Monoclonal antibodies to group II *Dermatophagoides* spp. allergens: Murine immune response, epitope analysis, and development of a two-site ELISA

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Background: *Group II allergens are a major cause of sensitization in patients allergic to mites. To facilitate the antigenic analysis of group II allergens and to develop improved methods of allergen detection, we compared IgG anti-group II antibody responses in inbred mouse strains and raised a panel of monoclonal antibodies (mAbs).*
Methods: *IgE antibody responses were compared by antigen-binding radioimmunoassay. Epitope specificity of the mAbs was analyzed by two-site binding assays and by cross-inhibition radioimmunoassays.*
Results: *Comparison of polyclonal IgG antibody responses in five BALB congenic strains showed that H-2^d mice had poor responses, whereas H-2^b and H-2^k mice had strong, cross-reactive, IgG anti-group II responses. The specificities of nine anti-*Der p *II IgE mAbs raised in A/J mice were compared with specificities of seven mAbs produced previously. Most mAbs (11 of 16) recognized common epitopes on* Der p *II and* Der f *II: three were specific to* Der p *II, and two showed high binding to* Der f *II. Epitope analysis showed that the mAbs defined four cross-reactive, nonoverlapping sites on the group II allergens. Binding of several combinations of mAbs was compared, and a two-site ELISA for group II antigens was developed. Linear regression analysis showed an excellent correlation between results of this assay and group II radioimmunoassay of house dust samples* ($n = 40$, $r = 0.85$, $p < 0.001$).
Conclusions: *There are multiple cross-reactive B-cell epitopes on group II allergens. The group II ELISA has several important applications, including assessment of environmental allergen exposure, monitoring of the efficacy of avoidance procedures, and standardization of commercial mite allergen extracts. (J ALLERGY CLIN IMMUNOL 1994;94:537-46.)*

Key words: *Monoclonal antibody, mite allergens, ELISA,* Dermatophagoides, *asthma*

Immunochemical and molecular studies have defined four groups of allergens from *Dermatophagoides pteronyssinus* and *D. farinae*: Group I (25 kd cysteine proteases), group II (14 kd proteins), group III (~30 kd serine proteases), and group IV (60 kd amylases). The group I and group II allergens are the most significant cause of sensitization, inducing IgG and IgE antibody responses in 80% to 95% of patients allergic to mites, and each of these allergens (*Der p* I, *Der f* I, *Der p* II, and *Der f* II) has been cloned and sequenced.[1] There is now strong evidence that sensitization and exposure to mite allergens are significant causes of asthma and associated causes of atopic dermatitis.[1-6] Much of this work has been based on clinical and epidemiologic studies in which immunoassays for group I allergens were used to detect environmental allergens. These

Abbreviations used
BSA: Bovine serum albumin
CFA: Complete Freund's adjuvant
mAb: Monoclonal antibody
PBS-T: Phosphate-buffered saline solution, pH 7.4, containing 0.05% Tween 20

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assays have used either *Der p* I– or *Der f* I–specific monoclonal antibodies (mAbs) or monospecific polyclonal antibodies in ELISA or radioimmunoassay or (RIA) techniques.[7-9] Assays for group I allergens have also been used to assess the quantities and distribution of mite allergens in the air and to assess the efficacy of physical and chemical procedures for mite avoidance.[1, 10-18]

Recent studies have compared the environmental distribution and airborne properties of group II allergens.[9, 12, 15, 19] Measurements of group II allergens have not been widely adopted, however, because relatively few anti-group II mAbs have been produced and it has only been possible to use these mAbs in RIA procedures. The first anti-group II mAb was raised against *Der p* II; three anti-*Der f* II mAbs were later produced.[20, 21] These mAbs recognized common epitopes on *Der p* II and *Der f* II and were used to develop affinity purification techniques and solid-phase RIAs for group II. Five other anti-*Der f* II mAbs have also recently been produced.[22]

In this article, we compare the immune responses to group I and group II allergens in inbred mouse strains and describe the production of a large panel of anti-*Der p* II mAbs. Analysis of the epitope specificities of the mAbs allowed a two-site ELISA to be developed. The results demonstrate differences in the magnitude and specificity of murine IgG antibody responses to mite allergens and show that multiple, nonoverlapping epitopes on group II allergens were defined by the mAbs. The group II ELISA showed a very good quantitative correlation with RIA. The results suggest that this assay will be useful in environmental studies, in assessing procedures for mite avoidance, and in the standardization of mite allergen extracts.

