DpI) was purified from a crude extract of *Dermatophagoides pteronyssinus* (D.p.) using Sephacryl S-200 gel filtration, DE52 ion exchange chromatography and isoelectric focusing. This antigen is not one of the major D.p. human allergens. Isoelectric focusing showed a single peak of PCA* activity at pI 4.7. Activity followed a broader banding pattern on polyacrylamide gel electrophoresis (PAGE). The molecular weight of DpI estimated from Sephadex G-150 chromatography was 1.7×10^5 daltons. Molecular weight calculated from SDS-gels (reducing), however, was 3.7×10^4 , which may indicate a molecule with a subunit composition. DpI contained no detectable hexose as measured by the phenol-sulfuric acid assay. PCA activity of DpI was stable at 98°C and to treatment with 0.1 M sodium metaperiodate, but destroyed by pronase. DpI represents approximately one per cent of the total protein of crude extracts. Purified DpI displays a specific activity increase of 40 times that of the crude extract with the PCA test. DpI is capable of inhibiting 40% of the binding between crude extract and human IgE, but only at extremely high concentrations. Human IgE was 700 times more sensitive to D.p. crude extract than purified DpI by enzyme immunoassay.

INTRODUCTION

Many workers have pointed out that *Dermatophagoides pteronyssinus* (D.p.)* and *D. farinae* (D.f.) are important allergens in house dust since the early reports by Voorhorst *et al.* (1964) (Miyamoto *et al.*, 1968; Morrison-Smith *et al.*, 1969; Biliotti *et al.*, 1972; Kabasawa *et al.*, 1976). Purification and characterization of these allergens is important for diagnosis and treatment of allergic asthma patients. Miyamoto *et al.* (1969) and subsequent workers (Romagnani *et al.*, 1972; Ishii *et al.*, 1973; Ishii & Miyamoto, 1975; Ricci *et al.*, 1975; Romagnani *et al.*, 1976; Nakagawa *et al.*, 1977; Baldo & Uhlenbruck, 1977; Chapman & Platts-Mills, 1978; Kabasawa & Ishii, 1979; Krilis *et al.*, 1979) have partially purified and characterized the allergens from house dust mites. Recently, a more defined allergen preparation has been isolated from D.p. (Chapman & Platts-Mills, 1980; Stewart & Turner, 1980a, b); however, a highly purified allergen from D.p. is necessary for characterization.

In our preliminary studies on IgE-suppression by D.p. allergens, when mice were immunized with the crude D.p. extract, the major IgE-inducing allergen in BALB/c mice was different from the major allergens in man. We have purified and characterized the major mouse allergen in D.p. crude extracts.

MATERIALS AND METHODS

Extraction of antigens from mites

Mite antigen was extracted by the method of Kabasawa & Ishii (1979). Briefly, D.p. separated from culture medium was homogenized with 0.12 M Tris-HCl buffer, pH 7.6. The homogenate was centrifuged at 60,000 *g* to obtain supernatant (crude extract). Protein concentration was measured by the method of Lowry *et al.* (1951) and by optical density at 280 nm with a Shimadzu UV-140 spectrophotometer (Shimadzu Ltd, Kyoto, Japan).

Preparation of antisera

A crude extract of D.p. in 0.85% (w/v) NaCl (20 µg protein/ml) was mixed with an equal volume of aluminum hydroxide gel (20 mg/ml). Aluminum hydroxide gel was prepared by the method of Levine & Vaz (1970). Ten male mice

* Abbreviations used: BSA, bovine serum albumin; D.f., *Dermatophagoides farinae*; D.p., *Dermatophagoides pteronyssinus*; EIA, enzyme immunoassay; PAGE, polyacrylamide gel electrophoresis; PAS, periodic acid-Schiff; PCA, passive cutaneous anaphylaxis; pI, isoelectric point; RAST, radioallergosorbent test; SDS, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane.

(BALB/c, 6–8 weeks) were each injected intraperitoneally with the crude extract-gel (0.2 ml) at weeks 0, 3 and 5. One week after the final injection blood from ten mice was pooled and the antiserum (M02) was stored at -20°C . Reciprocal PCA titer of this antiserum was 625.

A female rabbit (Japanese White, 3.8 kg) was injected five times at two week intervals with a crude extract (5 mg protein) in 0.85% NaCl (1 ml) emulsified with an equal volume of Freund's complete adjuvant (Difco Lab., Detroit, MI, U.S.A.). One week after the final injection, blood was collected and the antiserum (R802) was stored at -20°C .

