

Isolation and Characterization of Two Allergens from *Dermatophagoides farinae*

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Abstract. Two purified allergens, designated as DF1 and DF2, were isolated from the extract of the whole culture of *Dermatophagoides farinae* by a combination of ammonium sulfate precipitation and ion exchange, hydrophobic, chelate and gel chromatography. DF1 was isolated as a heat-sensitive acidic protein with an apparent molecular weight of 25,000 and an isoelectric point of 4.6-7.2. DF2 was isolated as a heat-stable basic protein with an apparent molecular weight of 15,000 and an isoelectric point of 7.8-8.3. No allergenic cross-reactivity was seen between DF1 and DF2. Both DF1 and DF2 were shown to be the major allergens of *D. farinae* by the results of radioallergosorbent test and histamine release assay.

Introduction

Allergy to house dust is an important cause of extrinsic asthma and allergic rhinitis in many countries. It has been well established that the mites of two species of genus *Dermatophagoides*, *D. pteronyssinus* and *D. farinae*, are the major source of house dust allergens [1-4]. Several investigations showed the existence of a high degree of allergenic cross-reactivity between these two species of mites [5, 6].

Recently, several groups of workers have described the characterization and the isolation of the allergens from *D. pteronyssinus* and *D. farinae* [7-16], and the major allergen of *D. pteronyssinus*, antigen P₁ [14] (identical to Dp42 and Dpt12 [17]), and the corresponding major allergen of *D. farinae*, Ag11 [16], have been isolated.

The present study describes the isolation and the immunochemical and physicochemical analyses of the two allergens, designated as DF1 and DF2, from the extract of the whole culture of *D. farinae*.

Materials and Methods

Preparation of Whole Culture Extract (WCE) of *D. farinae*

Mites were cultivated on a powdered animal food for laboratory mice and rats (Oriental Yeast Co., Ltd., Tokyo, Japan) according to the method of Sasa et al. [18]. After their 4-month growth, the

mites were frozen to death and the culture was lyophilized. Mite density was 10-20/mg. The lyophilized whole culture (200 g) was defatted with ether and extracted 1:10 (w/v) in 0.125 M ammonium bicarbonate containing 2 mM cysteine for 24 h at 4°C. After centrifugation (10,000 g), the supernatant was concentrated to 200 ml by the use of Amicon PM-10 filter (Amicon, Lexington, Mass., USA), dialyzed against 5 mM ammonium bicarbonate and lyophilized.

Mite body extract (MBE) prepared from isolated mite bodies of *D. farinae* [13] was kindly supplied by Torii & Co., Ltd., Tokyo, Japan.

Preparation of DF1 and DF2

The isolation procedure is summarized in figure 1. WCE obtained from 200 g of the whole culture was dissolved in 500 ml of 0.05 M Tris-HCl, pH 8.0, and separated by ammonium sulfate precipitation into two fractions at 0-60 and 60-95% saturation.

The ammonium sulfate precipitate at 0-60% saturation was dissolved in 80 ml of 0.05 M Tris-HCl, pH 8.0, dialyzed against the same buffer and applied to a DEAE-cellulose (DE-52, Whatman, Springfield Mill, Kent, England) column (2.6 × 20 cm) equilibrated with the same Tris buffer. The stepwise elution was then performed with 500 ml of 0.05 M Tris-HCl, pH 8.0, and 500 ml of 0.05 M Tris-HCl containing 0.2 M sodium chloride, pH 7.4. The eluates of this and subsequent chromatographies for the purification of DF1 were monitored by absorbance at 280 nm and by fused rocket immunoelectrophoresis (FRIE) [19] against rabbit anti-WCE serum. The fraction eluted with 0.05 M Tris-HCl containing 0.2 M sodium chloride, pH 7.4, (Fr. I) was concentrated to 40 ml by pressure filtration through Spectrapor membrane tubing 1 (Spectrum Medical Industries, Los Angeles, Calif., USA), dialyzed against 0.05 M sodium borate containing 2 M sodium chloride, pH 8.0, and fractionated on a Phenyl-Sepharose CL-4B (Pharmacia Fine Chemicals, Uppsala, Sweden) Column (1.5 × 20 cm), equilibrated with the

