0022-1767/86/1379-2841$02.00/0
THE JOURNAL OF IMMUNOLOGY

Vol. 137, 2841–2847, No. 9, November 1, 1986
Printed in U.S.A.

ANTIGEN *Der f* I FROM THE DUST MITE *DERMATOPHAGOIDES FARINAE*: STRUCTURAL COMPARISON WITH *Der p* I FROM *DERMATOPHAGOIDES PTERONYSSINUS* AND EPITOPE SPECIFICITY OF MURINE IgG AND HUMAN IgE ANTIBODIES[1]

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The physicochemical and antigenic properties of an allergen purified from *Dermatophagoides farinae*, *Der f* I, were compared with *Der p* I from *Dermatophagoides pteronyssinus*. On SDS-PAGE, *Der f* I migrated as a single polypeptide chain with the same m.w. as *Der p* I (24,000). Two isoallergenic peaks of *Der f* I were identified on preparative isoelectric focusing (pI 5.7 to 6.3 and pI 6.6 to 6.95). Fractions from each peak were shown to have an identical amino acid composition (which was similar but not identical to *Der p* I) and the same N-terminal amino acid sequence. There was a good correlation between quantitative intradermal skin tests to both purified allergens and to *D. farinae* extract in mite-allergic patients, with positive results when using as little as 10^{-5} μg/ml of *Der f* I. The majority of sera with detectable IgE antibody to *D. farinae* also had IgE antibody to *Der f* I both among children (29/42 = 69%) and adults (55/63 = 87%). By RAST, there was an excellent correlation between IgE antibody to *Der f* I and *Der p* I in sera from 42 mite-allergic children (n = 0.94, p <0.001). Polyclonal IgG antibodies from six mice immunized with *Der f* I showed preferential binding to that allergen, and most monoclonal antibodies (16 of 18) raised against *Der f* I did not bind *Der p* I. However, two monoclonal antibodies from this fusion showed cross-reactive binding to both allergens. Immunoabsorption experiments, using *D. pteronyssinus* and *D. farinae* extracts coupled to Sepharose, showed that a large proportion of murine antibodies (74% to *Der p* I and 60 to 93% to *Der f* I) could not be absorbed by the heterologous extract on the immunosorbent. In contrast, in sera from seven mite-allergic patients, most of the specific IgE and IgG antibody (i.e., ≥82%) was removed by either immunosorbent. Thus, *Der f* I and *Der p* I represent a homologous pair of major allergens which possess both cross-reacting and species-specific epitopes. The antibody response in mice immunized with either allergen in complete Freund's adjuvant was largely directed against species-specific epitopes, whereas in allergic humans, IgE- and IgG-specific antibodies bound predominately to cross-reacting epitopes.

In many parts of the world, mites of the genus *Dermatophagoides* are recognized as a major stimulus for human IgE antibody production (1, 2). Two species, *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*, are important and may coexist within the same household, although one or the other species is usually dominant (3–9). As judged by skin testing and the radioallergosorbent test (RAST),[4] there is extensive cross-reactivity or concordant sensitivity to these two species (10–15). At the molecular level, the antigenic relationship between allergens from these two species has primarily been examined by using polyclonal rabbit antibodies in immunodiffusion-based techniques (16–18). Despite the detection of common antigenic determinants, those studies have emphasized the importance of species-specific epitopes. Interestingly, recent studies on purified ragweed pollen allergens have also demonstrated that structurally similar proteins from different species show very little cross-reactivity (19).

We previously purified a major allergen from *D. pteronyssinus*, Antigen P_1 (*Der p* I),[5] a glycoprotein of m.w. 24,000 (20). We have also reported a series of 27 murine monoclonal antibodies to *Der p* I which showed no detectable binding to *D. farinae* extract (21). In the present study, we fully purified a major allergen, *Der f* I from *D. farinae*. On SDS-PAGE, this allergen and *Der p* I have identical m.w. In addition, the amino acid composition of *Der f* I is very similar to that of *Der p* I. The antigenic properties of these two structurally similar allergens has been studied by using murine polyclonal and monoclonal IgG and human IgE antibodies. In direct binding and immunoabsorption experiments, polyclonal and monoclonal antibodies to *Der f* I from mice were shown to recognize species-specific epitopes predominantly. In

Received for publication November 29, 1985.
Accepted for publication July 23, 1986.
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[1] This work was supported by the American Lung Association (Research Training Fellowship) and National Institutes of Health Grant A120565.
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[4] Abbreviations used in this paper: *Der f* I, antigen *Der f* I of *D. farinae*; *Der p* I, antigen *Der p* I of *D. pteronyssinus*; FrI, Fraction I; FrII, Fraction II; F_1a and F_1b, isoallergens of *Der f* I identified on preparative isoelectric focusing; RAST, radioallergosorbent technique/test; RIA, inhibition radioimmunoassay; IEF, isoelectric focusing; BBS, borate-buffered saline, pH 8; SAS, saturated ammonium sulfate; HSA, human serum albumin.

[5] Allergen nomenclature is based on that recently approved by the WHO/IUIS Allergen Nomenclature Subcommittee—D. G. Marsh, Allergen Nomenclature, Bulletin of the WHO. In press.

contrast, RAST and RAST absorption experiments showed that human IgE antibodies to both proteins are largely directed against common, or cross-reacting, determinants. Our results demonstrate that the epitope specificity of murine IgG antibodies is strikingly different from the epitope specificity of human IgE antibodies for these two major allergens.

