

Antigenic and structural analysis of group II allergens (*Der f II* and *Der p II*) from house dust mites (*Dermatophagoides* spp)

Peter W. Heymann, MD,* Martin D. Chapman, PhD,*
Robert C. Aalberse, PhD,** Jay W. Fox, PhD,*** and
Thomas A. E. Platts-Mills, MD, PhD*
Charlottesville, Va., and Amsterdam, The Netherlands

Monoclonal antibody affinity chromatography was used to purify two homologous mite allergens, Der f II from Dermatophagoides farinae and Der p II from D. pteronyssinus. They have the same molecular weight (MW) (15 kd) on sodium dodecyl sulfate-polyacrylamide gel electrophoresis, they have similar amino acid compositions, and their N-terminal amino acid sequences differ in only four of the first 35 residues. An excellent correlation was observed between IgE antibody to Der f II and Der p II measured in sera from 65 mite-allergic patients ($r = 0.94$; $p < 0.001$) and between quantitative intradermal skin tests to both allergens. A third allergen (Der f III, MW 29 kd) was purified from D. farinae by repeated gel filtration. In sera from 51 mite-allergic patients, IgE antibody to Der f II, Der f III, and previously purified Der f I (MW 24 kd) was detected in 92%, 16%, and 78% of the sera by radioimmunoassay, respectively. Most patients, 41/51 (80%), demonstrated IgE antibody to more than one allergen. With monoclonal antibodies fully cross-reactive with Der f II and Der p II, a two-site immunoassay was developed for measuring absolute quantities (nanograms or micrograms) of these allergens. In extracts rich in mite-fecal material ($n = 5$), Der f I and Der p I (group I allergens) and Der f II and Der p II (group II allergens) were measured in ratios of 11:1 to 35:1. Lower ratios (1:1 to 7:1) were observed in mite body extracts ($n = 6$). These experiments clearly define a second group of major dust mite allergens that demonstrate extensive structural and antigenic homology. (J ALLERGY CLIN IMMUNOL 1989;83:1055-67.)

Sensitization to allergens produced by house dust mites of the genus *Dermatophagoides* is commonly associated with symptoms of asthma and rhinitis in many parts of the world.^{1,2} Three species, *D. pter-*

onyssinus, *D. farinae*, and *D. microceras*, are recognized as important sources of allergen in house dust.^{3,4} With immunoelectrophoretic techniques (e.g., CRIE and Western blotting), a number of IgE-binding proteins have been identified in extracts of *D. pteronyssinus* and *D. farinae*.⁵⁻⁷ A limited number of these allergens, however, have been fully isolated. The first mite allergens to be purified to homogeneity were *Der p I* from *D. pteronyssinus* and *Der f I* from *D. farinae*.^{8,9} These allergens have the same MW (24,000) and are structurally homologous, as judged by amino acid composition and N-terminal amino acid sequence comparisons.¹⁰ The full cDNA sequence of *Der p I* has recently been published,¹¹ and we have obtained ~80% of the protein sequence for *Der f I* (unpublished data). Extensive cross-reactivity in binding human IgE and IgG abs has been demonstrated between *Der p I* and *Der f I*.¹⁰ *Der m I* (*Dm 6*), an homologous allergen produced by *D. microceras*, has also been reported.¹² Collectively, these allergens have been described as the group I mite allergens to which most mite-allergic patients (~80%) have measurable IgE abs.¹³ Strik-

From the *Division of Allergy and Clinical Immunology, Departments of Internal Medicine and Pediatrics, University of Virginia Medical Center, Charlottesville, VA.; **Department of Immunochimistry, Central Laboratory of The Netherlands Blood Transfusion Service and Laboratory for Experimental and Clinical Immunology, University of Amsterdam, Amsterdam, The Netherlands; and ***Department of Microbiology, University of Virginia, Charlottesville, Va.

Supported by National Institutes of Health Grants AI 24261, AI 24687, and AI 20565. Partial support was also provided by the Thomas F. and Kate Miller Jeffress Memorial Trust, by the Dutch Asthma Foundation Grant 79.25, and by an American Lung Association Research Training Grant.

Received for publication July 19, 1988.

Revised Nov. 18, 1988.

Accepted for publication Nov. 29, 1988.

Reprint requests: Peter W. Heymann, MD, Box 386, Department of Pediatrics, University of Virginia Medical Center, Charlottesville, VA 22908.

Abbreviations used

BBS:	Borate-buffered saline, pH 8.0
BSA:	Bovine serum albumin
CIE:	Crossed immunoelectrophoresis
CRIE:	Crossed radioimmunoelectrophoresis
Group I allergens:	<i>Der f</i> I, <i>Der p</i> I, and <i>Der m</i> I
Group II allergens:	<i>Der f</i> II and <i>Der p</i> II
IEF:	Isoelectric focusing
Mab:	Monoclonal antibody
PBS-T:	Phosphate-buffered saline, pH 7.2-Tween 20
SAS:	Saturated ammonium sulfate
ab:	Antibody
MW:	Molecular weight
RIA:	Radioimmunoassay
i.p.:	Intraperitoneally
SDS-PAGE:	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
CFA:	Complete Freund's adjuvant
pI:	Isoelectric point
HPLC:	High-performance liquid chromatography
TFA:	Trifluoroacetic acid
A ₂₈₀ :	Absorbance at 280 nm

ingly, most Mabs produced against the group I allergens are species specific, as are the Mab immunoassays that have been developed to measure these allergens in dust samples and extracts.^{14, 15}

Lind¹⁶ initially described the partial purification of a second *D. pteronyssinus* allergen, antigen DpX, which reacted strongly on CRIE with IgE abs in pooled sera from mite-allergic patients. In addition, Holck et al.¹⁷ and Yasueda et al.¹⁸ reported the purification of a major allergen from *D. farinae* (MW $\approx$ 14 to 15,000) that in skin tests and RAST analyses bound IgE abs in sera from $\geq$ 90% of mite-allergic patients. With Mab-affinity chromatography, we have purified two allergens having an identical MW (15,000), *Der f* II from *D. farinae* and *Der p* II from *D. pteronyssinus*. A third allergen, *Der f* III (MW 29,000), was purified by repeated gel filtration. The purification of these allergens to homogeneity has made it possible to make structural, antigenic, and allergenic comparisons of these proteins with the group I mite allergens. Mabs have been produced against *Der f* II and *Der f* III, and a Mab immunoassay for quantitative measurements of *Der f* II and *Der p* II in dust samples and extracts has been developed. Because *Der f* II and *Der p* II demonstrate extensive

structural and antigenic homology, they can be recognized as a second group (group II) of major mite allergens.

MATERIAL AND METHODS

Affinity purification of *Der f* II and *Der p* II

Mab used to affinity purify *Der f* II and *Der p* II was produced at Central Laboratory of The Netherlands Blood Transfusion Service in a BALB/b mouse immunized with lyophilized *D. pteronyssinus* extract (Beecham Research Laboratories, Worthing, U.K.).¹⁹ In CIE experiments, specificity of this Mab for antigen DpX (*Der p* II) was confirmed by Dr. Peter Lind (The Protein Laboratory, University of Copenhagen, Denmark). By RIA, this ab, Mab CLB-DpX, also bound allergen in a *D. farinae* extract and did not react with *Der p* I.¹⁹ We injected hybridoma cells producing this ab into irradiated BALB/c mice that had been pristane pretreated and exposed to 500 rad from a ¹³⁷cesium source (Gammacell-40, Atomic Energy of Canada, Ltd., Ottawa, Canada). Each mouse was injected i.p. with 4×10^6 cells in Iscove's modified Dulbecco's medium (Gibco, Grand Island, N.Y.). After harvesting ascites, an immunosorbent was prepared by coupling 200 mg of a 50% ammonium sulfate (SAS) fraction of Mab, CLB-DpX, rich ascites to 20 ml of swollen CNBr-activated Sepharose 4B (Pharmacia, Piscataway, N.J.) in 0.1 mol/L of NaHCO₃/0.5 mol/L of NaCl, pH 8.3, overnight at 4° C.