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same borate buffer. After the addition of the sample, the column was washed with 200 ml of the same borate buffer. The absorbed material was then eluted with 0.05 M sodium borate containing 50% (v/v) ethylene glycol, pH 8.0. The fraction eluted with 50% ethylene glycol (Fr. II) was concentrated to 20 ml, dialyzed against 0.05 M ammonium bicarbonate and applied on a Sephadex G-150 (Pharmacia) column (2.6 × 100 cm) equilibrated with 0.05 M ammonium bicarbonate. The DF1-containing fraction eluted at 360-460 ml (Fr. III) was concentrated to 5 ml, dialyzed against 0.05 M Tris-acetate containing 0.5 M sodium chloride, pH 7.5, and subjected to chelate chromatography on Chelating Sepharose 6B (Pharmacia) with the use of zinc as chelated ion. The column (1.0 × 15 cm) was chelated with zinc ion and equilibrated with the Tris-acetate buffer according to the manufacturer's instructions. The sample was applied to the column and the column was washed with 50 ml of the same buffer, after which the absorbed material was eluted with a gradient of decreasing pH of the Tris-acetate buffer from 7.5 to 5.0. The peak eluted at pH 5.7-5.2 (Fr. IV) was pooled and was further purified by gel chromatography twice on a Sephadex G-75 (superfine, Pharmacia) column (1.5 × 100 cm) equilibrated with 0.05 M ammonium bicarbonate. The fraction containing the isolated DF1 was pooled.

DF1 was further fractionated into subfractions by preparative isoelectric focusing in a sucrose density gradient with an LKB 8100-1 column (110 ml). The sample was focused at 4°C for 48 h at 600 V with 1.0% Ampholine (LKB Industries, Bromma, Sweden), which had a pH range of 3.5-10, 4-6 and 5-7 in the ratio of 4:1:1.

The ammonium sulfate precipitate at 60-95% saturation was dissolved in 40 ml of 0.05 M Tris-HCl, pH 8.0, dialyzed against the same buffer and passed through a DEAE-cellulose column (1.5 × 20 cm) equilibrated with the same Tris buffer. The eluates of this and subsequent chromatographies for the purification of DF2 were monitored by absorbance at 280 nm and by immunodiffusion against rabbit antiserum to partially purified DF2 (see below). The fraction eluted unretarded from the column (Fr. V) was concentrated to 20 ml, dialyzed against 0.01 M sodium acetate, pH 5.0, and applied to a CM-cellulose (CM-52, Whatman) column (1.5 × 20 cm) equilibrated with the same acetate buffer. The stepwise elution was then performed with 200 ml of the same acetate buffer and 150 ml of 0.1 M sodium phosphate containing 0.3 M sodium chloride, pH 6.5. The fraction eluted with the sodium phosphate buffer (Fr. VI) was concentrated to 4 ml and applied to a Sephadex G-75 (superfined) column (1.5 × 100 cm) equilibrated with 0.05 M ammonium bicarbonate. The DF2-containing fraction eluted at 110-122 ml (Fr. VII) was pooled, dialyzed against 0.01 M sodium acetate, pH 5.5, and fractionated on a CM-cellulose column (1.0 × 15 cm) equilibrated with the same acetate buffer. The column was eluted with a linear sodium chloride gradient from 0 to 0.2 M. The fraction containing the isolated DF2 was pooled.

Rabbit Antiserum

Antisera to WCE were prepared by immunizing rabbits with a mixture of WCE (10 mg in 1 ml saline) and 1 ml of complete Freund's adjuvant (Difco, Detroit, Mich., USA). The rabbits were injected subcutaneously four times at 3-week intervals and bled 10 days after the last injection. A portion of the antiserum was precipitated with ammonium sulfate at 33% saturation and the resulting IgG fraction was concentrated threefold compared to the original serum volumes.

Antiserum to partially purified DF2 was prepared by injecting a

rabbit subcutaneously with the antigen (200 µg in 0.5 ml saline) emulsified in 0.5 ml of complete Freund's adjuvant. Immunization was given four times at 4-week intervals. The rabbit was bled 10 days after the last injection. Partially purified DF2 was prepared from MBE by a combination of anion-exchange chromatography on DEAE-cellulose and gel chromatography on Sephadex G-75.