MATERIALS AND METHODS

Purification of Der f I. Purification of *Der f* I was achieved by using methods similar to those used to purify *Der p* I (20). The starting material for purification was whole *D. farinae* culture from which mite bodies had been removed. Three hundred forty grams of this culture (kindly provided by Hollister-Stier, Spokane, WA) were extracted overnight in 3.4 L of borate-buffered saline, pH 8 (BBS) at 4 °C. After centrifugation (4200 rpm × 30 min), the supernatant was stirred with an equal volume of saturated ammonium sulfate (SAS) for 1 hr at room temperature and was centrifuged (4200 × 30 min). The resulting ppt (50% SAS ppt) was resuspended in 75 ml BBS and was dialyzed extensively. Ten milliliters of this material were applied to an upward-flowing, 100 x 2.6-cm Sephacryl S-200 column, equilibrated in BBS. Eluted 5-ml samples were analyzed by direct RAST, optical density (OD) at 280 nm, and immunodiffusion against rabbit anti-*D. farinae*. In addition, samples were assayed by an inhibition radioimmunoassay (RIA) for cross-reacting determinants on *D. farinae* protein and *Der p* I. Samples rich in RAST activity and in cross-reactive protein were pooled as Fraction I (FrI) and were concentrated by negative pressure dialysis using Spectrapor 1 (MWCO 8000).

Preparative electrophoresis of 35 mg of FrI in barbital buffer (pH 8.6) was carried out by using a 50 x 50 x 1-cm block of Pevikon (Calbiochem Behring, San Diego, CA). Samples were electrophoresed for 24 hr at 60 mA. Samples were assessed by immunodiffusion, Lowry assay for protein, and RIA. Pooled samples were concentrated, were dialyzed against BBS, and were centrifuged (4200 rpm × 30 min). Eleven milligrams of this material were further purified by preparative isoelectric focusing (IEF) for 14 hr at 8 W in a gel containing 4% W/V Ultrodex and 5% Pharmalyte, pH 5 to 8 (Pharmacia Fine Chemicals, Piscataway, NJ), in 100 ml distilled water. Fractions off IEF were eluted and two major peaks of protein (F_1a and F_1b) were identified by RIA, immunodiffusion, and OD. To remove ampholines, both fractions were treated with 80% SAS, and the resulting precipitates were resuspended and were chromatographed separately over a 40 x 1-cm Sephadex G-100 column.

Antisera. Rabbit antiserum to *D. farinae* was obtained by repeated injection of New Zealand white rabbits with 4 mg of protein extracted from a different *D. farinae* culture (kindly provided by Bencard Ltd., Betchworth, UK) emulsified in complete Freund's adjuvant (CFA). Specifically purified antibodies to cross-reacting determinants on *Der p* I were obtained by passing 90 ml of rabbit anti-*D. farinae* serum over a *Der p* I immunosorbent column and eluting with glycine buffer, pH 2.8. Goat anti-serum to rabbit IgG was obtained by repeated immunization with rabbit IgG in CFA. Goat anti-IgE was obtained by immunizing goats with the Fc fragment of myeloma IgE and was specifically purified by elution from an IgE immunosorbent column (22).

Analytical gel electrophoresis and IEF. Fractions containing 5 to 10 μg of protein were analyzed by SDS-PAGE using a resolving gel containing 15% acrylamide. Electrophoresis at 30 mA was performed for 4 to 5 hr. Isoelectric point (pI) determinations were carried out in a vertical slab gel unit (Hoeffer, SE-600). The gel was 1.5 mm thick and contained 5% acrylamide and 3.3% Pharmalyte (pH 5 to 8); 10 to 20 μg of protein were focused at 20 W for 2.5 hr.

Amino acid analysis and N-terminal sequencing. Amino acid analyses using a Beckman 121 amino acid analyzer were performed on reduced and carboxyamidomethylated samples of purified F_1a and F_1b. Fifty micrograms of each were dissolved in 500 μl of 5.7 N HCl containing 0.3% phenol and 0.2% 2-mercaptoethanol. Hydrolyses at 110°C *in vacuum* were carried out in triplicate at 24, 48, and 72 hr. N-terminal sequences were obtained on 45-μg samples of both F_1a and F_1b by using an Applied Biosystems 470-A protein sequencer. PTA-amino acids were identified by using liquid chromatography on a Beckman 321 liquid chromatograph.

Radioimmunoassays (RIA). The inhibition RIA for cross-reactive determinants between *D. farinae* protein and *Der p* I is a modification of an RIA for *Der p* I (23), using antibodies to *D. farinae* specifically purified over a *Der p* I column as described above. In each assay, values were obtained by reference to a control curve of partially purified *D. pteronyssinus* extract which had been substandardized for *Der p* I content from the new WHO *D. pteronyssinus* International Standard (NIBSC 85/518) (24).

RAST and direct RAST were carried out by using CNBr-activated filter paper discs (Whatman Grade 541, 0.6 cm diameter) (25). For RAST, discs were coated with 2 μg/disc of either *Der f* I or *Der p* I or with equivalent amounts of these allergens contained in crude *D. farinae* or *D. pteronyssinus* extracts (derived from whole mite culture provided by Bencard) as determined in RIA. Control curves for each allergen preparation were obtained by using serial twofold dilutions of pooled sera from eight mite-allergic patients. This pool was arbitrarily allotted an activity of 1000 IgE antibodies RAST U/ml. For direct RAST, samples eluting from gel filtration or block electrophoresis were diluted (1/10 and 1/100) and were incubated with activated discs at 4°C overnight, followed by incubations with serum from mite-allergic patients and then ^{125}I-goat anti-IgE, sequentially.