The extracts from which *Der f* II and *Der p* II were purified were prepared from either *D. farinae* or *D. pteronyssinus* spent-growth medium (kindly provided by Hollister-Stier Laboratories, Spokane, Wash.), respectively. These cultures, from which mite bodies had been removed, were rich in fecal material and contained sufficient *Der f* II or *Der p* II for purification (see Table V). Two hundred grams of either *D. farinae* or *D. pteronyssinus* culture were extracted overnight in 2.0 L of BBS at 4° C and then centrifuged at 5500 g for 30 minutes. With a flow rate of 15 ml/hr, 100 ml of culture was adsorbed over a 20 ml column containing the CLB-DpX immunosorbent. Separate columns were used to purify *Der f* II from *D. farinae* or *Der p* II from *D. pteronyssinus*. Columns were then washed extensively with 400 ml of PBS-T, followed by the same volume of PBS per 0.5 mol/L of NaCl. Bound allergen was eluted with 0.1 mol/L glycine and 0.15 mol/L of NaCl (pH 11.0). Three milliliter fractions were collected, and samples were pooled based on protein content (A₂₈₀). The pooled allergen was then dialyzed extensively against PBS, lyophilized, redissolved in 1.0 ml of deionized water and stored at -70° C.

Purification of a third *D. farinae* allergen (*Der f* III)

Fifteen milliliters of a 50% to 80% SAS fraction of *D. farinae* spent-growth medium was applied to an upward flowing, 100 by 2.6 cm Sephacryl S-200 column equilibrated in BBS. Eluted 5 ml samples were analyzed by direct RAST with pooled sera from mite-allergic patients and by

optical density at 280 nm. Fractions were also analyzed by direct RAST with serum from a mite-allergic patient lacking IgE abs to *Der f* I. Samples rich in protein were pooled into four fractions (1 to 4) (see Fig. 2A), and then concentrated by negative pressure dialysis with Spectrapor 1 (Spectrum Medical Industries, Inc., Los Angeles, Calif.) (MW cutoff, 8000). Purified allergen (*Der f* III) was obtained by rechromatographing 5 ml of fraction 2 (29 mg of protein per milliliter) over Sephacryl S-200. Eluted samples (5 ml) were pooled, based on optical density (A_{280}) and analyzed on SDS-PAGE.

Production of Mabs to *Der f* II and *Der f* III

Mabs to *Der f* II were raised in an A/J mouse immunized i.p. with 40 μ g of affinity-purified *Der f* II in alum and boosted twice at intervals of 10 days. Two weeks after the final injection, a mouse whose serum demonstrated an IgG ab antigen-binding capacity of ≈ 80 μ g/ml by RIA was administered an intravenous boost of 40 μ g of *Der f* II. Spleen cells from this mouse were fused with Sp 2.0 - Ag 14 myeloma cells.²⁰ Antibody-producing hybrids were cloned by limiting dilution, and supernatants were screened by ELISA with microtiter plates coated with 2 μ g per well of a 50% to 80% SAS fraction of *D. farinae* culture. Clones demonstrating good ab titers by ELISA were rescreened for abs to radiolabeled *Der f* II. Anti-*Der f* II clones were then selectively expanded. Ascites containing anti-*Der f* II Mabs were obtained from Pristane pretreated CAF-1 mice injected i.p. with 2×10^6 cells in Iscove's modified Dulbecco's medium. Anti-*Der f* III Mabs were produced with spleen cells from an A/J mouse that had been immunized three times with 20 μ g of partially purified *Der f* III (see fraction 2, Fig. 2B) in CFA. Microtiter plates coated with 2 μ g per well of a 50% to 80% SAS fraction of *D. farinae* were also used to screen supernatants from ab producing hybrids from this fusion, since *Der f* III was demonstrated to precipitate from the *D. farinae* crude starting material at ammonium sulfate concentrations $>50\%$. Positive supernatants were then rescreened for abs to radiolabeled *Der f* III, and anti-*Der f* III clones were selectively expanded. Isotyping of Mabs was carried out, as previously described.²⁰ Both A/J and CAF-1 mice were ordered from Jackson Laboratories, Bar Harbor, Maine. A protocol for the care and handling of mice was approved by the Animal Research Committee, Department of Comparative Medicine, at the University of Virginia.

Physicochemical analyses of purified allergens

Fractions containing 20 to 30 μ g of protein were analyzed for purity on SDS-PAGE with a resolving gel containing 15% acrylamide. Before their use on RIAs, radiolabeled allergens were also analyzed on SDS-PAGE and were detected by autoradiography on Kodak X-Omat K film (Eastman Kodak Co., Rochester, N.Y.) in the presence of an image-intensifying screen at -70° C. Determinations of pI were carried out as previously described.¹⁰

Affinity-purified allergens (*Der f* II, *Der p* II, or *Der f*

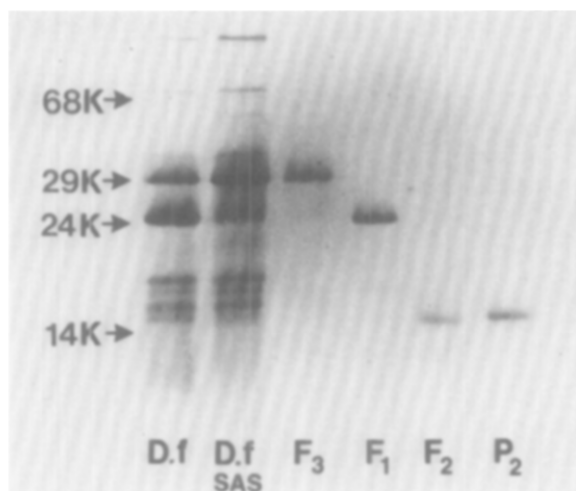


FIG. 1A. SDS-PAGE analysis of Mab affinity-purified mite allergens, *Df*, crude *D. farinae* extract; *Df* SAS, 50% to 80% SAS cut of *D. farinae*; *F*₃, *Der f* III (MW 29,000); *F*₁, *Der f* I (MW 24,000); *F*₂, *Der f* II (MW 15,000); *P*₂, *Der p* II (MW 15,000). Separate columns containing 20 ml of the Mab CLB-DpX immunosorbent were used to purify *Der f* II from *D. farinae* and *Der p* II from *D. pteronyssinus*.

III) were chromatographed with reverse-phase HPLC before amino acid analysis and *N*-terminal sequencing. Approximately 2 to 3 nmol of the allergen was loaded on a 5 μ m, 300Å C-8 column (Pierce Chemical Co., Rockford, Ill.) and developed with a 1% per minute gradient of buffer A (0.1% TFA in H₂O) to buffer B (0.1% TFA in acetonitrile [CHCN₃]) over 0% buffer B to 60% buffer B at a flow rate of 1 ml/min. The effluent was monitored at 214 nm, and the peaks were concentrated and analyzed by SDS-PAGE. For amino acid analysis, samples (in triplicate, 20 μ g each) were subjected to gas-phase hydrolysis by 5.7 N HCl containing 0.3% phenol and 0.2% 2-mercaptoethanol at 100° C for 24, 48, and 72 hours. The hydrolysates were derivatized with phenylisothiocyanate and the resulting phenylthiocarbamyl-amino acids were analyzed according to the method of Heinrikson and Meredith.²¹ *N*-terminal amino acid sequencing was performed on approximately 1 nmol of protein with an Applied Biosystems, Inc. (Foster City, Calif.) 470A gas-phase sequenator, and the PTH-amino acids were analyzed on an on-line Applied Biosystems, Inc. 120 analyzer.²²

Iodination of proteins

Iodination of Mabs, goat antihuman IgE, *Der f* I, and *Der f* III was carried out with a modification of the chloramine-T technique.²³ In each case, 20 μ g of protein was used for radiolabeling with 1 mCi of ¹²⁵I (Amersham Corp., Arlington Heights, Ill.), resulting in a specific activity of 20 to 30 μ Ci/ μ g of protein. Before radiolabeling, Mab CLB-DpX and 7A1 were purified on preparative IEF and had pI values of 6.5 and 6.2, respectively. *Der f* II was radiolabeled with ¹²⁵I-labeled Bolton and Hunter reagent