Crossed Immunoelectrophoresis (CIE) and

Crossed Radioimmuno-electrophoresis (CRIE)

CIE and CRIE were performed essentially as described by Lowenstein [19]. Electrophoresis was carried out on glass plates (94 × 84 mm) using a 1.0% agarose gel (Agarose C, Pharmacia) prepared in Tris-barbital, pH 8.6. The first dimension electrophoresis was performed at 10 V/cm for 40 min at 10°C, followed by a second dimension electrophoresis at 2 V/cm for 16 h into a gel containing IgG fraction of rabbit anti-WCE serum. The plates were pressed, washed and dried at room temperature. The plates were stained with Coomassie brilliant blue R after completion of autoradiography.

The unstained plates were incubated at 25°C for 24 h with pooled allergic serum, which was made from equal volumes of individual sera obtained from 5 highly mite allergic patients who had not been treated previously. Pooled allergic serum was 10-fold diluted in an incubation buffer (0.05 M sodium phosphate, 0.3% bovine serum albumin, 0.1% EDTA and 0.02% sodium azide, pH 7.5) to a volume of 10 ml. After washing with saline, the plates were incubated with ¹²⁵I-anti-IgE (Phadebas RAST isotope, Pharmacia), 3 × 10⁵ cpm, in 7 ml of incubation buffer at 25°C for 24 h. After washing four times with saline, the plates were dried and placed in X-ray cassettes on X-ray film (RX, Fuji Photo Film, Kanagawa, Japan). Exposure took place at -80°C for 12 days.

Electrophoretic and Chemical Analyses

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in 10% polyacrylamide gel and polyacrylamide gel isoelectric focusing (PAG-IEF) in 7.5% polyacrylamide gel were carried out as described previously [20]. The gels were stained with Coomassie brilliant blue R.

Total protein was determined by the method of Lowry et al. [21] using bovine serum albumin as standard.

Radioallergosorbent Test (RAST) and RAST Inhibition

Each preparation of mite allergens was coupled to cyanogen bromide activated paper discs according to the method of Ceska et al. [22]. An allergen disc was incubated for 3 h at room temperature with 50 µl of serum. When it was needed because of a high IgE antibody concentration, serum was diluted in a horse serum. The discs were then washed and 50 µl of ¹²⁵I-anti-IgE were added. After an overnight incubation, the discs were washed and counted in a gamma counter. The results were expressed in Phadebas RAST units per milliliter (PRU/ml) by the use of Phadebas RAST Reference System.

RAST inhibition was performed as described previously [20]. The results were expressed as percent inhibition, where counts per minute (cpm) obtained with 50 µl of pooled allergic serum in 50 µl of buffer and cpm obtained with only 100 µl of buffer were used in the calculations as the 0 and 100% inhibition value, respectively.

Histamine Release Assay

Histamine release experiments from washed leukocytes were

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carried out according to the method of Lichtenstein and Osler [23]. Washed leukocytes were obtained from the venous blood of mite allergic donors and suspended in PIPES buffer (25 mM piperazine-N, N'-bis-2-ethanesulfonic acid, 110 mM sodium chloride and 5 mM potassium chloride) containing 1 mM Ca^{++} , 1 mM Mg^{++} and 0.03% (w/v) human serum albumin, pH 7.4. Allergen preparations diluted in the same PIPES buffer were mixed at a series of concentrations with washed leukocytes and incubated at 37°C for 45 min.

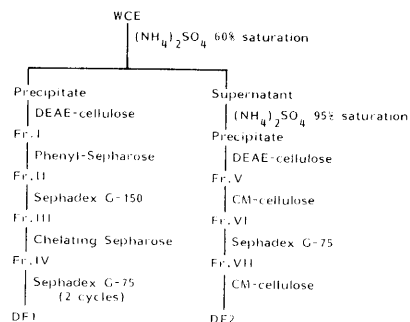


Fig. 1. Schematic diagram of the isolation procedure for DF1 and DF2.

The histamine released into the supernatant was measured by automated fluorometry [24] on a Technicon Autoanalyzer II (Technicon Instruments, Tarrytown, N.Y., USA).