Human antisera were collected and pooled (HS8001) from eight patients whose sera contained high levels of specific IgE to D.p. as measured by RAST.

Passive cutaneous anaphylaxis (PCA) reaction

The protocol for the PCA assay used in our experiments was based on the method of Mota & Wong (1969), as modified by Watanabe & Ovary (1977). Male rats (Sprague Dawley, 9–12 weeks) were sensitized intradermally with mouse antiserum M02 (0.05 ml, 1:300). Four hr after the sensitization one ml of 1% (w/v) Evans blue solution was injected intravenously. After 5–10 min serially diluted antigens (0.05 ml) were challenged at the same point. After 30 min the animals were killed and their dorsal skin was reflected, and the reaction sites were measured. A blue spot larger than 8×8 mm was estimated positive. Injection of only antigen (control) provoked a much smaller reaction.

Titer of antisera was determined as follows. Male rats were sensitized with serially diluted antisera (0.05 ml) as described above, except that one ml of a crude extract of D.p. (1 mg protein + 1% Evans blue) was injected intravenously. A blue spot larger than 5×5 mm was deemed positive. Every PCA reaction was performed in duplicate or triplicate and the geometric mean is presented in the results.

Radioallergosorbent test (RAST) and enzyme immunoassay (EIA) inhibition

The RAST assay was performed as described by Ceska & Ludkvist (1972). EIA inhibition represents a modification of the RAST assay. One half g of Toyo No. 6 paper discs (Toyo Roshii Co., Tokyo, Japan, ϕ 6 mm) were acti-

vated with cyanogen bromide (Katayama Chemical Co., Osaka, Japan) and mixed with D.p. crude extract (0.6 mg protein) in 1 M NaHCO_3 (20 ml) containing 0.02% (w/v) NaN_3 and incubated at 4°C for 4 days.

Anti-human IgE labeled with β -D-galactosidase and *o*-nitrophenyl- β -D-galactoside were donated as a kit (IgE[MITSUI]), from Kyushu Kainos Co., Fukuoka, Japan. A sample protein solution (0.05 ml) in 0.85% NaCl was incubated with human antiserum HS8001, (0.05 ml which had been diluted 1:40 with normal horse serum, Nakarai Co., Kyoto, Japan) at 30°C for 1 hr. A paper disc coupled with D.p. crude extract was incubated with the mixture described above at 30°C for 3 hr. The discs were then washed three times with 0.1% (v/v) Tween 20–0.85% NaCl and once with EIA-buffer (1% BSA, 0.9% NaCl, 0.1% Tween 20 and 0.05% NaN_3 in 0.01 M phosphate buffer, pH 7.4). The washed paper disc was then incubated with enzyme-labeled anti-human IgE solution (0.05 ml, 1% BSA, 0.1 M NaCl, 1 mM MgCl_2 and 0.05% NaN_3 in 0.01 M phosphate buffer, pH 7.0) at 25°C for 15–20 hr and then washed four times with 0.1% Tween 20–0.85% NaCl. The paper discs were then incubated with 0.1 ml of substrate solution (0.1% *o*-nitrophenyl- β -D-galactoside, 0.1% BSA, 0.1 M NaCl, 1 mM MgCl_2 and 0.5% H_3BO_3 in 0.01 M phosphate buffer, pH 7.0) at 36°C for 60 min. The enzyme reaction was stopped by the addition of 0.3 ml of 0.1 M Na_2CO_3 . Optical density at 420 nm was measured. Inhibition rate (In) was obtained as follows:

$$\text{In}(\%) = \frac{[\text{OD standard}] - [\text{OD sample}]}{[\text{OD standard}] - [\text{OD blank}]} \times 100.$$

The standard represents OD₄₂₀ of sample minus allergen; the blank contains normal horse serum without allergen and antiserum HS8001. The sample contains buffer, allergen and antiserum HS8001.

Isoelectric focusing

Isoelectric focusing was carried out according to the method of Vesterberg (1971), with Ampholine, pH 3.5–10 (LKB, Bromma, Sweden), on a modified LKB model 8101 (110 ml) column.

Polyacrylamide gel electrophoresis (PAGE)

PAGE was performed by the method of

Davis (1964) on 7.5% polyacrylamide gel slabs. Strips from gel lanes were stained with 0.25% (w/v) Coomassie Brilliant Blue R-250 [Meyer & Lamberts (1965), Nakarai Co., Kyoto, Japan] or reacted with periodic acid-Schiff [PAS, Zacharius *et al.* (1969)]. A third gel strip was cut into 5 mm pieces. Each piece was then allowed to stand for 72 hr in phosphate-buffered saline and the supernatant was used for PCA testing.