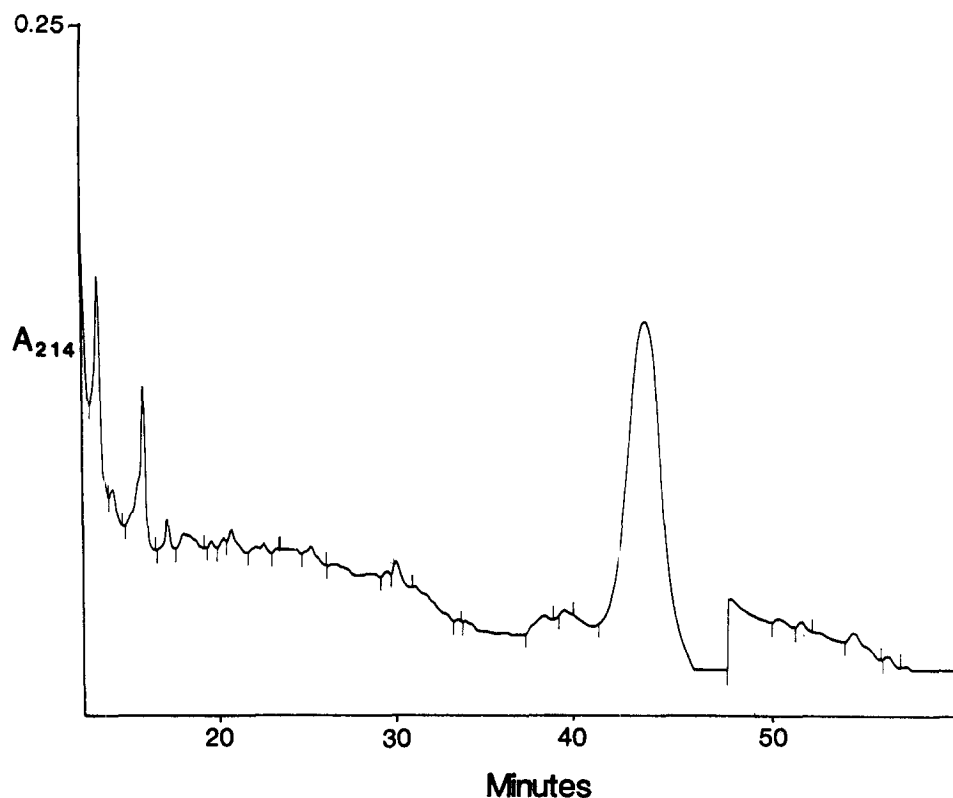


FIG. 1B. Reverse-phase HPLC of affinity-purified *Der p* II. Column, C8, 5 μ m; buffer A, 0.1% TFA in H_2O ; buffer B, 0.1% TFA in $CHCN_3$; gradient, 0% buffer B to 100% buffer B at 1% per minute; flow rate, 1 ml/min.

(~ 2000 Ci/mmol) (Amersham Corp.). Ten micrograms of *Der f* II in 10 μ l of 0.1 mol/L of borate buffer, pH 8.5, was labeled as described,²⁴ and the specific activity of labeled allergen was 12 to 18 μ Ci/ μ g.

Skin testing

As judged by the allergen-specific Mab immunoassays, purified preparations of *Der f* II and *Der p* II used for quantitative skin testing lacked any *Der f* I or *Der f* III. Similarly, *Der f* I was also demonstrated to lack *Der f* II and *Der f* III. Each allergen, sterilized as previously described,¹⁰ and a *D. farinae* whole body extract (Hollister-Stier Laboratories) was prepared in serial tenfold dilutions (10^{-1} through 10^{-6} μ g/ml) in 0.05% human serum albumin in phenol saline. Test sites were examined 15 minutes after injecting 0.05 ml intradermally. Skin testing of patients was approved by the Human Investigation Committee of the University of Virginia.

Assays for serum IgE and IgG abs

Sera from mite-allergic patients were obtained from patients with asthma and/or rhinitis who gave an immediate skin response (≥ 4 by 4 mm wheal in ≤ 15 minutes) to prick tests with 1:20 wt/vol of *D. farinae* (Hollister-Stier Laboratories) (containing 37 μ g/ml of *Der f* I and 6.2 μ g/ml of *Der f* II) and *D. pteronyssinus* (Bencard, Ltd., Brentford,

Middlesex, U.K.) (containing 82 μ g/ml of *Der p* I and 31.5 μ g/ml of *Der p* II) glycerinated extracts. Both IgE and IgG abs to *Der f* I, *Der f* II, or *Der f* III were determined by antigen-binding assay.²⁵ Serum dilutions of 1/6 and 1/24 in 1% of bovine serum albumin/PBS-T were used in the IgE ab assays, and dilutions of 1/12 and 1/36 were used to measure IgG ab binding. Sera binding $>50\%$ of radio-labeled *Der f* I, *Der f* II, or *Der f* III in the IgG ab assays were used to establish control curves for the quantification of each allergen in binding activity units per milliliter.

For RAST determinations of antimitic IgE ab, CNBr-activated filter paper disks (grade 541, 0.6 cm in diameter, Whatman International Ltd., Maidstone, U.K.) were coated with *D. farinae* or *D. pteronyssinus* extracts.²⁶ Results were quantitated relative to a reference serum pool University of Virginia 87-01, which had been substandardized from the National Institute for Biological Standards and Control serum pool (82/528) in units described previously.²⁷ In separate experiments, disks were coated with 2 μ g per disk of either *Der f* II or *Der p* II purified by Mab affinity chromatography followed by reverse-phase HPLC. Serial two-fold dilutions of serum pooled from eight patients with high titers of IgE anti-*Der f* II ab were used to form a control curve to measure IgE ab to *Der f* II or *Der p* II. For measurements of mite allergen eluting from gel filtration experiments, 0.1 ml from each fraction (diluted 1/10 and 1/100

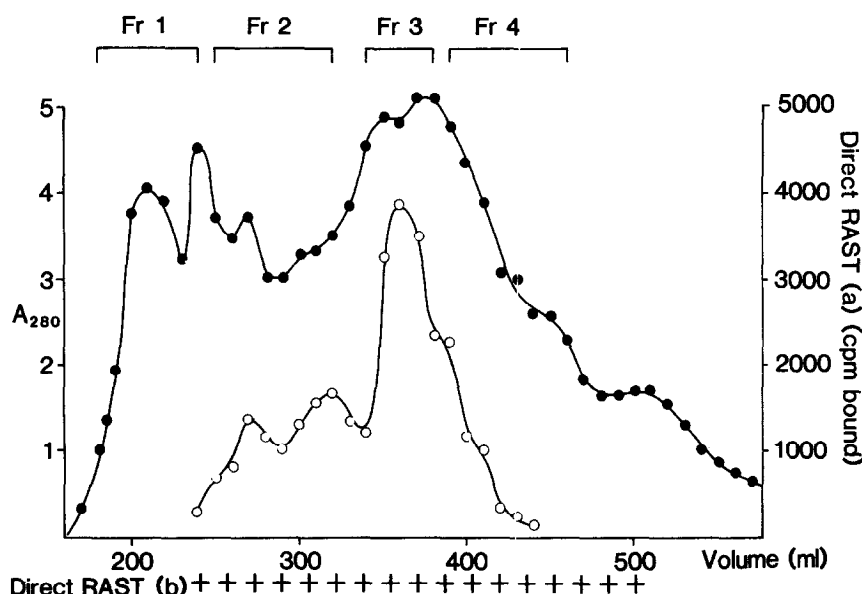


FIG. 2A. Gel filtration of a 50% to 80% SAS fraction of *D. farinae* over Sephacryl S-200. The elution pattern of proteins (A_{280}) (●) is illustrated. The concentration of allergen distinct from *Der f* I was assessed by direct RAST (a) with serum from a mite-sensitive patient with no detectable IgE ab to radiolabeled *Der f* I (○). With pooled serum from mite-allergic patients, direct RAST (b) demonstrated fractions that were strongly positive for total mite allergen (+). Fractions (Fr) 1, 2, 3, and 4 were pooled, as indicated, and samples were applied to SDS-PAGE for analysis (Fig. 2B).

in 0.1 mol/L of NaHCO_3 , pH 8.3) was incubated with a CNBr-activated disk overnight at 4° C.