Results

Isolation of DF1 and DF2

In preliminary experiments, it has been indicated that WCE had two highly allergenic fractions. One was precipitable at 60% saturation of ammonium sulfate and had an acidic isoelectric point with approximately 20,000 to 30,000 molecular weight. The other was not precipitable at 60% saturation of ammonium sulfate and had a basic isoelectric point with less than 20,000 molecular weight. Thus, the first step of the fractionation of WCE was achieved by ammonium sulfate precipitation at 60% saturation (fig. 1).

DF1 was isolated from the ammonium sulfate precipitate of WCE at 60% saturation by a combination of anion-exchange chromatography on DEAE-cellulose, hydrophobic chromatography on Phenyl-Sepharose, gel chromatography on Sephadex G-150 and chelate chromatography on Chelating Seph-

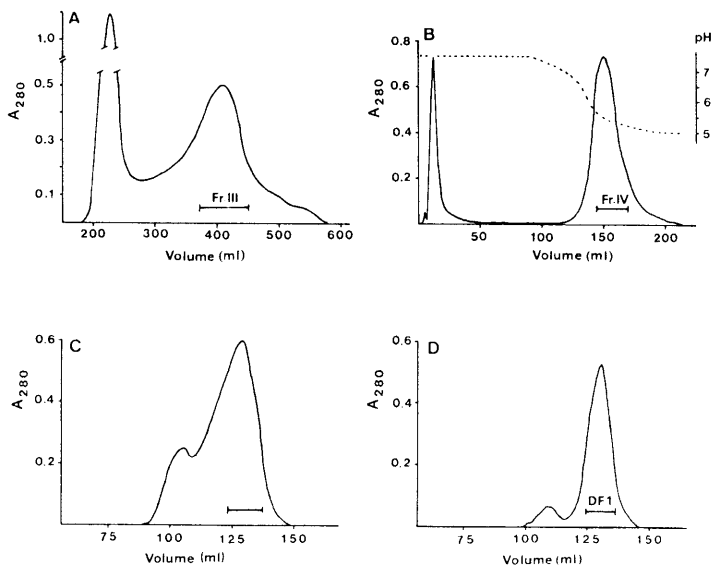


Fig. 2. Major chromatographic steps in the purification of DF1. A Gel chromatography of Fr. II on a Sephadex G-150 column (2.6 × 100 cm) equilibrated with 0.05 M ammonium bicarbonate. B Chelating chromatography of Fr. III on Chelating Sepharose. The column (1.0 × 15 cm) was chelated with zinc ion and equilibrated with 0.05 M Tris-acetate containing 0.5 M sodium chloride, pH 7.5. The sample was eluted with a gradient of decreasing pH of the buffer from 7.5 to 5.0. C Gel chromatography of Fr. IV on a Sephadex G-75 (superfine) column (1.5 × 100 cm) equilibrated with 0.05 M ammonium bicarbonate. D Re-chromatography of the DF1-containing fraction shown in C on the same Sephadex G-75 column.

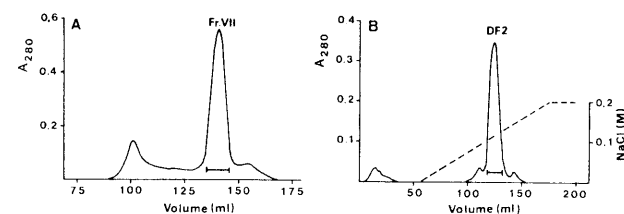


Fig. 3. Major chromatographic steps in the purification of DF2. A Gel chromatography of Fr. VI on a Sephadex G-75 (superfine) column (1.5 × 100 cm) equilibrated with 0.05 M ammonium bicarbonate. B Cation-exchange chromatography of Fr. VII on a CM-cellulose column (1.0 × 15 cm) equilibrated with 0.01 M sodium acetate pH 5.5. The sample was eluted with a linear sodium chloride gradient from 0 to 0.2 M.