METHODS

Mite allergens

Group I and group II allergens were purified from aqueous extract of *D. pteronyssinus* or *D. farinae* culture by mAb affinity chromatography.[21] Immunosorbents were prepared with mAb 4C1 for *Der p* I and *Der f* I and with mAb αDpX or mAb 6D6 for *Der p* II and *Der f* II, respectively. Allergens were eluted with 0.005 mol/L glycine in 50% ethylene glycol, pH 10.0.[21]

Mouse immunizations

Comparison of IgG antibody responses to Der p *I and* Der p *II.* Groups of five animals of six different mouse strains (BALB/c, C57B16, C3H, CBA, A/J, and AKR) were immunized intraperitoneally three times at 14-day intervals with 10 μg *Der p* I or 10 μg *Der p* II in complete Freund's adjuvant (CFA). Mice were bled from the tail vein 14 to 21 days after the final injection, and IgG antibodies in the serum samples were measured by antigen-binding RIA with iodine 125–labeled group I or group II allergens.

Comparison of antibody responses Der p *II in different BALB strains.* Groups of 3 to 5 mice (BALB/c, BALB.B, BALB.K, BALB.A10, or BALB.BD.2R) were immunized intraperitoneally three times at 14-day intervals with 5 μg *Der p* II in CFA or at 15-day intervals with allergen adsorbed to 250 μg aluminum hydroxide gel (Maalox, Rorer Pharmaceuticals, Fort Washington, Pa.). IgG antibody responses to *Der p* II or *Der f* II were determined by antigen-binding RIA.

Mice were obtained either from Hilltop Lab Animals, Inc. (Scottdale, Pa.) or from Dr. David Sachs, Harvard University (BALB strains; currently available from Hazelton Washington, Vienna, Va.). The use of animals for this study was approved by the Animal Research Committee of the University of Virginia under guidelines for the use and care of animals formulated by the National Council for Medical Research.

Monoclonal antibodies to group II allergens

Six A/J mice were immunized with 60 μg *Der p* II in CFA. At 2-week intervals, mice were boosted twice with 30 μg *Der p* II. A final intrasplenic injection of 50 μg *Der p* II was given to the mouse demonstrating the highest IgG antibody titer by mAb RIA (1/13,000). Three days later this mouse was killed, and its spleen cells were fused with SP_2/O myeloma cells in 37% polyethylene glycol as described previously.[23, 24] Two-week postfusion hybrids were screened by ELISA with 50% to 80% saturated ammonium sulfate fraction of *D. pteronyssinus* extract (containing 1300 μg/ml *Der p* II). Hybrids producing IgG anti-mite antibodies were tested for binding to ^{125}I-labeled *Der p* II and ^{125}I-labeled *Der f* II and cloned by limiting dilution. Positive clones were expanded and injected into pristine primed mice for ascites production. Nine anti-*Der p* II mAbs were derived from this fusion. Three anti-*Der f* II mAbs were also studied (clones 6D6, 7A1, and 4E5) and four mAbs were kindly provided either by Dr. R. Aalberse (αDpX) or by Dr. H. Okudaira (13A4, 15E11, and 18G8).[20-22]

Antigen-binding RIA

The antigen-binding RIA for measuring murine IgG antibody to mite allergens has been described previously.[23, 24] Essentially, 100 μl diluted mouse antiserum or mAb was incubated for 4 hours with 100 μl ^{125}I-allergen and IgG antibody-antigen complexes were precipitated by the addition of 100 μl goat anti-mouse IgG. The precipitates were washed three times with borate-buffered saline solution, pH 8.0, and counted in a gamma counter.

Iodine 125 labeling of proteins

Purified mAb or mite allergens were iodinated with a modified chloramine-T technique in which 20 μg protein was labeled with 18.5 MBq (0.5 mCi) ^{125}I (Amer-

sham Corp., Arlington Heights, Ill.).[25] The specific activity of ^{125}I-*Der p* II or ^{125}I-*Der f* II was 0.25 MBq/μg protein; that of the ^{125}I-labeled mAbs or group I allergens was 1.1 to 1.9 MBq/μg.

Epitope analysis of mAbs

The epitope specificities of mAbs raised against *Der p* II were compared by solid-phase RIA. Cyanogen bromide–activated paper disks were coupled with mAbs (1 μg/disk), and each disk was incubated for 2 hours with 0.1 ml of a 50% to 80% saturated ammonium sulfate fraction of *D. pteronyssinus* or *D. farinae* extract containing 1 μg/ml *Der p* II or *Der f* II, respectively. Disks were washed five times with phosphate-buffered saline solution containing 0.05% Tween 20 (PBS-T) and incubated with 500 ng cold mAb together with approximately 5 ng ^{125}I-mAb for 4 hours. After washing, the disks were counted in a gamma counter. All assays were carried out in duplicate, and the sample diluent was 1% bovine serum albumin (BSA) in PBS-T. Uninhibited binding of ^{125}I-mAb to *Der p* II or *Der f* II was calculated from the mean counts per minute of four wells incubated with 1% BSA in PBS-T. The percent inhibition of binding of ^{125}I-mAb to *Der p* II by cold mAb was calculated as previously described.[24] Epitope specificity was also analyzed by two-site binding RIA with the use of a capture mAb to "present" *Der p* II to a second ^{125}I-mAb. Sixteen solid-phase mAbs were each coupled to CNBr-activated paper disks and incubated for 4 hours with 100 μl *D. pteronyssinus* or *D. farinae* extract. After washing, 5 ng of each of six ^{125}I-mAbs (100,000 cpm) was incubated with the disks for 2 hours, and the disks were washed and counted.