Epitope specificity of Mabs

The epitope specificities of Mab CLB-DpX and Mabs raised against *Der f* II were compared in a solid-phase RIA for binding to group II allergens. Polyvinyl chloride microtiter plates (Dynatech, Alexandria, Va.) were coated with serial dilutions (10^{-1} to 10^{-7}) of ascites containing Mabs in 0.1 mol/L of bicarbonate buffer, pH 9.6, overnight at 4° C. Residual binding sites were blocked with 0.1 ml 1% of bovine serum albumin/PBS-T. Each well was then incubated for 2 hours with 0.1 ml of a 50% to 80% SAS fraction of *D. farinae* or *D. pteronyssinus* containing 1 μg per well of *Der f* II or *Der p* II, respectively. After washing five times with PBS-T, wells were incubated with 0.1 ml of 3 ng ^{125}I -labeled Mab (either CLB-DpX or anti-*Der f* II, 7A1) for 2 hours. Wells were then washed, dried, and counted on a gamma counter (Micromedic 4/200, Micromedic Systems, Inc., Horsham, Pa.). The epitope specificity of the Mabs was established from the antibody combinations giving positive binding in the two-site assay. Epitope analysis of Mab to *Der f* III was carried out by cross-inhibition RIA, as previously described.¹⁴

Mabs were also used in a modification of the RAST technique (Mab-modified RAST) to detect human IgE ab to specific mite allergen. CNBr-activated disks were coated with 10 mg of a 50% SAS fraction of ascites per gram of disks in 0.1 mol/L of NaHCO_3 , pH 8.3. As judged by the quantities of purified Mab obtained on preparative IEF, the

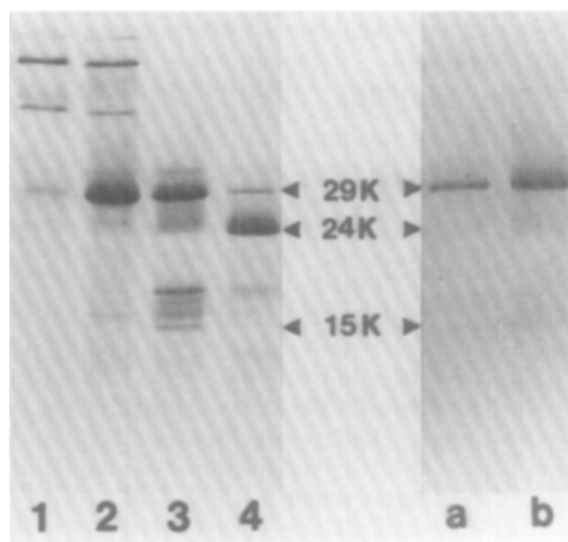


FIG. 2B. SDS-PAGE analysis of pooled fractions (1 to 4) from gel filtration (Sephacryl S-200) of the 50% to 80% SAS cut of *D. farinae*. Fractions 1, 2, 3, and 4 are pooled from gel filtration (see Fig. 2A); (a) *Der f* III after repeated gel filtration of fraction 2 over Sephacryl S-200; (b) Mab affinity-purified *Der f* III.

50% SAS cuts of ascites have been calculated to contain ~7 mg/ml of Mabs.²⁰ Each Mab-coated disk was washed, blocked, and then incubated for 2 hours with 0.1 ml of a 50% to 80% SAS fraction of *D. farinae* or *D. pteronyssinus*

TABLE I. Specificity of Mabs to *D. farinae* allergens*

Clone	Allergen†	<i>Der f I</i>	<i>Der f II</i>	<i>Der f III</i>
4C1‡	<i>Der f I</i>	27,546	656	1,121
6A8‡	<i>Der f I</i>	15,175	984	1,195
CLB-DpX§	<i>Der p II</i>	1,399	5,465	848
7A1§	<i>Der f II</i>	586	4,512	974
4E5§	<i>Der f II</i>	749	11,681	1070
6D6§	<i>Der f II</i>	655	4,106	987
5A12	<i>Der f III</i>	775	596	10,767

*Results in counts per minute of ¹²⁵I-labeled allergen bound by Mab-diluted 10⁻⁴.

†Allergens to which Mabs were raised.

‡Previously reported Mabs produced against *Der f I*.⁹ Sixteen Mabs (represented by 6A8) are species specific for *Der f I*. Two Mabs (represented by 4C1) also bind *Der p I* from *D. pteronyssinus*.

§Also bind the homologous allergen, *Der p II*, from *D. pteronyssinus*.

||Seven Mabs (represented by 5A12) were raised against *Der f III*. Antibody 5A12 also binds *D. pteronyssinus* allergen, as judged by Mab-modified RAST.

extract (containing 1 µg of either *Der f II* or *Der p II*, respectively) per disk. After washing disks five times with PBS-T, 0.1 ml of sera (diluted 1/4 and 1/10) from mite-allergic patients was added. After 4 hours, disks were washed again, and radiolabeled goat antihuman IgE ab was added to detect bound allergen. Serial twofold dilutions of pooled sera from patients known to contain IgE ab to the bound allergen were used to establish a control curve. Serum pools were arbitrarily defined as containing 1000 IgE ab units per milliliter.

Mab two-site RIAs

Mab RIAs for measuring *Der f I* and *Der p I* allergens were carried out as previously described.¹⁵ A two-site monoclonal immunoassay for measuring group II allergens was developed with an anti-*Der f II* Mab, 7A1, coupled to CNBr-activated filter-paper disks, together with a second radiolabeled anti-*Der f II* Mab, 6D6, of different epitope specificity or radiolabeled anti-*Der p II* Mab, CLB-DpX, to detect bound allergen. Disks used in this assay were coated with 1 µg per disk of a 50% SAS cut of Mab 7A1 ascites, as described above for the Mab-modified RAST assay. Weighed samples of affinity-purified *Der f II* or *Der p II* were used to establish standards for quantitation of this assay. Control curves were formed with doubling dilutions of either the *Der f II* or *Der p II* standard (range 0.4 to 400 ng/ml).

RESULTS

Purification of *Der f II* and *Der p II* (group II allergens)

Der f II was affinity purified from an aqueous extract of *D. farinae* culture with Mab CLB-DpX coupled to CNBr-activated Sepharose. On SDS-PAGE, *Der f II* had the same MW (15 kd) as *Der p II* that was purified from *D. pteronyssinus* with the same technique (Fig. 1A). As determined by RIA, with a two-site Mab assay for group II allergens, this single-step procedure resulted in 2.1 mg of *Der f II* purified from 100 ml of

D. farinae extract (containing 6.4 mg of *Der f II*). Similarly, 3.4 mg of *Der p II* was purified from 100 ml of *D. pteronyssinus* extract (containing 8.8 mg of *Der p II*). Reverse-phase HPLC of affinity-purified *Der f II* or *Der p II* demonstrated a single prominent peak of protein eluting at 43% CHCN₃ (Fig. 1B).

Purification of a third *D. farinae* allergen

When a 50% to 80% SAS cut of *D. farinae* was applied to a Sephacryl (S-200) column, eluted samples were pooled into four fractions (Fig. 2A). By direct RAST, fractions 2, 3, and 4 contained samples that were strongly positive for mite allergen. Fraction 4 demonstrated a strong band of protein at MW 24 kd on SDS-PAGE (Fig. 2B) and was rich in *Der f I*, as judged by immunodiffusion against rabbit anti-*Der f I* serum (data not presented). Fraction 3 contained the highest concentration of allergen detected by RAST with serum from a mite-allergic patient with no detectable IgE to *Der f I*. This serum was subsequently demonstrated to contain a high IgE ab titer to *Der f II*. When fraction 3 was radiolabeled with the chloramine-T technique, the fraction bound IgE ab in 11 of 29 (41%) additional sera selected from mite-allergic patients lacking IgE ab to *Der f I* (data not presented). Autoradiographic analysis of radiolabeled fraction 3 demonstrated that most of the radiolabel bound to a 29 kd protein. This protein was present in purer form in fraction 2 (Fig. 2B). By rechromatographing fraction 2 over Sephacryl S-200, this protein (referred to as *Der f III*) was further purified and demonstrated a single band on SDS-PAGE (Fig. 2B). Seven Mabs to *Der f III* were raised from spleen cells from an A/J mouse immunized with fraction 2. With one of these Mabs, 5A12 (Table I), coupled to CNBr-activated Sepharose, *Der f III* was affinity purified

Der f I^a = T¹ S A C R I N S V N V P S E L D L R S L²⁰
M R T V T P I R M²⁹

Der f II = D¹ Q V D V K D ? A N N E I K K V M V D G²⁰
? H G S D P ? I I H R G K P F³⁵

Der p II = D¹ Q V D V K D ? A N H E I K K V L V P G²⁰
? H G S E P ? I I H R G K P F Q L E A V⁴⁰

Der f III = I¹ V G G V K A L A G D ? P Y E I S L E V²⁰

FIG. 3. *N*-terminal amino acid sequence analysis of *D. farinae* allergens, *Der f I*, II, and III, and *Der p II* from *D. pteronyssinus*. Data previously reported (a) for *Der f I*.⁹ Residues in the sequence that could not be identified are indicated by a question mark (?). *Der f II* and *Der p II* differ in sequence at only four residues that are enclosed in squares.