RAST absorption was performed as described by Gleich and Jacob (26). CNBr-activated Sepharose 4B (Pharmacia) was coupled with a 50% SAS precipitate of *D. farinae* or *D. pteronyssinus* whole culture extract (10 mg/g gel, protein content determined approximately by measuring OD at 280 nm and assuming 1 OD = 1 mg/ml) or with human serum albumin (HSA; 10 mg/g gel). Whole culture was provided by Bencard. The quantity of *Der f* I and *Der p* I coupled to the Sepharose 4B gel was measured by RIA on the extracts before and after binding and was estimated to be 6.9 mg and 7.3 mg per gram of gel, respectively. One milliliter of dilutions (1/4 and 1/10) of serum from six mite-sensitive patients was absorbed with 0.25 ml 40% Sepharose beads coupled with *D. farinae*, *D. pteronyssinus*, or HSA protein. Five of the six patients had never been on immunotherapy. An established reference pool of sera (NIBSC, 82/528) (27) from patients allergic to *D. pteronyssinus* was absorbed in the same fashion. Measurements of total IgE, IgE against *D. farinae* and *D. pteronyssinus* allergens by RAST, and IgE or IgG binding to *Der f* I were carried out on each dilution post-absorption, using described methods (28). Similarly, dilutions (1/10) of serum from five mice immunized with *Der f* I as well as of serum pooled from 10 mice immunized with *Der p* I were absorbed in the same fashion. Residual IgG binding to either ^{125}I *Der p* I or ^{125}I *Der f* I was measured after absorption.

Iodination of proteins was carried out by using a modification of the chloramine-T technique (29). Twenty micrograms of protein were used for radiolabeling with 1 mCi ^{125}I which, in the case of *Der f* I, resulted in a specific activity of 20 to 30 μCi/μg.

Skin testing. Skin testing of patients was approved by the Human Investigation Committee of the University of Virginia. *Der p* I and *Der f* I were sterilized by filtration through 0.22-μm Millipore filters (Millipore Corp., Bedford, MA). Both antigens and *D. farinae* whole body extract (Hollister-Stier, Spokane, WA) were prepared in serial 10-fold dilutions (10^1 through 10^{-6} μg/ml) in 0.05% of HSA in phenol saline. Skin test sites were examined 15 min after an injection of 0.05 ml intradermally.

Production of monoclonal antibodies. Seven BALB/c mice were immunized i.p. with 10 μg of FrI from S-200 gel filtration and were then boosted twice at intervals of 10 days. Two weeks after the final injection, serum from each mouse was assayed for binding activity to ^{125}I *Der p* I and ^{125}I FrI. A mouse with an antibody titer of 1:10,000 and good binding to both antigens was boosted with 20 μg FrI. Spleen cells from this mouse were used for fusion as described (21). In brief, fusion was carried out with Sp2/O-Ag14 myeloma cells, using 37% polyethylene glycol. The cells were plated out in 96-well microtiter plates (Costar, Boston, MA). Two weeks post-fusion, the wells were screened for antibody production by enzyme-linked immunosorbent assay (ELISA). Supernatants positive in ELISA were then tested for binding activity to ^{125}I *Der p* I and ^{125}I Fr-I, and positive clones were selectively expanded. Isotyping by immunodiffusion using ascites diluted 1/1000 was carried out for each clone against rabbit anti-mouse subclass antisera (Nordic, El Toro, CA).

RESULTS

Purification of Der f I. When a 50% SAS cut of *D. farinae* extract was chromatographed over a calibrated Sephacryl S-200 column, direct RAST using a serum pool from 10 mite-allergic patients showed that the allergen eluted with an m.w. range of 5000 to 30,000 (Fig. 1). By using an RIA specific for protein cross-reactive with *Der p* I, a peak (FrI) was identified from which samples were pooled, were concentrated, and were dialyzed. Eluted samples were also tested in a direct RAST assay using serum from a mite-sensitive patient which showed good binding to *D. farinae* and *D. pteronyssinus* extracts by

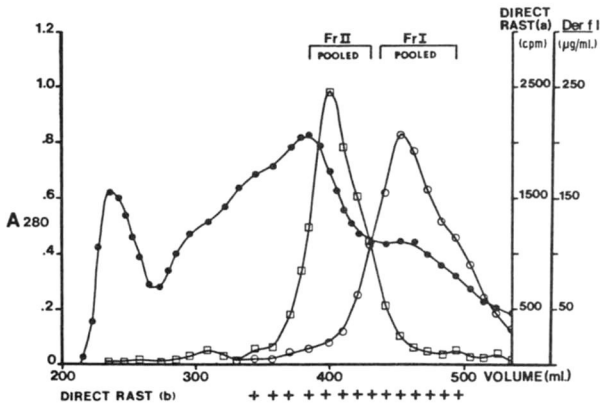

Figure 1. Gel filtration of *D. farinae* over Sephacryl S-200. The elution pattern of proteins (absorbance at 280 nm) (●) and *Der f* I content as measured by RIA (○) is shown. The concentration of mite allergen distinct from *Der f* I was assessed by direct RAST (a) using serum from a mite-sensitive patient with no demonstrable binding to ^{125}I *Der p* I (□). By using pooled serum from mite-allergic patients, direct RAST (b) showed which fractions were strongly positive for total mite allergen (+); FrI and FrII were pooled as indicated.