Estimation of molecular weight

Purified DpI solution (2 mg/4 ml) was chromatographed on a Sephadex G-150 (Pharmacia, Uppsala, Sweden) column (ϕ 1.6 $\times$ 66.5 cm) with 0.15 M NaCl 0.01 M phosphate buffer, pH 6.6. Standard proteins (1 mg): aldolase, BSA, ovalbumin and cytochrome *c* (Sigma Co., St Louis, Mo., U.S.A.), Blue Dextran 2000 (0.3 mg, Pharmacia) and NaCl (30 mg) were dissolved in the buffer (1.3 ml) and applied to the column. Apparent partition coefficients (K_{av}) were obtained as follows

$$K_{av} = \frac{V_e - V_o}{V_t - V_o},$$

where V_t = elution volume of NaCl, and V_e and V_o represent the elution volume of protein and Blue Dextran 2000 respectively.

PAGE with sodium dodecyl sulfate (SDS) was carried out by the method of Weber & Osborn (1969). Sample and standard proteins were treated with 1.7% (v/v) 2-mercaptoethanol at 100°C for 1 hr and electrophoresed on 10% gels. Molecular weights were determined using bovine gamma globulin (Miles, Elkhart, Ind., U.S.A.), BSA ovalbumin, α -chymotrypsinogen A (Sigma), cytochrome *c* and bromphenol blue as standards.

Immunoelectrophoresis

Immunoelectrophoresis was performed according to the methods of Ouchterlony & Nilsson (1978) on 1.2% (w/v) agarose (Nakarai, Kyoto, Japan) in barbital buffer (pH 8.6, μ = 0.025).

Pronase digestion

A sample protein solution (13 μg in 0.25 ml) was mixed with pronase (5 μg , Kaken Chemical, Tokyo, Japan) in 0.1 M phosphate buffer,

pH 7.2 (0.25 ml), containing 0.01% NaN_3 and incubated at 37°C for 24 hr. PCA activities of sample solution and control solutions without pronase or sample protein were measured.

Periodate oxidation

Sample protein (110 μg) in 0.08 M acetate buffer, pH 5.2 (0.3 ml), was mixed with 0.2 M sodium metaperiodate (0.3 ml, Wako Pure-Chemical, Osaka, Japan) and incubated at room temperature for 24 and 70 hr in the dark. Samples were dialyzed and PCA activity was measured. A control sample protein without the metaperiodate was similarly treated.

Phenol-sulfuric acid method

Hexose concentration was determined by the method of Dubois *et al.* (1951).

Amino acid analysis

Freeze-dried DpI (60–80 μg) was hydrolyzed in vacuum with 6 N HCl or 4 N methanesulfonic acid (Simpson *et al.*, 1976) at 110°C for 24 hr. The hydrolysates were analyzed with a Hitachi 835F analyzer (Hitachi Co., Tokyo, Japan).

RESULTS

Purification of a main IgE-inducing antigen (DpI)

The crude D.p. extract was chromatographed on a Sephacryl S-200 (Pharmacia, Uppsala, Sweden) column. Binding activities of various fractions to mouse IgE and human IgE were measured by the PCA reaction and a direct-RAST respectively. Fractions used in the test were diluted (1:500) with 0.85% NaCl. Figure 1 indicates that a major PCA positive component was different in apparent molecular weight from the major RAST active components. The PCA active fraction (fraction 1, Fig. 1) was collected and concentrated on an Amicon UM-10 (Amicon, Lexington, Mass., U.S.A.) membrane filter. The concentrated material was then dialyzed against 0.014 M Tris-HCl buffer, pH 7.7, for 12 hr. This material was then applied to a DE52 (Whatman, Maidstone, Kent, England) anion exchange column. With the DE52 column,