TABLE II. Amino acid analysis of *Der f I*, *Der f II*, *Der p II*, and *Der f III* (residues per 100 residues of amino acids)

Amino acids	<i>Der f I</i> [*]	<i>Der f II</i> [†]	<i>Der p II</i> [†]	<i>Der f III</i>
Aspartic acid	12.1	13.5	11.3	11.6
Threonine	5.9	6.1	4.0	5.9
Serine	6.8	4.9	4.6	8.8
Glutamic acid	7.9	11.8	12.4	9.7
Proline	5.9	6.1	7.4	5.2
Glycine	11.0	8.5	8.9	11.4
Alanine	7.4	8.1	8.6	6.6
½ Cystine	0.7	NA	NA	NA
Valine	6.9	8.7	10.6	6.7
Methionine	1.5	1.7	1.4	2.1
Isoleucine	8.7	7.4	8.0	6.3
Leucine	5.4	8.8	7.3	7.4
Tyrosine	7.8	1.7	2.0	3.8
Phenylalanine	1.4	3.6	2.8	2.3
Histidine	2.2	2.9	3.5	2.2
Lysine	1.4	3.4	3.7	6.6
Arginine	6.9	2.6	2.4	3.4

NA, Not analyzed.

^{*}Previously reported analysis of a purified *Der f I* fraction that concentrated on IEF at pI 6.6 to 6.95.⁹

[†]With the equation proposed by Marchalonis and Weltman²⁸ for proteins of equal size, *Der f II* and *Der p II* differ by 19 SΔQ units and are judged to be closely related.

under the same elution conditions used to purify the group II allergens (Fig. 2B).

Amino acid composition and *N*-terminal amino acid sequences of purified allergens

The amino acid compositions of *Der f II* and *Der p II* were very similar (Table II), and their *N*-

terminal amino acid sequences (Fig. 3) differed in only four of the first 35 amino acid residues (comprising approximately one third of the full sequence based on MW). By contrast, the amino acid compositions of *Der f I*, *Der f II*, and *Der f III* demonstrate significant differences, and comparison of their *N*-terminal amino acid sequences suggests that they are

TABLE III. Quantitative skin tests with *D. farinae*, *Der f I*, *Der f II*, and *Der p II*

Patient	Quantitative intradermals ($\mu\text{g/ml}$)*			
	<i>D. farinae</i>	<i>Der f I</i>	<i>Der f II</i>	<i>Der p II</i>
C. W.	10^{-6}	$>10^{-1}$	10^{-3}	10^{-2}
P. J.	10^{-3}	$>10^{-1}$	$>10^{-1}$	10^{-1}
B. P.	10^{-7}	10^{-5}	10^{-6}	10^{-4}
M. A.	10^{-6}	10^{-5}	10^{-5}	10^{-4}
M. H.	10^{-5}	10^{-4}	10^{-3}	10^{-2}
M. H.	10^{-8}	10^{-6}	10^{-5}	10^{-4}
S. F.	10^{-7}	$>10^{-1}$	10^{-4}	10^{-3}
V. D.	10^{-8}	10^{-5}	10^{-7}	10^{-7}
K. K.	10^{-7}	10^{-6}	10^{-6}	10^{-5}
P. T.	10^{-7}	10^{-4}	10^{-4}	10^{-5}
F. M.	10^{-6}	10^{-6}	10^{-5}	10^{-4}
M. W.	10^{-7}	10^{-5}	10^{-4}	10^{-3}
Control subjects $\times 3$ ‡	$>1 \mu\text{g}$	$>1 \mu\text{g}$	$>1 \mu\text{g}$	$>1 \mu\text{g}$

*Values represent end point dilutions eliciting an 8 by 8 mm wheal. Negative skin tests are noted as $>1 \mu\text{g}$.

†*D. farinae* whole body extract (Hollister-Stier Laboratories) containing 37 μg of *Der f I* and 6.2 μg of *Der f II* by Mab immunossays for group I and group II allergens, respectively.

‡Control subjects are nonallergic individuals.

TABLE IV. IgE ab to *D. farinae* allergens

	MW	No. positive*	%	<i>r</i> †	Single allergen specificity‡
<i>Der f I</i>	24,000	40	78	0.68	1
<i>Der f II</i>	15,000	48	94	0.81	8
<i>Der f III</i>	29,000	8	16	—	1

*Number of sera with IgE ab specific for radiolabeled allergen (^{125}I -labeled *Der f I*, *II*, or *III*) identified by screening 51 sera from mite-allergic patients. No IgE abs to these three allergens was detected in sera from 10 skin test-negative control subjects.

†Correlation coefficient determined by linear regression analysis of IgE abs to purified allergen and *D. farinae* extract.

‡Number of sera with IgE abs specific for one allergen, but not the other two allergens.

structurally distinct (Fig. 3). Analysis on IEF demonstrated pI ranges of 5.5 to 8.2 for *Der f II*, 4.1 to 4.7 for *Der f III*, and, by comparison, 5.7 to 6.95 for *Der f I*.

IgE abs to *Der f I*, *II*, and *III*

Quantitative intradermal skin testing in 12 adult mite-allergic patients demonstrated a good correlation between end points for *Der f II* and *Der p II* (Table III). Positive reactions were obtained in nine of 12 patients to *Der f I* and 11 of 12 patients to *Der f II* at concentrations $\leq 10^{-3} \mu\text{g/ml}$.

In sera from 51 mite-allergic patients, IgE ab to *Der f I*, *Der f II*, and *Der f III* were measured by RIA.

Autoradiography of these allergens radiolabeled was used to confirm their purity before their use in RIAs. *Der f I*, *Der f II*, and *Der f III* demonstrated single bands of radioactivity at 24, 15, and 29 kd, respectively (Fig. 4). IgE abs to *Der f I* and *Der f II* were detected in 40 of 51 (78%) and 48 of 51 (94%) sera, respectively (Table IV). Linear regression analysis of IgE abs to these allergens demonstrated an excellent correlation between IgE abs to *Der f II* and a *D. farinae* crude extract ($r = 0.81$; $p < 0.001$) and between *Der f I* and *D. farinae* ($r = 0.68$; $p < 0.001$). IgE abs to *Der f III* were demonstrated in eight of the 51 sera (16%). Most patients (i.e., 41/51) demonstrated IgE abs to more than one allergen. An IgE ab response to only one of the three allergens (i.e., single allergen specificity) was demonstrated in one patient to *Der f I*, eight patients to *Der f II*, and one patient to *Der f III* (Table IV).