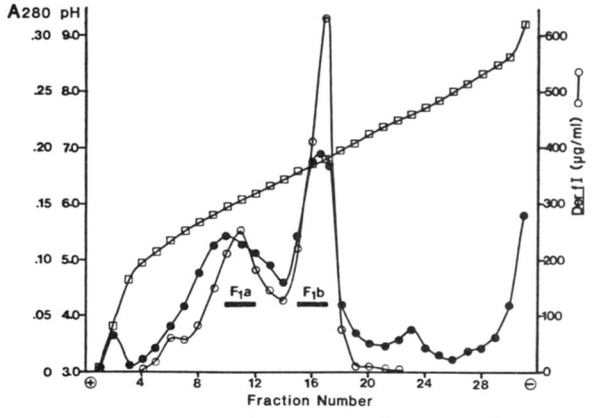

Figure 2. Preparative IEF of partially purified *Der f* I after preparative block electrophoresis. The pH (□), protein content (absorbance at 280 nm) (●), and *Der f* I concentration (○) of each fraction are shown. F_1a and F_1b were pooled as indicated.

RAST but which lacked IgE binding to ^{125}I *Der p* I. A peak of allergen distinct from Fr I was identified by this serum. This peak (FrII) contained a separate protein, m.w. 29,000, the further characterization of which is in progress.

Block electrophoresis of Fr I produced a peak of protein which was cross-reactive with *Der p* I by RIA and which reacted strongly in direct RAST using the *D. farinae* allergic serum pool. On preparative IEF, this material could be detected over a wide pI range but was concentrated in two peaks, pI 5.7 to 6.3 (F_1a) and pI 6.6 to 6.95 (F_1b) (Fig. 2). Pooled fractions from these peaks were rechromatographed over Sephadex G-100 yielding 1.4 mg and 2 mg of purified F_1a and F_1b, respectively. Sequential purification leading to these fractions of *Der f* I was monitored on SDS-PAGE (Fig. 3*A*). Once purified, both fractions (F_1a and F_1b) gave single cross-reacting lines on immunodiffusion against rabbit antisera to *D. farinae* and also gave single lines on SDS-PAGE with a relative m.w. of 24,000 (Fig. 3*B*). The amino acid compositions of F_1a and F_1b (Table I) were judged to be "identical" by using the SΔQ equation proposed by Marchalonis and Weltman (30). In addition, sequence analysis for the first 20 N-terminal amino acids from F_1a and F_1b were essentially identical (Fig. 4). Thus, these fractions show immunochemical identity and appear to be isoallergens of *Der f* I.

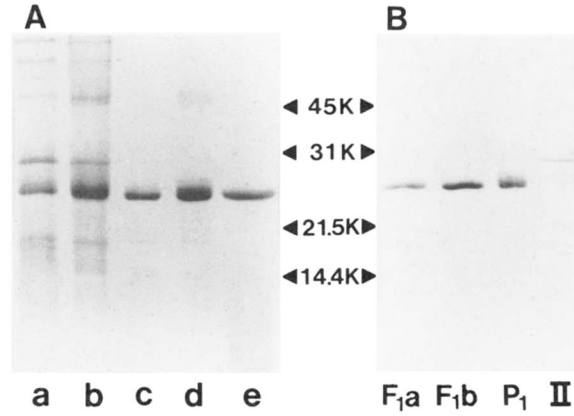

Figure 3. A, Stages in the purification of *Der f* I analyzed on SDS-PAGE. (a) Crude *D. farinae* extract; (b) 50% SDS precipitate; (c) gel filtration over S-200; (d) preparative block electrophoresis; and (e) preparative IEF. *B*, Comparison of purified isoallergen fractions of *Der f* I (F_1a and F_1b) and *Der p* I. Also shown is FrII from gel filtration over S-200 containing allergens distinct from *Der f* I.

TABLE I

Amino acid analysis of F_1a, F_1b, and Der p I (residues/100 residues of amino acids)

Amino Acids	F_1a	F_1b	*Der p* I[a]
ASX	11.8	12.1	14.3
THR	5.8	5.9	4.6
SER	7.6	6.8	7.6
GLX	9.0	7.9	12.2
PRO	5.7	5.9	4.9
GLY	10.9	11.0	11.6
ALA	6.6	7.4	9.5
½CYS	1.2	0.7	2.0
VAL	6.3	6.9	4.2
MET	1.3	1.5	1.2
ILEU	8.2	8.7	6.3
LEU	4.9	5.4	4.3
TYR	7.6	7.8	5.2
PHE	1.6	1.4	2.3
HIS	3.2	2.2	2.4
LYS	1.6	1.4	2.1
ARG	6.7	6.9	5.4

[a] Previously reported data of Fr.5 (pI 4.7 to 5.4) of Antigen P_1 (20).

F_1a NH_2 - Thr - Ser - Ala - Cys[a] - Arg - Ile - Asn - Ser - Val - ^{10}Asn -

F_1b NH_2 - Thr - Ser - Ala - Cys - Arg - Ile - Asn - Ser - Val - Asn -

F_1a - Val - Pro - Ser - Glu - Leu - Asp - Leu - Arg - Ser - ^{20}Leu

F_1b - Val - Pro - Ser - Glu - Leu - Asp - Leu - Arg - Ser - Leu

Figure 4. Sequence of N-terminal amino acids comparing F_1a and F_1b from preparative IEF. The Cys residue in position 4, F_1a, is an estimate, but was positively identified in F_1b under reduced and alkylated conditions.

Comparison of Der f I and Der p I: m.w. and amino acid composition. On SDS-PAGE, *Der f* I and *Der p* I had the same apparent m.w. of 24,000. A comparison of the amino acid composition of these allergens show that F_1a and F_1b differ from *Der p* I by 45 and 57 SΔQ units, respectively, and therefore appear to be closely related but not identical (Table I). The most notable differences are in the contents of aspartate, glutamine, alanine, valine, isoleucine, and tyrosine.