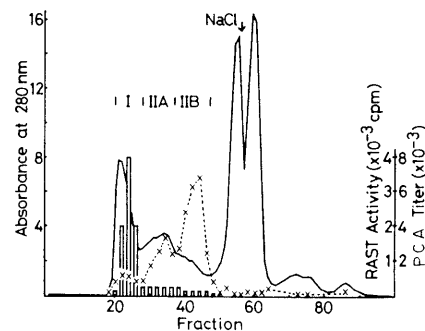


Fig. 1. Gel filtration of crude extract on Sephacryl S-200 column. Crude extract (830 mg protein) dissolved in 0.15 M NaCl, 0.06 M Tris-HCl buffer, pH 7.6 (22 ml), was applied on a Sephacryl S-200 column (ϕ 2.5 $\times$ 95 cm). Fractions (9.75 ml) were collected at a flow rate of 12 ml/hr. Absorbance at 280 nm (—), PCA titer (---) and RAST activity (· · · · ·) are shown. Eluted position of NaCl (I) was estimated by a conductivity meter (Type CD-35M II, M & S Instruments Inc., Osaka, Japan).

PCA activity could be effectively separated from most non-active proteins. The major PCA positive component (fraction c, Fig. 2) was eluted at 0.25 M NaCl, concentrated and dialyzed as described above. Ten mg of fraction c protein was dissolved in 0.014 M phosphate buffer, pH 6.4 (6 ml), and rechromatographed on DE52 equilibrated with the same buffer. Two major protein peaks were eluted at 0.14 M and 0.2 M NaCl (data not shown). PCA ac-

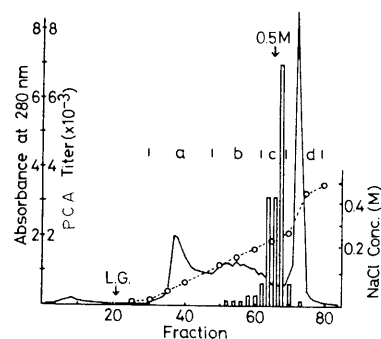


Fig. 2. Chromatography of fraction I on DE52 column. Fraction I (145 mg protein) dissolved in 0.01 M Tris-HCl buffer, pH 7.7 (23 ml) was chromatographed on a DE52 column (ϕ 1.6 $\times$ 25 cm) equilibrated with the same buffer. Fractions (5.85 ml) were collected at a flow rate of 23 ml/hr. Linear concentration gradient to 0.3 M NaCl in the buffer (total 300 ml) was applied at arrow L.G. The buffer containing 0.5 M NaCl was applied at arrow 0.5 M. Absorbance at 280 nm (—), PCA titer (---) and NaCl concentration (· · · · ·) are shown.

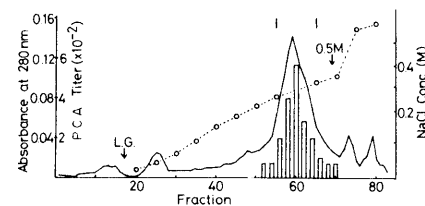


Fig. 3. Chromatography of fraction 2 on DE52 column. Fraction 2 (2 mg protein) dissolved in 0.02 M phosphate buffer, pH 6.2 (13 ml) was chromatographed on a DE52 column (ϕ 0.9 $\times$ 20 cm). Fractions (2.55 ml) were collected at a flow rate of 27 ml/hr. Linear concentration gradient to 0.3 M NaCl in the buffer (total 140 ml) was applied. Indications are the same as in Fig. 2.

tivity off this column was detected in the second major peak, which we called fraction 2. Fraction 2 was then pooled from several columns, concentrated and dialyzed, and rechromatographed on the DE52 ion exchange column, as before. Figure 3 shows that the major protein peak was coincident with the symmetrical distribution PCA activity. This PCA-positive material was designated DpI. Approximately 9 mg of DpI was obtained with a starting concentration of 3.2 g of crude extract protein. Based on the 280 absorbance of PCA positive fractions and the PCA negative fractions, DpI constitutes about 1% of the total protein in the crude extract. Protein concentrations at PCA end point for crude extract and DpI were 5.3 μ g/ml and 0.13 μ g/ml respectively. This indicates an increase in specific activity for DpI of about 40 times over that of crude extract.

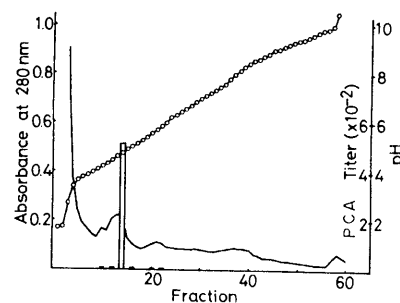


Fig. 4. Isoelectric focusing of DpI. Purified fraction containing DpI (0.6 mg) was dissolved in 0.6% NaCl (3 ml) and applied on an isoelectric focusing column. Electric power (3.1 W) was supplied for 18 hr at 1°C. After fractions (2 ml) were collected, and absorbance at 280 nm (—), pH (· · · · ·) and PCA titer (---) of dialyzed fraction were measured.