Cross-reactivity between *Der f II* and *Der p II*

In keeping with their structural homology, the quantitative correlation between IgE ab to *Der f II* and *Der p II* by RAST ($n = 28$) was close to unity ($r = 0.94$; $p < 0.001$). Similarly, in a Mab-modified RAST with the CLB-DpX cross-reacting Mab coupled to CNBr-activated disks, the same correlation ($r = 0.94$; $p < 0.001$) was observed with sera from 65 mite-allergic patients (Fig. 5). When disks coated with the same Mab were incubated with *D. microceras* extract, the correlation of IgE ab to *Der f II*, *Der p II*, and *D. microceras* allergen by multiple regression analysis

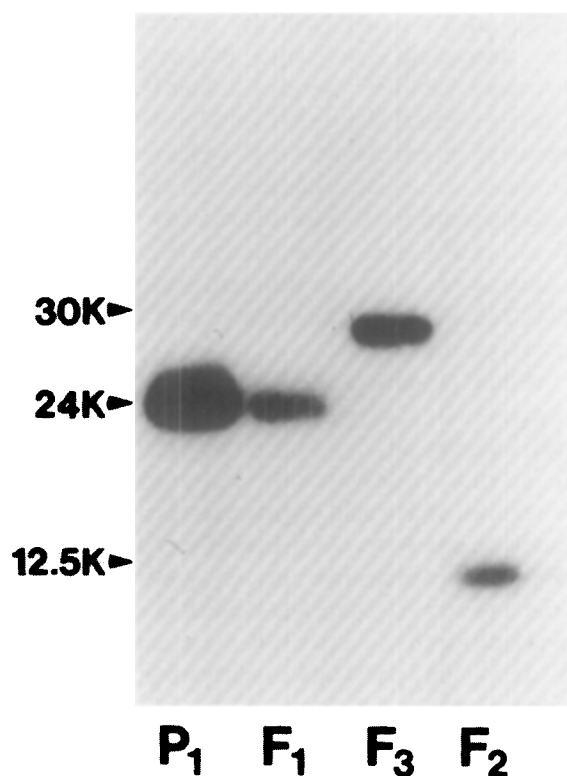


FIG. 4. Autoradiograph of radiolabeled (^{125}I) allergens analyzed on SDS-PAGE. P_1 , *Der p* I; F_1 , *Der f* I; F_3 , *Der f* III; f_2 , *Der F* II.

was $r = 0.80$ and $p < 0.001$, with the same 65 sera. On immunodiffusion, *Der f* II and *Der p* II demonstrated complete cross-reactivity with polyclonal antisera from A/J mice hyperimmunized with affinity-purified *Der f* II (data not presented). When quantitative intradermal skin tests with purified *Der f* II and *Der p* II were compared in 12 mite-allergic patients, similar end points for both *Der f* II and *Der p* II were noted (Table III).

Mab two-site RIAs for measuring mite allergens

CLB-DpX, which was raised against *Der p* II, also demonstrated binding to *Der f* II and *D. microceras* allergen in both the Mab-modified RAST and two-site monoclonal assay for group II allergens (data not presented). Three Mabs raised against *Der f* II also bind *Der p* II, and two of these Mabs (6D6 and 7A1) define two separate epitopes distinct from that recognized by Mab CLB-DpX.

A two-site RIA for quantitative measurements of group II allergens was developed with anti-*Der f* II Mab, 7A1, on CNBr-activated paper disks and radiolabeled anti-*Der p* II Mab, CLB-DpX, to detect bound allergen. This assay elicited parallel control

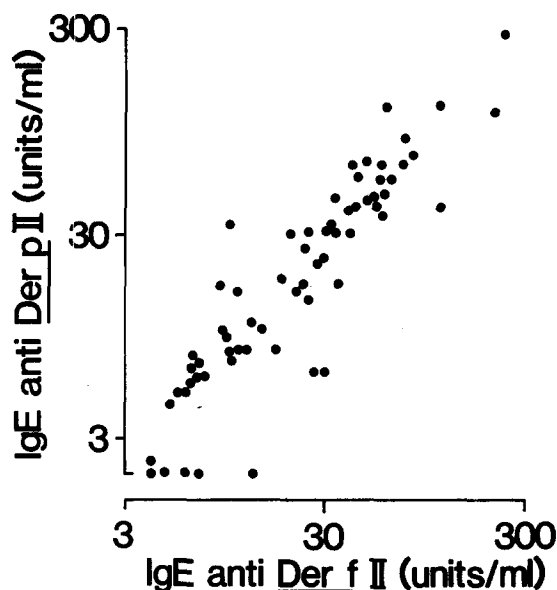


FIG. 5. Comparison of human IgE abs to *Der f* II and *Der p* II with sera from 65 mite-allergic patients. Determinations were made with the Mab-modified RAST assay. Activated (CNBr) disks coated with a cross-reacting anti-group II Mab were incubated with a 50% to 80% SAS fraction of either *D. farinae* or *D. pteronyssinus* to measure IgE abs to *Der f* II or *Der p* II, respectively. IgE abs to *Der f* II and *Der p* II were undetectable (<2 units per milliliter) in sera from six patients who were skin test negative to mite allergen.

curves for dilutions of purified *Der f* II and *Der p* II (Fig. 6). Ratios of group I to group II allergens were compared in extracts from isolated *D. farinae* fecal particles and isolated mite body extracts from both *D. farinae* and *D. pteronyssinus* with two-site Mab assays specific for allergen in each group (Table V). Nine other extracts from different source materials were also analyzed. Group I allergens were more concentrated than group II allergens in all extracts. In whole culture, spent culture, and fecal particle extracts ($n = 5$), group I and group II allergens were measured in ratios of 11:1 to 35:1. In isolated mite and mite body extracts ($n = 6$), group I to group II allergens were detected in ratios of 1.1:1 to 7:1.

DISCUSSION

Our study demonstrates the purification of *Der f* II and *Der p* II dust mite allergens with the use of Mab-affinity chromatography. The Mab used to affinity purify these allergens was previously demonstrated to bind antigen DpX by CIE.¹⁹ In the present studies, we have demonstrated that this Mab and the Mabs to *Der f* II also bind the *D. farinae* allergen, Ag 19/20, purified and provided to our laboratory by Dr. Siri Dale (Oslo, Norway).¹⁷ Immunochemical analyses of

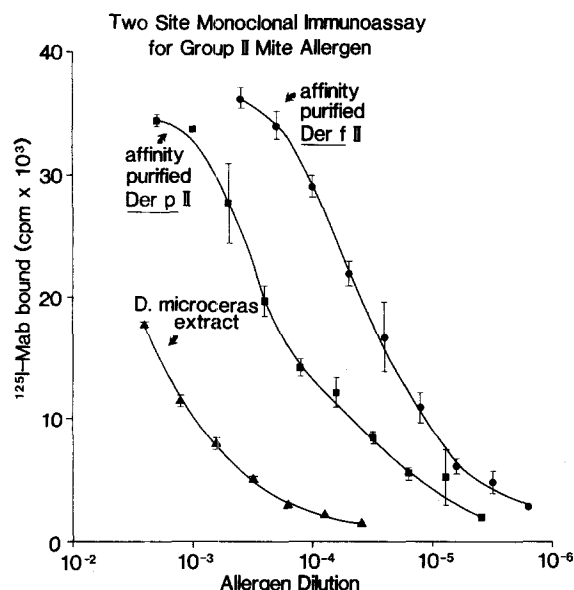


FIG. 6. Binding curves for affinity-purified *Der f* II (2 mg/ml), with *Der p* II (0.5 mg/ml), and *D. microceras* crude extract with the Mab two-site assay for group II allergens. Serial twofold dilutions of allergen were tested with CNBr-activated disks coated with Mab, 7A1, and radiolabeled Mab CLB-DpX, to detect bound allergen. Dilution concentrations of *Der f* II and *Der p* II were transformed by natural logarithm to linearize the relationship between the counts per minute of ^{125}I -labeled Mab bound and the allergen concentration per dilution. Least-squares linear regression was performed to estimate slopes. Pair-wise significance of differences between the slopes was determined by Z statistic demonstrating no significant difference between slopes for *Der f* II and *Der p* II ($p < 0.05$).

purified *Der f* II and *Der p* II in our experiments establish that they are structurally and antigenically homologous, and, therefore, they can be recognized as a second group of major mite allergens (group II allergens).

The structural and biochemical properties of *Der f* II and *Der p* II are clearly different from the group I allergens. On SDS-PAGE, the group I allergens have MWs of 24,000, whereas the group II allergens have apparent MWs of 15,000.^{8, 10, 17, 18} On gel filtration, *Der f* II migrates at a higher MW (~20 to 22,000) in keeping with the findings of Lind¹⁶ who demonstrated overlapping elution patterns for DpX and *Der p* I and reported apparent MWs of 18,000 for both allergens, as judged by gel filtration. Higher ammonium sulfate concentrations ($\approx 80\%$) are required to precipitate the group II allergens than the group I ($\leq 50\%$) allergens.¹⁶ Although the pI ranges for both groups are heterogeneous and overlap an IEF, the group II allergens focus over a broader pI range, up to 8.3.^{10, 17, 18} *Der f* II, which has a low tyrosine content (1.7/100

amino acid residues), did not radiolabel well with the chloramine-T technique but was successfully iodinated with the ^{125}I -labeled Bolton-Hunter reagent. Clear differences between *Der f* I and *Der f* II were apparent in their amino acid compositions and N-terminal amino acid sequences. The complete amino acid sequence for *Der p* I has recently been deduced from a cDNA clone,¹¹ and our data on the amino acid sequence of *Der f* I protein indicate approximately 80% homology between these group I allergens (unpublished data.)