Skin testing and comparative RAST studies. Quantitative intradermal skin testing in adult mite-allergic patients showed similar endpoints for *D. farinae* crude extract and *Der f* I in 10 of 13 patients (Table IIA). Seven

TABLE II

Quantitative skin tests with D. farinae, Der f I, and Der p I[a]

(A)	Quantitative Intradermals (μg/ml)[b]		(B)	Quantitative Intradermals (μg/ml)[b]	
Patient	*D. farinae*	*Der f* I	Patient	*Der p* I	*Der f* I
J.A.	10^{-5}	10^{-5}	J.L.	10^{-5}	10^{-4}
D.S.	10^{-5}	10^{-4}	A.S.	10^{-5}	10^{-4}
M.C.	$10^{-5.5}$	10^{-6}	C.H.	10^{-4}	$10^{-3.5}$
L.Y.	$10^{-4.5}$	10^{-3}	B.W.	10^{-3}	10^{-3}
S.B.	$10^{-6.5}$	10^{-6}	A.A.	10^{-2}	$10^{-1.5}$
M.W.	10^{-6}	10^{-5}	D.B.	10^{-5}	10^{-4}
S.R.	10^{-5}	$10^{-4.5}$	R.H.	10^{-5}	10^{-5}
M.H.	10^{-6}	10^{-5}	J.G.	$10^{-4.5}$	10^{-4}
S.P.	$10^{-5.5}$	10^{-5}	S.S.	$10^{-4.5}$	10^{-4}
M.W.	10^{-5}	$10^{-4.5}$	Y.F.	$10^{-3.5}$	10^{-4}
L.C.	10^{-2}	>1 μg	M.L.H.	10^{-3}	10^{-3}
J.E.	10^{-1}	>1 μg	E.G.	>1 μg	>1 μg
L.B.	10^{-2}	≥1 μg			
Controls × 6[c]	>1 μg	>1 μg	Controls × 4[c]	>1 μg	>1 μg

[a] All patients and controls were also skin tested with 10 μg/ml of culture medium provided by Hollister-Stier. No positive skin reactions were observed.

[b] Intradermal skin tests were carried out by using 0.05 ml of serial 10-fold dilutions of allergen. Values represent endpoint dilutions giving a 6 x 6-mm wheal. Negative skin tests are noted as >1 μg.

[c] Controls = nonallergic individuals.

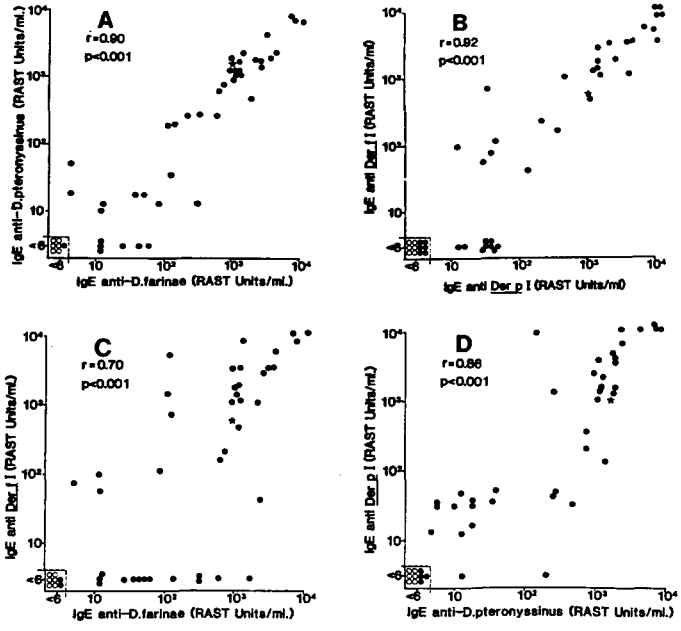

Figure 5. Results of RAST determinations comparing human IgE ab to: (*A*) *D. farinae* vs *D. pteronyssinus* crude extracts; (*B*) *Der f* I vs *Der p* I; (*C*) *D. farinae* crude extract vs *Der f* I; and (*D*) *D. pteronyssinus* crude extract vs *Der f* I. Assays were done by using sera from 42 mite-allergic children and six skin test-negative controls. • = reference pool of sera used as standard NIBSC (82/568) control for patients allergic to *D. pteronyssinus* (27).

patients had a positive skin reaction with as little as 10^{-5} μg/ml of both *D. farinae* and *Der f* I, whereas six control patients did not react with 1 μg/ml of either material. In addition, 12 patients with positive prick test responses to *D. farinae* (wheal >5 x 5-mm) showed similar endpoints for both *Der f* I and *Der p* I (Table IIB). None of these patients reacted with a 1 μg/ml extract of the culture medium used for growing these mites.

RAST assays were carried out on sera from 42 children positive by prick test (wheal ≥3 x 3-mm) to *D. farinae* and/or *D. pteronyssinus* and six skin test-negative controls (Fig. 5). Each serum was tested in four different assays using discs coated with crude extracts of either *D. farinae* or *D. pteronyssinus* or with *Der f* I or *Der p* I. Linear regression analysis showed an excellent correlation betwen human IgE antibody to extracts of *D. farinae* and *D. pteronyssinus* ($r = 0.90$, $p < 0.001$) (Fig. 5*A*) and between the IgE antibody response to *Der f* I and *Der p* I ($r = 0.94$, $p < 0.001$) (Fig. 5*B*). When RAST results were compared between *D. farinae* and *Der f* I (Fig. 5C) and *D. pteronyssinus* and *Der p* I (Fig. 5*D*), the correlation coefficient was lower but still significant, $r = 0.70$ ($p < 0.001$) and $r = 0.86$ ($p < 0.001$), respectively. A few sera showed good binding to the crude extracts but not to either purified allergen, suggesting that these sera contain IgE antibodies against other mite allergen(s). In addition, in sera from 63 RAST-positive mite-allergic adults, it was shown that 55 (87%) had IgE antibody to *Der f* I by antigen binding assay. The results of quantitative skin tests, RAST, and antigen binding assays strongly suggest that *Der f* I is a major allergen of *D. farinae*.