Table 1. Amino acid composition of DpI

	μ mol/g atom	Molar ratio
Asp	728	30.00 ^a (30)
Thr	218	8.98 (9)
Ser	370	15.25 (16-17)
Glu	1969	81.14 (81)
Pro	51.9	2.14 (2)
Gly	185	7.62 (7-8)
Ala	848	34.95 (35)
1/2Cys	9.3	0.38 (1 or more)
Val	317	13.06 (13)
Met	238	9.81 (10)
Ile	362	14.92 (15)
Leu	735	30.29 (30)
Tyr	86.2	3.55 (4)
Phe	82.6	3.40 (3-4)
Lys	713	29.38 (29-30)
His	106	4.37 (4-5)
Trp	0 ^b	0 (0)
Arg	601	24.77 (25)
NH ₃		About 40
Total amino acid		(314-319)
Minimal mol. wt		36,798 ^c

^aAsp was used as standard.

^bTrp was obtained by another hydrolysis in methanesulfonic acid.

^cThe molecular weight of subunit of DpI was 37,000 by SDS polyacrylamide gel electrophoresis.

Fig. 5. Polyacrylamide gel disc electrophoresis of DpI. Purified fraction containing DpI (10-20 μ g) in 35% (w/v) sucrose solution (0.1 ml) was applied to one lane of a 7.5% polyacrylamide gel plate (pH 9.4). Constant current (1.6 mA/cm) was applied for 3.5 hr. Bromophenol blue was used as a marker.

Physico-chemical properties of DpI

As can be seen in Fig. 4, DpI is expressed as a sharp PCA positive band at pI 4.7 on an isoelectric focusing column. However, DpI represents a broad band on PAGE (pH 9.4, Fig. 5). PAS staining of the purified DpI fraction indicates no positive PAS reaction coincident with the PCA positive region of the gel.

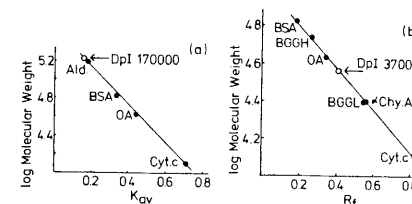


Fig. 6. Estimation of molecular weight by (a) gel filtration on a Sephadex G-150 column and (b) SDS polyacrylamide gel electrophoresis. Standard proteins were aldolase (Ald., 158,000), bovine serum albumin (BSA, 67,000), ovalbumin (OA, 43,000), cytochrome c (Cyt.c, 13,000), bovine gamma globulin (heavy chain: BGGH, 55,000; light chain: BGGL, 25,000) and α -chymotrypsinogen A (α -Chy.A, 25,000).

Molecular weights were estimated by gel-filtration on Sephadex G-150 and SDS-PAGE (reducing) gels (Fig. 6). The molecular weight of DpI estimated by gel filtration and by SDS-PAGE were 1.7×10^5 and 3.7×10^3 respectively.

The amino acid analysis of DpI presented in Table 1 explains the acidic isoelectric point. The low extinction coefficient ($E_{280}^{1\%} = 4.1$) can be explained by the lack of tryptophan residues. As estimated by the phenol-sulfuric acid method, DpI contains less than 0.6% hexose.

Table 2 indicates that PCA activity of DpI was stable to heating at 98°C and to treatment

Table 2. Stability of DpI on some treatments

Treatment	PCA titer
Heating, 98°C, 30 min	90
Heating, 98°C, 60 min	52
Control	90
Pronase ^a , 37°C, 24 hr	0
Control	256
0.1 M NaIO ₄ , 24 hr	47
0.1 M NaIO ₄ , 70 hr	47
Control	27

^aThe enzyme was mixed with substrates (1:2.6) in 0.1 M phosphate buffer, pH 7.2.

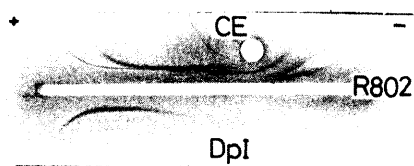


Fig. 7. Immunoelectrophoresis of DpI. DpI (20 μ g) and crude extract (CE, 100 μ g) were applied on a gel plate (7.5 $\times$ 2.5 cm). Constant current (6 mA) was supplied for 40 min. After a center basin was filled with rabbit anti-serum R802 the gel plate was kept at room temperature for 24 hr.

with 0.1 M sodium metaperiodate. However, its PCA activity was lost by incubation with pronase.