A third *D. farinae* allergen, *Der f* III, was initially purified from *D. farinae* by repeated gel filtration and, subsequently, by Mab-affinity chromatography. In judging its MW, amino acid composition, and N-terminal amino acid sequence, this protein is structurally unrelated to *Der f* I and *Der f* II. On immunodiffusion, serum from an A/J mouse immunized with a mixture of *Der f* I, II, and III demonstrated no evidence for cross-reactivity between any two of these three proteins, and Mabs to each were allergen specific. Although the extent of homology and cross-reactivity between *Der f* III and an equivalent *D. pteronyssinus* allergen has yet to be established, one anti-*Der f* III Mab, 5A12, does bind protein in *D. pteronyssinus*, and sera from patients in London who were exposed predominately to *D. pteronyssinus* contain IgE abs that bind *Der f* III (data not presented).

Measurements of IgE abs to the three *D. farinae* allergens in mite-allergic patients revealed that most individuals had IgE abs to *Der f* I and *Der f* II. Individual differences in IgE ab binding to these allergens were also noted, and most sera contained IgE abs to more than one mite allergen. In RAST-absorption experiments, IgE ab to *Der p* I has been demonstrated to account for a high proportion of anti-*D. pteronyssinus* IgE abs in highly selected sera from mite-allergic patients.⁸ We are now in a position to compare the proportion of antimitte IgE ab to each allergen, to define further their relative importance in stimulating IgE ab, and to assess whether other important mite allergens remain. In studies of cellular immune responses, a proliferative response to *Der p* I has been demonstrated in peripheral blood mononuclear cells, predominately of the helper T cell phenotype, from *D. pteronyssinus*-allergic patients.³⁰ In addition, T cell recognition of a lower MW, *D. farinae* polypeptide, which migrates with serologically defined *Der f* II, has recently been described.³¹ Comparative studies of T cell recognition and proliferative responses to the group I and group II allergens should therefore be possible. Such studies are of interest in examining factors that may influence differences in specific IgE ab production to mite allergens in indi-

TABLE V. Concentrations of group I and group II allergens in *D. farinae* (Df) and *D. pteronyssinus* (Dpt) extracts*

	Protein (mg/ml)	Group I (μg/ml)	Group II (μg/ml)	Ratio Gpl/Gpll
Extracts rich in fecal material†				
Fecal particles (I)	ND	38	2.8	13/1
Fecal particles (II)	0.2	18.5	1.7	11/1
NIBSC (82/518) (Dpt)	0.48	14	0.4	35/1
Dpt spent culture‡	>10.0	1134	96	12/1
Df spent culture‡	9.1	1450	75	19/1
Extracts rich in mite bodies§				
Df mite bodies	6.9	621	87	7/1
Dpt mite bodies	1.3	187	36.5	5/1
Dpt (OBRR/FDA E 1-Dp)	2.3	46	41	1.1/1
Df (OBRR/FDA E 1-Df)	2.0	35	21	1.7/1
Dpt whole body	5.6	111	39	2.8/1
Df whole body	4.2	122	18	6.8/1
Commerical mite extracts				
Dpt	2.6	82	31.5	2.6/1
Df¶	3.1	37	6.2	6/1
Mab affinity-purified allergens				
Der p I	ND	2000	<0.1	>2000/1
Der f I	ND	2000	<0.1	>2000/1
Der f II	ND	<0.1	2000	<1/2000
Der p II	ND	<0.1	500	<1/500
Der f III	2.0	<0.1	<0.1	
<i>T. putrescentiae</i> #	2.5	<0.1	<0.1	
<i>A. siro</i> #	2.7	<0.1	<0.1	

Gpl/Gpll, Groups I and II; ND, not determined; OBRR, Office of Biologics Research and Review; FDA, Food and Drug Administration.

*Allergen concentrations were determined with two-site monoclonal immunoassays specific for group I (*Der f* I or *Der p* I) and group II (*Der f* II and *Der p* II) allergens. Protein concentrations (milligrams per milliliter) were determined with the Bradford assay.²⁹

†Fecal particles were identified under a dissecting microscope in a *D. farinae* culture grown in our laboratory. Their identification was confirmed in a sample of isolated particles by electron microscopy. National Institute for Biological Standards and Control (82/518) is the World Health Organization/International Union of Immunological Societies international reference preparation of *D. pteronyssinus*.³⁰ Spent-culture extracts (i.e., mite cultures from which mite bodies have been removed) were obtained from Hollister-Stier Laboratories.

‡Same as the spent-growth media used for the purification of *Der p* II or *Der f* II, described in Material and Methods.

§*D. farinae* and *D. pteronyssinus* mite bodies were kindly provided by Dr. Wen (Shanghai Medical University, China) and Commonwealth Serum Laboratories (Parkville and Melbourne, Australia), respectively. Mite bodies (50 mg) were homogenized (overhead stirrer, Wheaton Instruments, Millville, N.J.) and extracted in 2.5 ml of BBS in 50% glycerol. Reference preparations of *Dpt* and *Df* whole body extracts were obtained from the Office of Biologics Research and Review, Bethesda, Md. Two other whole body extracts were obtained from Hollister-Stier Laboratories.

||*Dpt* and *Df* extracts are prick test reagents obtained from Bencard, Ltd. and Hollister-Stier Laboratories, respectively.

¶Same as the *D. farinae* extract used for intradermal skin testing (Table III).

#*Tyrophagus putrescentiae* and *Acarus siro* extracts were provided by Dr. W. Rolfsen, Pharmacia, Uppsala, Sweden.¹⁴

vidual patients and may include (1) differences in the structural properties of each allergen, (2) genetic or immunoregulatory differences among individual patients, and (3) exposure to different concentrations of each allergen.

In studies with CIE, CRIE, and electroblotting, differences between extracts rich in mite bodies versus mite fecal material have been demonstrated with respect to their composition of allergens and antigens.^{5, 7} Previous measurements of mite allergen in

extracts evaluated during the selection of an international standard for *D. pteronyssinus* suggested that DpX (i.e., *Der p* II) may be relatively more concentrated in whole mite bodies than in fecal material.³² With the two-site Mab immunoassays, group I allergens were demonstrated to be considerably more concentrated in extracts rich in mite-fecal material than the group II allergens. By contrast, the group II allergens were found to be relatively more concentrated in whole body extracts. Because of the variability

observed in the group I to group II allergen ratios in these extracts, it may be necessary to measure both groups in the process of assessing the allergen potency of extracts derived from different source materials. The Mabs used in the two-site assay to measure group II allergens bind strongly to allergens within the group. In contrast, the Mab immunoassays for the group I allergens are species specific for measurements of either *Der f I* and *Der p I*.¹⁵ Thus, species identification of dust mites in epidemiologic studies can be accomplished with the Mab assays for the group I allergens that is less time consuming than identifying mites microscopically and does not require a detailed understanding of mite morphology. In contrast, the Mab assay for group II allergens, because it is cross-reactive, may have greater usefulness in monitoring mite-allergen exposure irrespective of which *Dermatophagoides* species is present.

Human IgE ab from mite-allergic patients has previously been demonstrated to cross-react extensively with the group I allergens, *Der f I* and *Der p I*.¹⁰ Similarly, in skin tests and antigen-binding assays, an excellent correlation between IgE ab to the group II allergens is apparent in keeping with the structural homology demonstrated between *Der f II* and *Der p II*. In contrast, the murine IgG ab response to the group I allergens is primarily species specific and is strikingly different from the cross-reactive immune response observed for polyclonal and monoclonal murine ab raised against the group II allergens. In addition to these differences in epitope specificity in mice, the behavior of the group I and group II allergens is clearly different with respect to immunogenicity. When BALB/c mice were immunized with 10 µg of either *Der p I* or *Der f I* in CFA, high titers ($\geq 1/10,000$) of IgG ab to the immunizing allergen were detected. We were unable, however, to detect a significant immune response to 40 µg of *Der f II* in BALB/c mice with either alum or CFA (data not presented). Mabs raised against *Der f II* and *Der p II* were raised in A/J and BALB/b mice, respectively. Further structural analyses (e.g., full amino acid sequencing, x-ray crystallography, molecular modeling) and antigenic analyses of purified peptide fragments should provide a better understanding of the molecular determinants of humoral and cellular immune responses to these allergens.