Binding characteristics of polyclonal antisera and murine monoclonal antibodies to Der f I and Der p I. By using antiserum from a rabbit hyperimmunized with *D. farinae* crude extract, *Der f* I and *Der p* I were partially cross-reactive on immunodiffusion. Antisera from six mice immunized with FrI showed preferential binding to *Der f* I in each case; however, sera from three mice (Nos. 2, 3, and 5) showed good binding to *Der p* I (Table IIIa). Spleen cells from mouse number 2 were used to raise monoclonal antibodies. The binding of these antibodies to each purified allergen is presented in Table IIIb. Sixteen of 18 monoclonal antibodies bound only to *Der f* I; however, two antibodies from hybrid 4C1 bound well to both *Der f* I and *Der p* I. Isotyping showed that all clones belonged to the IgG1 subclass. In addition, all of the monoclonal antibodies bound equally well to serial dilutions of ^{125}I F_1a and ^{125}I F_1b (data not shown), adding further evidence that these iso-allergens are antigenically

TABLE IIIa

Mouse antisera to Der f I

Serum No.	*Der f* I Bound (cpm)[a]	*Der p* I Bound (cpm)[a]
1	5,828	777
2	8,573	3,494
3	6,786	1,950
4	10,182	897
5	9,331	2,681
6	553	123
NMS[b]	120	82

TABLE IIIb

Specificity of monoclonal antibodies derived from mouse no. 2 immunized with Der f I

Clone	*Der f* I Bound (cpm)[c]	*Der p* I Bound (cpm)[c]
3E2-E10	20,076	485
3E2-H12	16,345	455
5B5-E6	15,509	489
5G5-A4	20,680	448
6A8-E8	9,760	546
6A8-B10	16,052	491
6A9-D7	13,848	424
4C1-B8	14,794	9,175
4C1-C6	1,800	1,063
Fel d I[d]	601	537
NMS[b]	584	486

[a] Values are the mean of duplicate assays using polyclonal mouse antisera (10^{-2} dilution). Total radioactivity added = 59,723 cpm for ^{125}I *Der f* I and 53,247 cpm for ^{125}I *Der p* I.

[b] NMS, Normal mouse serum.

[c] Values are the mean of duplicate assays using mouse ascites containing monoclonal antibody (10^{-4} dilution). Total radioactivity added = 103,019 cpm for ^{125}I *Der f* I and 87,689 cpm for ^{125}I *Der p* I.

[d] Monoclonal antibody to cat allergen *Fel d* I.

identical.

Absorption of mouse IgG and human IgE allergen-specific antibodies by using D. farinae and D. pteronyssinus immunosorbents. The proportion of mouse IgG and human IgE and IgG antibodies directed against *Der f* I and *Der p* I was compared by immunoabsorption. Sera from five immunized mice (Table IIIa, mice Nos. 1 to 5) and seven mite-allergic patients were absorbed with either *D. farinae* or *D. pteronyssinus*. Figure 6*A* shows that ≥90% of murine antibodies binding *Der f* I were removed by the *D. farinae* immunosorbent; however, a large proportion (61 to 93%) of these antibodies to *Der f* I were not absorbed by *D. pteronyssinus*. Likewise, 73% of IgG antibodies to *Der p* I remained after absorption with *D. farinae* using a pool of sera from 10 mice immunized with *Der p* I.

When sera from six mite-allergic patients and the NIBSC reference pool of sera were absorbed, most of the IgE antibody to *Der f* I (≥82%) was removed by *D. pteronyssinus* (Fig. 6*B*). Similarly, the majority of IgG antibodies to *Der f* I were removed by either immunosorbent from sera of two of these patients. Taking mite exposure into account, a modest species-specific IgE antibody response to *Der f* I was noted after absorption with *D. pteronyssinus* in sera from patients D.R., I.M., and S.B. All three patients were known to be exposed to *D. farinae*, and almost exclusively so in the case of D.R. and I.M. (Table IV). In the sera from these patients, the IgE antibody to *Der f* I was not fully absorbed by *D. pteronyssinus* (Fig. 6*B* and Table IV). These results suggest that a small proportion of the IgE antibody induced by *Der f* I was directed against species-specific epitopes. On direct RAST, six of the unabsorbed sera bound well to both extracts; however, serum from pateint I.M. bound *D. farinae* preferentially (Table IV). In keeping with this finding, a large proportion of the IgE antibodies to *D. farinae* in the serum from this patient was not absorbed by *D. pteronyssinus*. Interestingly, this was the only patient who had received immunotherapy.