Immunochemical properties of DpI

Hyperimmune rabbit anti-crude extract serum was used to assess the immunochemical purity of DpI. Immunoelectrophoresis shows that DpI is not the major precipitating antigen in the crude extract. However, only one precipitin line develops against anti-crude extract rabbit serum.

DpI reaction with human IgE

To determine whether DpI, the main IgE-inducing antigen in the mouse, reacts with D.p.-sensitized human IgE, an EIA inhibition assay was performed. Figure 8 shows that BSA does not inhibit binding of human IgE, while DpI does inhibit binding by 40%. However, the concentration of DpI necessary to exhibit this 40% inhibition was 700 times the concentration

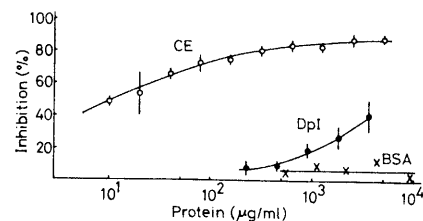


Fig. 8. Inhibitory activity of DpI on the binding of crude extract to human IgE. Three assays (mean $\pm$ S.D.) were carried out for crude extract (CE) and DpI, and one assay for BSA.

of the crude extract necessary to express the same inhibition.

DISCUSSION

Because the mouse serum PCA titer was reduced from 625 to less than 5 after heating (56°C for 2 hr) we conclude that the antibody class involved is entirely IgE (Ovary *et al.*, 1975).

DpI appears to be a heat-stable protein. This is supported by the findings that PCA positive molecules could not be absorbed to Concanavalin A-Sepharose (data not shown) and PCA activity was stable to periodate oxidation. Only minor amounts of hexose were detected by the phenol-sulfuric acid assay. This may have been due to slight contamination by minor components. Minor impurities were noted (Fig. 5) on PAGE, along with a broad banding of PCA-active molecules. This banding on PAGE is in apparent contrast to the homogeneous banding apparent in isoelectric focusing.

Nakagawa *et al.* (1977) have shown that partially purified crude extract from D.f. was heat-stable, periodate-resistant and pronase-sensitive by PCA reaction in the mouse-rat system, using mouse antiserum obtained by immunization with the antigen. They reported that the molecular weight of the most potent allergenic fraction was approximately 20,000–30,000. The PCA-active components of D.f. are similar to DpI except in molecular weight. A detailed comparison, however, is impossible, as these authors did not describe their exact method of extraction. Complexity of composition in crude extracts depends on the method of extraction, as has been reported by Chapman & Platts-Mills (1980). Stewart & Turner (1980a) have reported that a low molecular weight allergen of D.p. (Dpt 12, M.W. 24,000, pI 6.6) appears to be the major allergen in man. This antigen Dpt 12, may correspond to the antigen P₁ (M.W. 24,000, pI 4.7–5.4 and 6.6–7.1) described by Chapman & Platts-Mills (1980). Thus, the major allergens in man should be contained in fraction IIB (Fig. 1) from our experiments. The molecular weight of the main RAST positive components (fraction IIB) was 2×10^4 by estimation on a Sephadex G-75 column (Abe T., unpublished data).

Although most of the PCA activity in the crude extract was attributable to DpI, lower molecular components did express PCA activity to some extent. The PCA-active com-

ponents in fraction IIB (Fig. 1), however, did not represent DpI or its subunit. Furthermore, they appear to be different from the major allergens in man (Abe T., unpublished data).

O'Donnell & Mitchell (1978) reported that the active allergenic components of *Ascaris lumbricoides* body fluid were different by two types of assay, that is, by the RAST with sera from humans naturally infected and by the PCA reaction with sera from mice sensitized by nasal inhalation. They determined that only one relatively pure fraction (M.W. 360,000, pI 8.0–8.4) was active with mouse antiserum when assayed by PCA reaction.