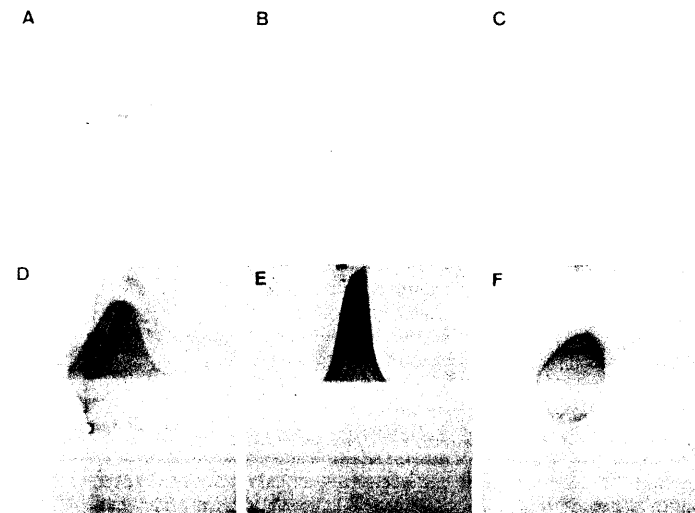


Fig. 4. CIE (A-C) and corresponding CRIE (D-F) analysis of WCE (A and D, 200 µg), DF1 (B and E, 4 µg) and DF2 (C and F, 4 µg). Electrophoresis was performed for 40 min at 10 V/cm in the first dimension and for 16 h at 2 V/cm in the second dimension. The second dimension gel contained 6 µl/cm² of IgG fraction of rabbit anti WCE serum. Pooled allergic serum was used for CRIE analysis.

arose. In each chromatography, identification of DF1 in column eluates was achieved by FRIE with the use of rabbit anti-WCE serum. DF1 gave a clear precipitate with intense staining in FRIE. Major chromatographic steps in the purification of DF1 are shown in figure 2.

DF2 was isolated from the ammonium sulfate pre-

cipitate of WCE at 60–95% saturation by a combination of anion-exchange chromatography on DEAE-cellulose, cation-exchange chromatography on CM-cellulose and gel chromatography on Sephadex G-75. Because DF2 was not visualized in FRIE against anti-WCE serum, DF2 in column eluates was identified by immunodiffusion analysis with the use of rabbit an-

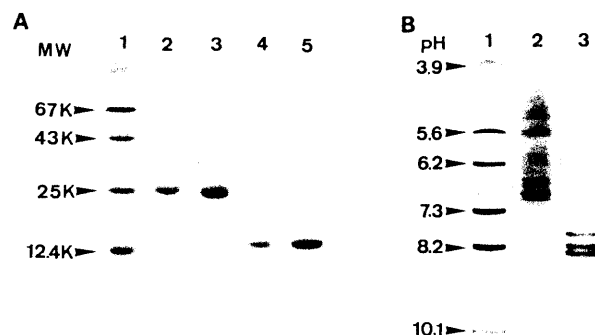


Fig. 5. Electrophoretic analysis of DF1 and DF2. A SDS-PAGE: (1) molecular weight (MW) markers containing bovine serum albumin (MW 67,000), ovalbumin (MW 43,000), chymotrypsinogen A (MW 25,000) and cytochrome C (MW 12,400); (2) DF1 (4 µg); (3) DF1 (20 µg); (4) DF2 (4 µg); (5) DF2 (20 µg). B PAG-IEF: (1) pI marker (Oriental Yeast Co., Ltd.); (2) DF1 (50 µg); (3) DF2 (20 µg).

tiserum to partially purified DF2. Figure 3 shows major chromatographic steps in the purification of DF2. The yields of DF1 and DF2 from 200 g of the whole culture were 6.0 and 4.5 mg, respectively.

Homogeneity and Physicochemical Properties

WCE and the two isolated allergens, DF1 and DF2, were analyzed by the CIE/CRIE system with the use of rabbit anti-WCE serum and pooled allergic serum (fig. 4). WCE gave at least 14 precipitates in CIE. Three precipitates with intense radiostaining were found in CRIE of WCE. Single precipitates with intense staining in both CIE and CRIE were obtained for DF1. DF2 was not visualized in CIE but gave a single precipitate with intense radiostaining in CRIE.

The homogeneity of DF1 and DF2 with respect to molecular size was shown by SDS-PAGE. DF1 and DF2 both showed single bands and were found to have apparent molecular weights of 25,000 and 15,000, respectively. No contaminants were visible at protein loads up to 20 µg (fig. 5A). On the other hand, both DF1 and DF2 were shown to be heterogeneous with respect to molecular charge. On PAG-IEF, DF1 showed multiple bands in the pH range 4.6–7.2, and DF2 showed three bands with pIs of 7.8, 8.1 and 8.3 (fig. 5B).