We thank Hollister-Stier Laboratories for generous supplies of *D. farinae* and *D. pteronyssinus* culture, Dr. William M. Sutherland of the University of Virginia Lymphocyte Culture Center for assistance with production of monoclonal antibodies against *Der f II* and *Der f III*, and Dr. Siri Dale, Oslo, Norway, for providing a sample of purified Ag 19/20. We are also very grateful to Annette G. Whelchel,

Richard E. Berkey, and Marcus St. Raymond for their technical assistance, to Madeleine Watkins for drawing the figures, and Arlene Trader for preparing the manuscript.

REFERENCES

1. Voorhorst R, Spijksma FTM, Varekamp H. House dust atopy and the house dust mite. Leiden: Stafleu's Scientific Publishing, 1969.
2. van Bronswijk JEMH, Sinha RN. Pyroglyphid mites (Acari) and house dust allergy. *J ALLERGY* 1971;47:31-52.
3. Wharton GW. House dust mites. *J Med Entomol* 1976;12:577-621.
4. Griffiths DA, Cunnington AM. *Dermatophagoides microceras* sp.n.: a description and comparison with its sibling species. *D. farinae*, Hughes, *J Stored Prod Res* 1971;7:1-14.
5. Arlian LG, Bernstein IL, Geis DP, Vyszenski-Moher DL, Gallagher JS, Martin B. Investigations of culture medium-free house dust mites. III. Antigens and allergens of body and fecal extract of *Dermatophagoides farinae*. *J ALLERGY CLIN IMMUNOL* 1987;79:457-66.
6. Krilis S, Baldo BA, Basten A. Antigens and allergens from the common house dust mite *Dermatophagoides pteronyssinus*. Part II. Identification of the major IgE-binding antigens by crossed radioimmuno-electrophoresis. *J ALLERGY CLIN IMMUNOL* 1984;74:142-6.
7. Tovey ER, Baldo BA. Comparison by electroblotting of IgE-binding components in extracts of house dust mite bodies and spent mite culture. *J ALLERGY CLIN IMMUNOL* 1987;79:93-102.
8. Chapman MD, Platts-Mills TAE. Purification and characterization of the major allergen from *Dermatophagoides pteronyssinus*-antigen P₁. *J Immunol* 1980;125:587-92.
9. Dandeu JP, LeMas J, Lux M, Rabillon J, David B. Antigens and allergens in *Dermatophagoides farinae* mite. II. Purification of Ag 11, a major allergen in *Dermatophagoides farinae*. *Immunology* 1982;46:679-87.
10. Heymann PW, Chapman MD, Platts-Mills TAE. Antigen *Der f I* from the dust mite *Dermatophagoides farinae*: structural comparison with *Der p I* from *D. pteronyssinus* and epitope specificity of murine IgG and human IgE antibodies. *J Immunol* 1986;137:2841-7.
11. Chua KY, Stewart GA, Thomas WR, Simpson RJ, Dilworth RJ, Plozza TM, Turner KJ. Sequence analysis of cDNA coding for a major house dust mite allergen, *Der p I*. *J Exp Med* 1988;167:175-82.
12. Lind P. Demonstration of close physicochemical similarity and partial immunochemical identity between the major allergen, Dp 42, of the house dust mite, *D. pteronyssinus* and corresponding antigens of *D. farinae* (Df 6) and *D. microceras* (Dm 6). *Int Arch Allergy Appl Immunol* 1986;79:60-5.
13. Platts-Mills TAE, Chapman MD. Dust mites: immunology, allergic disease, and environmental control [CME article]. *J ALLERGY CLIN IMMUNOL* 1987;80:755-75.
14. Chapman MD, Heymann PW, Platts-Mills TAE. Epitope mapping of two major inhalant allergens, *Der p I* and *Der f I*, from mites of the genus *Dermatophagoides*. *J Immunol* 1987;139:1479-84.
15. Chapman MD, Heymann PW, Wilkins SR, Brown J, Platts-Mills TAE. Monoclonal immunoassays for the major dust mite (*Dermatophagoides*) allergens, *Der p I* and *Der f I*, and quantitative analysis of the allergen content of mite and house dust extracts. *J ALLERGY CLIN IMMUNOL* 1987;80:184-94.
16. Lind P. Purification and partial characterization of two major allergens from the house dust mite *Dermatophagoides pteronyssinus*. *J ALLERGY CLIN IMMUNOL* 1985;76:753-61.

17. Holck A, Dale S, Stetten K. Purification and characterization of a major allergen from the house dust mite *Dermatophagoides farinae*. *Allergy* 1986;41:408-17.
18. Yasueda H, Mita H, Yiu Y, Shida T. Isolation and characterization of two allergens from *Dermatophagoides farinae*. *Int Arch Allergy Appl Immunol* 1986;81:214-23.
19. van der Zee JS, van Swieten P, Jansen HM, Aalberse RC. Skin tests and histamine release with P₁-depleted *Dermatophagoides pteronyssinus* body extracts and purified P₁. *J ALLERGY CLIN IMMUNOL* 1988;81:884-96.
20. Chapman MD, Sutherland WM, Platts-Mills TAE. Recognition of two *Dermatophagoides pteronyssinus*-specific epitopes on antigen P₁ using monoclonal antibodies: binding to each epitope can be inhibited by sera from dust mite-allergic patients. *J Immunol* 1984;133:2488-95.
21. Heinrikson RL, Meredith SC. Amino acid analysis by reverse-phase high-performance liquid chromatography: precolumn derivatization with phenylisothiocyanate. *Anal Biochem* 1984;136:65-74.
22. Hewick RM, Hunkapiller MW, Hood LE, Dreyer WJ. A gas-liquid solid-phase peptide and protein sequenator. *J Biol Chem* 1981;256:7990-7.
23. Klinman NR, Taylor RB. General methods for the study of cells and serum during the immune response: the response to dinitrophenol in mice. *Clin Exp Immunol* 1969;4:473-87.
24. Bolton AE, Hunter WM. The labelling of proteins to high specific radioactivities by conjugation to a ¹²⁵I-containing acylating agent. *Biochem J* 1973;133:529-39.
25. Platts-Mills TAE, Snajdr MJ, Ishizaka K, Frankland AW. Measurement of IgE antibody by an antigen-binding assay: correlation with PK activity and IgG and IgA antibodies to allergens. *J Immunol* 1978;120:1201-10.
26. Chapman MD, Platts-Mills TAE. Measurement of IgG, IgA, and IgE antibodies to *Dermatophagoides pteronyssinus* by antigen-binding assay, using a partially purified fraction of mite extract (F₂P₁). *Clin Exp Immunol* 1978;34:126-36.
27. Pollart SM, Reid MJ, Fling JA, Chapman MD, Platts-Mills TAE. Epidemiology of emergency room asthma in northern California: association with IgE antibody to ryegrass pollen. *J ALLERGY CLIN IMMUNOL* 1988;82:224-30.
28. Marchalonis JJ, Weltman JK. Relatedness among proteins: a new method of estimation and its application to immunoglobulins. *Comp Biochem Physiol* 1971;38B:609-25.
29. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976;72:248-54.
30. Rawle FR, Mitchell EB, Platts-Mills TAE. T cell responses to the major allergen from the house dust mite *Dermatophagoides pteronyssinus*, antigen P₁: comparison of patients with asthma, atopic dermatitis, and perennial rhinitis. *J Immunol* 1984;133:195-201.
31. O'Hehir RE, Young DB, Kay AB, Lamb JR. Cloned human T-lymphocytes reactive with *Dermatophagoides farinae* (house dust mite): a comparison of T- and B-cell antigen recognition. *Immunol* 1987;62:635-40.
32. Ford AW, Rawle FC, Lind P, Spiekma FTM, Lowenstein H, Platts-Mills TAE. Standardization of *Dermatophagoides pteronyssinus*: assessment of potency and allergen content in ten coded extracts. *Int Arch Allergy Appl Immunol* 1985;76:58-67.