An important question is why the main IgE-inducing components were different in the mouse and man. Within the limits of our experimental data, we describe here some possible explanations to this question; (1) the mucosal membrane must be a major entrance of naturally occurring house dust mite allergens. Due to restrictions in size or chemical composition, DpI may not be able to cross these mucosal membranes for exposure; (2) most respiratory allergens of house dust mites seem to be their excreta (Tovey *et al.*, 1981). DpI may not be excreted from the mite; it may, for example, represent a structural protein. This could represent another reason for a lack of absorption into the human body; (3) DpI may not induce IgE responses in the human body in spite of being absorbed, because an adjuvant may be essential for IgE induction with DpI; (4) the response of IgE-forming cells to the components of D.p. may be genetically different in the two species; (5) the dose of human IgE-active components in the immunized fraction may be insufficient for induction of IgE in the mouse. These explanations mentioned above must be tested experimentally before definitive conclusions can be drawn.

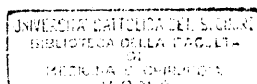
As DpI is a potent IgE-inducing antigen in the mouse, it may be an effective material to study the mechanism of allergy in an animal model. Our results, however, indicate that studies on allergens in an animal model must be carefully interpreted according to the purity of antigens, methods of sensitization and assay systems used.

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REFERENCES

- Baldo B. A. & Uhlenbruck G. (1977) Selective isolation of allergens I. Reaction of house dust mite extracts with tridacnin and concanavalin A and examination of the allergenicity of the isolated components. *Clin. Allergy* **7**, 429–443.
- Biliotti G., Passaleva A., Romagnani S. & Ricci M. (1972) Mites and house dust allergy. I. Comparison between house dust and mite (*Dermatophagoides pteronyssinus* and *D. farinae*) skin reactivity. *Clin. Allergy* **2**, 109–113.
- Ceska M. & Lundkvist U. (1972) A new and simple radioimmunoassay method for the determination of IgE. *Immunochimistry* **9**, 1021–1030.
- Chapman M. D. & Platts-Mills T. A. E. (1978) Measurement of IgG, IgA and IgE antibodies to *Dermatophagoides pteronyssinus* by antigen-binding assay, using a partially purified fraction of mite extract (F₄P₁). *Clin. exp. Immun.* **34**, 126–136.
- Chapman M. D. & Platts-Mills T. A. E. (1980) Purification and characterization of the major allergen from *Dermatophagoides pteronyssinus*-antigen P₁. *J. Immun.* **125**, 587–592.
- Davis B. J. (1964) Disk electrophoresis-II. Method and application to human serum proteins. *Ann. N.Y. Acad. Sci.* **121**, 404–427.
- Dubois M., Gilles K., Hamilton J. K., Rebers P. A. & Smith F. (1951) A colorimetric method for the determination of sugars. *Nature, Lond.* **168**, 167.
- Ishii A. & Miyamoto J. (1975) Enzymatic hydrolysis of allergens extracted from the house dust mite, *Dermatophagoides farinae*. *Jap. J. exp. Med.* **45**, 263–268.
- Ishii A., Noda K., Nagai Y., Ohsawa T., Kato I., Yokota M., Nakamura S., Matsushashi T. & Sasa M. (1973) Biological and biochemical properties of the house dust mite extract. *Dermatophagoides farinae*. *Jap. J. exp. Med.* **43**, 495–507.
- Kabasawa Y. & Ishii A. (1979) Studies on the physicochemical properties of house dust mite (*Dermatophagoides pteronyssinus*) allergens involved in reaginic reaction. *Jap. J. exp. Med.* **49**, 51–57.
- Kabasawa Y., Ishii A., Murata H. & Takaoka M. (1976) Clinical significance of the house dust mite (*Dermatophagoides pteronyssinus*) in asthmatic children in Japan. *Acta allerg.* **31**, 442–454.
- Krill S., Baldo B. A. & Basten A. (1979) Dialyzable allergens from the common house dust mites. *Naturwissenschaften* **66**, S. 475–476.
- Levine B. B. & Vaz N. M. (1970) Effect of combinations of inbred strain, antigen, and antigen dose on immune responsiveness and reagin production in the mouse. *Int. Archs Allergy appl. Immun.* **39**, 156–171.
- Lowry O. H., Rosebrough N. J., Farr A. L. & Randall R. J. (1951) Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193**, 265–275.
- Meyer T. S. & Lamberts B. L. (1965) Use of Coomassie brilliant blue R250 for the electrophoresis of microgram quantities of parotid saliva proteins on acrylamide-gel strips. *Biochim. biophys. Acta* **107**, 144–145.
- Miyamoto T., Oshima S. & Ishizaki T. (1969) Antigenic relationship between house dust and a dust mite, *Dermatophagoides farinae* Hughes 1961, by a fractionation method. *J. Allergy* **44**, 282–291.
- Miyamoto T., Oshima S., Ishizaki T. & Sato S. (1968) Allergenic identity between the common floor mite (*Dermatophagoides farinae* Hughes, 1961) and house dust as a causative antigen in bronchial asthma. *J. Allergy* **42**, 305–319.
- Morrison-Smith J., Disney M. E., Williams J. D. & Geols Z. A. (1969) Clinical significance of skin reactions to mite extracts in children with asthma. *Br. med. J.* **2**, 723–726.
- Mota I. & Wong D. (1969) Homologous and heterologous passive cutaneous anaphylactic activity of mouse anti-