Separation of DF1 by Preparative Isoelectric Focusing

Since DF1 showed multiple bands on PAG-IEF over a wide pH range, fractionation of DF1 by pre-

parative isoelectric focusing was performed. After focusing, column eluates were analyzed by FRIE and separated into three subfractions, DF1a (pI 4.6–5.3), DF1b (pI 5.4–6.4) and DF1c (pI 6.5–7.2), respectively (fig. 6). By FRIE with the use of rabbit anti-WCE serum, only a single precipitate which was almost in parallel with protein peak (monitored by absorbance at 280 nm) was observed. Allergenic cross-reactivity among the three isolated subfractions of DF1 was assessed by RAST inhibition. DF1b was coupled to activated paper discs and the ability of the three subfractions of DF1 to inhibit the binding of IgE antibodies in pooled allergic serum to the discs was tested. As shown in figure 7, inhibition curves of the three subfractions were almost identical. These results indicate that the multiple molecular forms of DF1 with different isoelectric points are antigenically identical and also seemed to be allergenically identical.

Cross-Reactivity between DF1 and DF2

RAST cross-inhibition experiments between DF1 and DF2 were carried out to assess cross-allergenicity or cross-contamination between the two allergen preparations (fig. 8). Binding of IgE antibodies in pooled allergic serum to either DF1-coupled disc or DF2-coupled disc was well inhibited by homologous antigen. The inhibitory capacity of heterologous antigen was less than 0.01% of that of homologous antigen, suggesting that there was no or little allergenic cross-reactivity between the two allergen preparations.

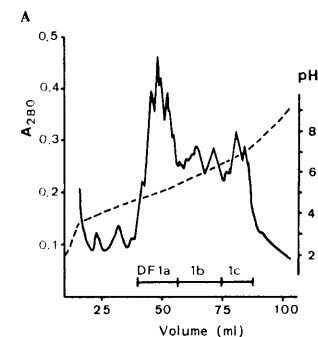


Fig. 6. Column isoelectric focusing of DF1 in a sucrose density gradient. The sample load was 5 mg. The column eluates were monitored by absorbance at 280 nm (A) and analyzed by FRIE with the use of rabbit anti-WCE serum (B). Three subfractions were pooled as indicated.

Allergenic Activity of DF1 and DF2

The allergenic activities of DF1 and DF2 were measured by RAST-binding assay as well as histamine release assay and the results were compared with those obtained using WCE and MBE. IgE antibody levels to each of the allergen preparations in sera from 20 untreated mite allergic patients measured by RAST-binding assay are shown in table I. In 20 sera, all of which had detectable IgE antibodies to

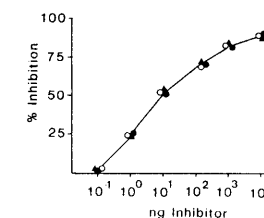


Fig. 7. RAST inhibition comparing the relative abilities of DF1a (●), DF1b (○) and DF1c (▲) to inhibit the binding of IgE antibodies in pooled allergic serum to DF1b-coupled disc.

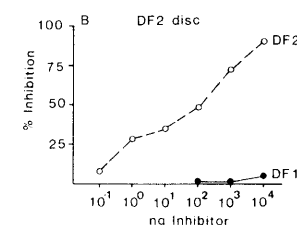
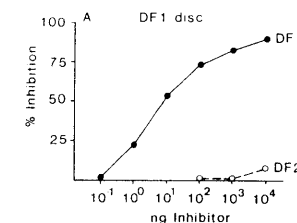


Fig. 8. RAST cross-inhibition between DF1 and DF2. Binding of IgE antibodies in pooled allergic serum to DF1-coupled disc (A) or DF2-coupled disc (B) was inhibited by either DF1 (●) or DF2 (○).

both MBE and WCE, 18 had detectable IgE antibodies to both DF1 and DF2, one (No. 20) had detectable IgE antibodies to DF1 and not to DF2, and one (No. 18) had no detectable IgE antibodies to either DF1 or DF2.