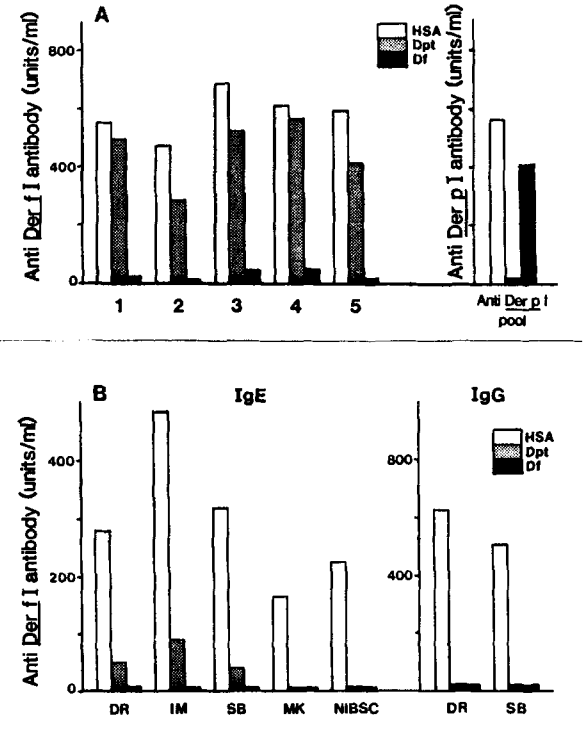

Figure 6. A, Immunoabsorption of mouse polyclonal IgG antibodies to *Der f* I and *Der p* I. Immunosorbents used were HSA (open bars), 50% SAS *D. pteronyssinus* (hatched bars), and 50% SAS *D. farinae* (black bars). Binding to ^{125}I *Der f* I is shown after absorption of sera from BALB/c mice (1–5) immunized with *Der f* I. Binding to ^{125}I *Der p* I is shown after absorption of sera pooled from 10 mice immunized with *Der p* I. *B*, Immunoabsorption of human IgE and IgG antibodies to *Der f* I. Immunosorbents used were the same as described for *A*. Binding to ^{125}I *Der f* I was carried out as described (28). In dust samples from homes of patients D.R. and I.M., >90% of mites identified were *D. farinae*. The NIBSC reference pooled sera (27) and serum from M.K. were from London patients exposed predominately to *D. pteronyssinus*.

DISCUSSION

By using physicochemical techniques similar to those used to purify *Der p* I from *D. pteronyssinus* (20), we have purified a homologous allergen, *Der f* I, from *D. farinae*. On immunodiffusion, *Der f* I is identical to the previously reported *D. farinae* allergens, Df6 and Ag11, using rabbit antisera raised independently against these allergens (18, 31). It was shown to be a major allergen of *D. farinae* because 69% of mite-allergic children and 87% of *D. farinae* RAST-positive adults had *Der f* I-specific IgE antibody in their serum. In addition, quantitative intradermal skin testing using *Der f* I and *D. farinae* whole body extract in parallel showed positive skin tests with as little as 0.05 ml of 10^{-5} μg of *Der f* I/ml.

Der f I was indistinguishable from *Der p* I on SDS-PAGE and was similar, but not identical, in its composition of amino acids. Multiple iso-allergens were demonstrated for both allergens and were concentrated in two peaks on IEF. Fractions purified from each peak (F_1a and F_1b) were identical in their amino acid composition and in the sequence of their first 20 N-terminal amino acids.

On immunodiffusion using rabbit antisera, *Der f* I and *Der p* I showed partial cross-reactivity (23), suggesting the presence of both cross-reacting and species-specific epitopes on the two molecules. Polyclonal antisera from six mice immunized with *Der f* I all showed preferential binding to this allergen, and sera from 10 mice immunized with *Der p* I showed much stronger binding to *Der p* I than *Der f* I (21). In keeping with this finding, monoclonal antibodies raised against these allergens were predominately directed against species-specific epitopes (i.e., 27/27 clones raised against *Der f* I (21) and 16/18 clones raised against *Der f* I). Furthermore, using *D. pteronyssinus* and *D. farinae* immunosorbents, the majority of murine antibodies could not be absorbed by the heterologous allergen extract.

The epitope specificity of human IgE antibodies was strikingly different from the antibody response in mice. Previous studies have established an excellent correlation between human IgE antibody responses to *D. farinae* and *D. pteronyssinus* (10–15). In our experiments using RAST, most patients with IgE antibodies to *D. farinae* and *D. pteronyssinus* extracts also had IgE antibodies to the purified allergens. In addition, the quantitative correlation between IgE antibodies to *Der f* I and *Der p* I by RAST was close to unity (r = 0.94), strongly suggesting extensive cross-reactivity between these allergens. The results of RAST absorption experiments establish that the major antigenic determinants recognized by human IgE antibodies are common to both allergens.

The ability of some sera from mite-allergic patients to inhibit murine monoclonals to *Der p* I has been reported previously (21). In those experiments, two separate epi-

TABLE IV

Absorption of human IgE antibodies specific for mite allergens by D. farinae and D. pteronyssinus extracts

Mite Exposure	PT.	Unabsorbed Serum			D.f. Immunosorbent[c] (% absorbed)			D.pt. Immunosorbent[c] (% absorbed)		
		Total IgE[b]	D.f.RAST[a] U/ml	D.pt.RAST[a] U/ml	D.f.RAST[a]	D.pt.RAST[a]	^{125}I *Der f* I	D.f.RAST[a]	D.pt.RAST[a]	^{125}I *Der f* I
D.f.[d]	D.R.	480	2,300	3,000	94	>95	>95	90	>95	82
	I.M.[g]	1,116	985	80	82	>95	>95	44	>95	82
D.pt.[e]	M.K.	1,065	427	685	>95	88	>95	>95	>95	>95
	J.L.	962	840	1,350	>95	80	>95	>95	>95	>95
	NIBSC[h]	1,872	1,287	1,480	89	82	>95	>95	>95	>95
N.D.[f]	S.B.	2,238	5,433	5,667	>95	89	>95	76	94	87
	M.H.	591	787	650	95	>95	>95	94	>95	>95