- sera during the course of immunization. *Life Sci.* **8**, 813-820.
- Nakagawa T., Kudo K., Okudaira H., Miyamoto T. & Horiuchi Y. (1977) Characterization of the allergenic components of the house dust mite, *Dermatophagoides farinae*. *Int. Archs Allergy appl. Immun.* **55**, 47-53.
- O'Donnell I. J. & Mitchell G. F. (1978) An investigation of the allergens of *Ascaris lumbricoides* using a radioallergen sorbent test (RAST) and sera of naturally infected humans: comparison with an allergen for mice identified by a passive cutaneous anaphylaxis test. *Aust. J. Biol. Sci.* **31**, 459-487.
- Ouchterlony Ö. & Nilsson L.-Å. (1978) Immunodiffusion and immunoelectrophoresis. In *Handbook of Experimental Immunology in Three Volumes* (Edited by Weir D. M.). Ch. 19. Blackwell Scientific Publications, Oxford.
- Ovary Z., Caiazza S. S. & Kojima S. (1975) PCA reactions with mouse antibodies in mice and rats. *Int. Archs Allergy appl. Immun.* **48**, 16-21.
- Ricci M., Biliotti G. & Romagnani S. (1975) Antigenic and allergenic constituents of *Dermatophagoides pteronyssinus* extract. *Dev. Biol. Standard* **29**, 123-131.
- Romagnani S., Biliotti G., Passaleva A. & Ricci M. (1972) Mites and house dust allergy. II. Relationship between house dust and mite (*Dermatophagoides pteronyssinus* and *D. farinae*) allergens by fractionation methods. *Clin. Allergy* **2**, 115-123.
- Romagnani S., Boccaccini P., Amadori A. & Ricci M. (1976) Studies on allergens of *Dermatophagoides pteronyssinus* by direct and indirect RAST. *Int. Archs Allergy appl. Immun.* **50**, 525-535.
- Simpson R. J., Neuberger M. R. & Liu T. Y. (1976) Complete amino acid analysis of proteins from a single hydrolysate. *J. Biol. Chem.* **251**, 1936-1940.
- Stewart G. A. & Turner K. J. (1980a) Physicochemical and immunochemical characterization of the allergens from the mite *Dermatophagoides pteronyssinus*. *Aust. J. exp. Biol. med. Sci.* **58** (Pt. 3), 259-274.
- Stewart G. A. & Turner K. J. (1980b) Physicochemical and immunochemical characterization of the high molecular weight allergens from *Dermatophagoides pteronyssinus* with particular reference to the tridacnin, Con-A and S107 reactive components. *Aust. J. exp. Biol. med. Sci.* **58** (Pt. 3), 275-288.
- Tovey E. R., Chapman M. D. & Platts-Mills T. A. E. (1981) Mite faeces are a major source of house dust allergens. *Nature, Lond.* **289**, 592-593.
- Vesterberg O. (1971) Isoelectric focusing of proteins. In *Methods in Enzymology* (Edited by Jakoby W. B.). Vol. XXII, p. 389. Academic Press, New York.
- Voorhorst R., Spieksma-Boezeman M. I. A. & Spieksma F. Th. M. (1964) Is a mite (*Dermatophagoides* sp.) the producer of the house dust allergen? *Allergic Asthma* **10**, 329-334.
- Watanabe N. & Ovary Z. (1977) Antigen and antibody detection by *in vivo* methods: a reevaluation of passive cutaneous anaphylactic reactions. *J. Immun. Meth.* **14**, 381-390.
- Weber K. & Osborn M. (1969) The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. *J. Biol. Chem.* **244**, 4406-4412.
- Zacharius R. M., Zeli T. E., Morrison J. H. & Woodlock J. J. (1969) Glycoprotein staining following electrophoresis on acrylamide gels. *Analyt. Biochem.* **30**, 148-152.



6 MAR 1982