Comparable histamine release assays were carried out with leukocytes from mite allergic patients. The concentration required to induce 50% histamine release is a reflection of the cell sensitivity to the test an-

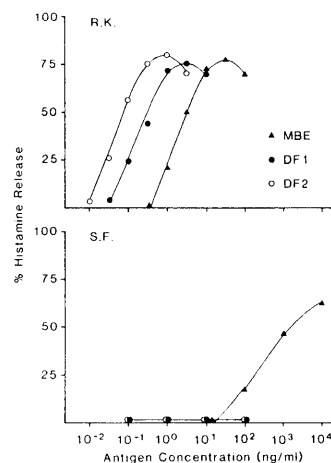


Fig. 9. Typical dose-response curves on histamine release assay with leukocytes of 2 different allergic patients.

tigen and is a measure of allergenicity [25]. The results obtained from 16 patients' leukocytes which released more than 50% of total histamine by all of the test antigens are summarized in table II. In all patients, leukocytes required a smaller concentration of the purified allergens (DF1 and DF2) than the crude extracts (MBE and WCE) to release 50% of total histamine. From the geometric mean values of the cell sensitivity in this group of patients, the purified allergens were 20–60 times more active than the crude extracts.

Typical dose-response curves of histamine release assay obtained with 2 patients are shown in figure 9. Patients R.K. and S.F. were donors of serum No. 1 and 18 in table I, respectively. Leukocytes from patient R.K., whose serum had high IgE antibody levels to DF1 and DF2, released histamine well by all of the test antigens. Leukocytes from patient S.F., whose serum had no detectable IgE antibodies to either DF1 or DF2, released no histamine by either DF1 or DF2. But his leukocytes released histamine well by MBE, to which his serum had detectable IgE antibodies, sug-

Table I. RAST-binding assays with *D. farinae* allergens

Serum No.	IgE antibody, PRU/ml			
	MBE	WCE	DF1	DF2
1	120	58	30	78
2	104	82	57	112
3	82	57	45	35
4	80	65	53	80
5	80	53	30	46
6	60	50	33	45
7	45	36	27	23
8	36	39	22	31
9	36	21	10.0	11.0
10	34	34	28	26
11	28	29	19.0	22
12	28	18.0	9.0	19.5
13	23	22	17.5	18.0
14	22	15.5	8.5	13.5
15	16.5	13.0	7.3	9.0
16	13.0	18.5	5.6	17.5
17	12.5	8.0	5.0	6.3
18	10.0	4.6	<0.35	<0.35
19	3.2	4.0	1.7	2.3
20	2.0	1.1	2.2	<0.35
Geometric mean	27.3	21.1	12.1	14.9

Table II. Histamine release assays of *D. farinae* allergens

Patient No.	Concentration of antigen required for 50% histamine release, ng/ml			
	MBE	WCE	DF1	DF2
1	0.7	3.1	0.18	0.037
2	1.0	1.8	0.71	0.010
3	1.5	4.0	0.057	0.40
4	3.1	6.5	0.40	0.071
5	5.4	9.5	0.32	0.080
6	5.7	2.8	0.098	0.064
7	5.8	7.0	0.26	0.090
8	6.0	8.8	0.074	0.080
9	8.0	11	0.58	0.14
10	8.2	5.4	0.36	0.16
11	8.3	7.2	0.53	0.12
12	10	7.2	0.30	0.20
13	18	29	0.88	0.88
14	28	22	0.54	0.45
15	31	15	0.30	0.25
16	84	270	7.5	1.4
Geometric mean	6.9	9.0	0.35	0.14

Table III. Effect of heating (100°C, 10 min) on the allergenic activity of DF1 and DF2

Patient No.	Concentration of antigen required for 50% histamine release, ng/ml			
	DF1		DF2	
	native	heated	native	heated
17	0.36	1,400	ND	ND
18	0.30	300	ND	ND
19	0.94	1,400	ND	ND
20	0.074	700	0.082	0.17
21	1.6	4,200	0.26	0.35
22	ND	ND	0.50	0.63
23	ND	ND	0.20	0.34
24	ND	ND	0.49	0.61
Geometric mean	0.41	1,100	0.25	0.38

ND = Not done.

gesting that he was probably sensitized with different allergen(s) in the crude mite extract.