[a] Results were obtained from a control curve and are expressed in arbitrary U/ml of binding activity.
[b] Determined by double antibody inhibition RIA on unabsorbed samples. One unit of IgE approximately equivalent to 2.4 ng.
[c] 10 mg lyophilized 50% SAS *D. farinae* or *D. pteronyssinus*/g of Sepharose 4B swollen gel. D.f. and D.pt. RAST as well as binding to ^{125}I *Der f* I expressed as a percent of binding following absorption with HSA immunosorbent.
[d] >90% of mites identified in dust samples from these patients' homes were *D. farinae*.
[e] >90% of mites identified in dust samples from these patients' homes were *D. pteronyssinus*.
[f] N.D., not determined.
[g] Serum absorbed × 3.
[h] An established reference pool of sera, NIBSC, 82/528 (27).

topes could be inhibited by allergic sera, although only four of 14 sera showed >50% inhibition at a serum dilution of 1/5. When sera from mite-allergic patients were tested for their ability to inhibit monoclonal antibody binding to *Der f* I, modest inhibition (10 to 31% inhibition of the cross-reacting monoclonal 4C1-B8 and 8 to 20% inhibition of the species-specific clone 6A8-B10 using sera from five of nine and four of nine allergic patients, respectively) was observed (data not shown). Although some of the human polyclonal antibodies may be species-specific and thus block the binding of the monoclonal antibodies, other nonspecific factors may explain these results. First, some of the cross-reacting epitopes recognized by human antibodies may overlap the species-specific epitopes detected by the monoclonals. The monoclonal 4C1-B8, which binds to both allergens, can inhibit >90% of the binding of one of the species-specific monoclonals to *Der p* I (10B9 F6) (data not shown). Secondly, the size of *Der f* I and *Der p* I may limit the number of antibodies which can bind to their surface simultaneously. The binding of human polyclonal antibodies may thus, in part, block the binding of murine monoclonals by steric hindrance rather than by binding to identical sites. Finally, serum dilutions of 1/2 were required to observe levels of inhibition of monoclonal antibodies which were significantly above the nonspecific background for nonallergic sera.

Whether mite allergens other than *Der f* I and *Der p* I exhibit cross-reactivity in regard to human IgE binding remains to be established. In reviewing RAST and antigen-binding results on a large series of adult mite-allergic patients, we identified 20 patients who had no detectable binding activity to *Der f* I. These sera also showed an excellent correlation of IgE antibodies to *D. farinae* and *D. pteronyssinus* by RAST ($r = 0.98$, $p < 0.001$). This finding supports the belief that cross-reactivity exists between other allergens produced by these two species.

In conclusion, our results reveal a dichotomy between the IgG antibody response in mice and the IgE antibody response in humans with respect to epitope specificity on two homologous mite allergens. Although there is convincing evidence that the entire surface of proteins foreign to the host may be antigenic (32), in any individual host the antibody response may only be directed against a subset of antigenic sites. Several factors may explain the dichotomy in our results. First, it is possible that antibody isotypes (e.g., IgE vs IgG) differ in epitope specificity. However, *D. pteronyssinus* and *D. farinae* immunosorbents removed the majority of both IgE and IgG antibodies from two human sera used in our studies. Furthermore, studies in patients with ragweed hayfever indicate that allergen-specific IgE and IgG responses may be under the same genetic control (33). Thus, there is little evidence that different isotypes could explain the differences in epitope specificity. Secondly, we presume that the human IgE antibody response is polyclonal because these allergens can cross-link IgE antibodies on mast cells in the skin and on basophils in vitro (34). Whether the binding of human antibodies to epitopes on *Der f* I and *Der p* I is more, or less, diverse than the polyclonal response in mice is not clear. Thus far, only two mouse monoclonals have been shown to bind both allergens. Further analysis of this problem may well require additional monoclonal antibodies directed against cross-reacting epitopes and structural data including the amino acid sequences. Third, it is well established that host regulatory factors including genetic controls (e.g., *Ir* genes, the host's antibody gene repertoire, etc.) are important in determining which epitopes are dominant in any one immunization (35). At present, there is very little data on the genetics of the human response to mite allergens. Finally, the route and dosage of antigen presentation and the adjuvant used to stimulate an antibody response are well known to influence the extent of the IgE antibody response to protein antigens (36–38). In our experiments, mice were hyperimmunized by using approximately 80 μg of allergen in CFA, whereas mite-allergic humans are naturally exposed to only microgram quantities of inhaled mite allergen per year (39). This difference might well be an important factor in influencing epitope specificity. Additional experiments comparing the epitope specificity of antibodies from different strains of mice immunized either conventionally with allergen in CFA or with repeated low doses of allergen in alum might provide answers to some of these questions. The results from such experiments may also stimulate interest in examining whether the epitope specificity of antibodies produced by atopic individuals can be altered by immunotherapy.

Acknowledgments. We are grateful to Dr. J. P. Dandeu, Institute Pasteur, Paris, France, and Dr. Peter Lind, Co-

penhagen, Denmark, for providing antisera to Ag11 and Df6, respectively. We also thank Hollister-Stier Ltd. and Bencard Ltd. for generous supplies of *D. farinae* and *D. pteronyssinus* culture; Dr. Jay Fox for carrying out amino acid and N-terminal sequence analyses; Dr. William M. Sutherland for assistance with production of monoclonal antibodies against *Der f* I; Dr. Rob Aalberse for providing monoclonal antibody to *Fel d* I; Susan R. Wilkins for her valuable technical assistance and help in drawing figures; and Martha D. Waring and Nancy Malone for their secretarial assistance.

Note Added in Proof. Further purification of Fr II shows that it contains two distinct *D. farinae* allergens, *Der f* II (m.w. ~ 11,500 on SDS-PAGE) and *Der f* III (m.w. 29,000). We have shown that *Der f* II shares antigenic determinants with the *D. pteronyssinus* allergen DpX (40). *Der f* II, like DpX, also appears to be a major allergen.

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