Thermostability of DF1 and DF2

The antigens were heated at 100°C for 10 min and the allergenicity of the heat-treated antigens were compared to that of the native antigens with histamine release assay. The concentrations of the native and heat-treated antigens to induce 50% histamine release from individual leukocytes are shown in table III. The heat-treated DF1 was about 0.001–0.0001 as active as the native antigen. On the other hand, the heat-treated DF2 retained 0.5–0.8 of the activity of the native antigen. This result indicates that the allergenicity of DF1 is heat-sensitive and that of DF2 is essentially heat-stable.

Discussion

Two allergens were isolated from the extract of the whole culture of *D. farinae*. The culture medium used in this study was a powdered animal food for laboratory mice and rats [18], which consists of many kinds of materials such as fish powder, wheat flour and wheat germ. Some of these materials contain allergens. In fact, about 20% of mite allergic patients showed positive RAST to the culture medium extract,

although IgE binding to the culture medium extract was invariably less than that to WCE or MBE. Thus, WCE which contains the bulk of culture medium components is not suited for diagnostic or therapeutic use. However, the use of the whole culture of mite cultivated in an animal food is seemed to be suited for the purpose of the isolation of purified mite allergens in a large scale, since an animal food is easy to obtain and the preparation of a kilogram scale of the whole culture is quite simple.

It has been demonstrated that the majority of the major allergen of *D. pteronyssinus*, antigen P₁, in the whole culture was associated with fecal particles [26]. However, the full repertoire of important allergens of *D. pteronyssinus*, including antigen P₁, was identified in both extracts of the whole culture and of the isolated mite body [11]. Mite allergens have been isolated from a wide variety of starting materials including whole culture extract [14], mite body extract [13], extract of spent growth medium [15] and acetone precipitate of whole culture extract [16]. WCE was chosen as starting material in this study, since all of the extractable allergens in the whole culture associated with both fecal particles and mite bodies were included in WCE.

PAGE-IEF analysis of DF1 and DF2 showed that both of them were heterogeneous with respect to molecular charge. In particular, DF1 revealed multiple molecular forms over a wide pH range (pI 4.7–7.2). Preparative isoelectric focusing of DF1 and the following FRIE and RAST inhibition (fig. 6, 7) suggested that those molecular forms of DF1 were antigenically and allergenically identical. DF2 revealed at least three molecular forms with the pI values differing by less than 1 pI unit. The isoallergenicity of three molecular forms of DF2 remains to be determined.

RAST-binding assay and histamine-release assay showed that both DF1 and DF2 were the major allergens of *D. farinae*. IgE antibodies to DF1 and DF2 were found in sera of the majority of mite allergic patients, and leukocytes from mite-allergic patients had higher cell sensitivity to DF1 and DF2 than to WCE and MBE. However, all mite allergic individuals are not sensitive to DF1 and/or DF2 as indicated by 1 subject (S.F.) who was not sensitized with either DF1 or DF2 but was sensitized with other allergen(s) in the crude mite extract (table I; fig. 9).

Our finding that there are at least two major allergens in *D. farinae* is in accord with the finding of Dale and Landmark [12]. They characterized the allergens

in the mite body extract of *D. farinae* by means of the CIE/CRIE system and identified a total of 11 allergens. They have shown that the majority of patients' sera gave specific IgE binding to 2 (Ag 19/20 and Ag 22) of those 11 allergens. The presence of two major allergens in *D. pteronyssinus* has also been demonstrated [10].

RAST cross-inhibition experiments showed that there was no or little allergenic cross-reactivity between DF1 and DF2. This finding contrasts with those obtained with the two major allergens of short ragweed, antigens E and K, where it was demonstrated by either the histamine-release assay [25] or RAST inhibition [27] that antigen K shared some allergenic determinants with antigen E.

DF1 is probably equivalent to the major allergen of *D. pteronyssinus*, antigen P₁, isolated by Chapman and Platts-Mills [14], since several physicochemical properties of DF1, including the apparent molecular weight on SDS-PAGE, distribution of isoelectric points over a wide pH range and the heat-sensitive nature of its allergenicity, are very similar to those of antigen P₁ reported.

Currently isolation studies of allergens from *D. pteronyssinus*, which are equivalent to DF1 and DF2, are being undertaken in our laboratory to investigate the cross-reactivity of individual allergens between the two species of genus *Dermatophagoides*.

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