Biotinylation of mAbs

Anti-*Der p* II mAbs, 1D8-C4 or 4G7-F1, were purified from ascites by high-performance liquid chromatography on a 5 ml recombinant protein G Sepharose column (Gamma-Bind Plus; Pharmacia, Piscataway, N.J.) and dialyzed against 0.1 mol/L sodium bicarbonate, pH 8.4, overnight at 4° C. For biotinylation, 7.25 mg mAb in a volume of 10.35 ml (0.7 mg/ml) was mixed with 1.24 ml Enzotin (EAB-40 G; Enzo Biochem, Inc., N.Y.) in dimethyl sulfoxide for 4 hours at room temperature.[7] Enzotin was prepared at the same concentration as the mAbs. Unreacted Enzotin was removed by dialysis against 4 L phosphate-buffered saline solution at 4° C. Biotinylated mAbs were titrated with *D. pteronyssinus* (UVA 92/01) and *D. farinae* (UVA 92/02) extracts to determine the optimal for binding in the ELISA (1/15,000). Biotinylated mAbs were stored in the dark, at 4° C, diluted 1:15 in 50% glycerol, 1% BSA phosphate-buffered saline solution, 0.01% thimerosal. Each aliquot contained 42 μg/ml biotinylated mAb.

Measurement of group II allergens by ELISA

The group II ELISA was modified from the previously published assay for *Der p* I and *Der f* I.[7] Plastic microtiter plates (Dynatech Immunlon 2; Dynatech Laboratories, Inc., Chantilly, Va.) were coated overnight with 1 μg/well of a 50% saturated ammonium sulfate cut of mAb 7A1 ascites in 0.05 mol/L bicarbonate buffer, pH 9.6, at 4° C. The plates were washed three times with PBS-T, incubated with 1% BSA in PBS-T for 1 hour, washed, and incubated with twofold dilutions of allergen or dust extracts. Allergen extracts (100 μl) were assayed at dilutions of 1:100 to 1:3200, and house dust extracts were assayed at 1:10 to 1:80. Bound allergen was detected by adding 100 μl 1:1000 dilution of biotinylated mAb 1D8 (containing 4.2 ng mAb) and, after further washing, 100 μl 1:1000 dilution of streptavidin-peroxidase (Sigma Chemical Company, St. Louis, Mo.). The enzyme substrate was 2,2′-azinobis-3-ethyl-benzthiazoline sulfonic acid 100 μl 1 mmol/L in 70 mmol/L citrate phosphate buffer, pH 4.2, containing 0.03% hydrogen peroxide, and the green color reaction was read at 405 nm in an ELISA microplate reader (Titertek; ICN Biomedicals, Costa Mesa, Calif.).[7]

The ELISA was quantified with doubling dilutions of *D. pteronyssinus* or *D. farinae* extracts to form control curves from 0.5 to 250 ng/ml. These extracts were prepared by Miles Laboratories (Spokane, Wash.) from whole mite bodies and designated UVA 92/01 *(D. pteronyssinus)* and UVA 92/02 *(D. farinae)*. Each extract had been substandardized against the Center for Biologics Evaluation and Research mite references, E1-Dp and E1-Df, which were estimated to contain 50 μg/ml or 20 μg/ml *Der p* II or *Der f* II, respectively. Stock solutions of the UVA references were prepared at 5 μg/ml in 50% glycerol, phosphate-buffered saline solution, 1% BSA, and 0.01% thimerosal and were used at a 1:20 dilution to form the first dilution of the control curve (250 ng/ml).

To validate the results of the ELISA, 40 house dust extracts from 20 houses in São Paulo, Brazil were assayed, and the results were compared with those previously obtained by RIA.[19] House dust samples were obtained by vacuuming 1 m^2 of carpet for 2 minutes with a handheld vacuum cleaner (Electrolux, São Paulo, Brazil). The samples were sieved through an 0.3 mm mesh screen, and 100 mg fine dust was extracted overnight at 4° C in 2 ml BBS.[19] The group II ELISA and RIA results were compared by linear regression analysis.

RESULTS

Murine IgG antibody responses to group I and group II allergens

Six inbred mouse strains were compared for IgG antibody responses to *Der p* I or *Der p* II (Table I). All responding mouse strains immunized with *Der p* I showed a predominantly species-specific response, and in most cases IgG anti-*Der f* I antibody was less than 20% of the anti-*Der p* I antibody. These results are in keeping with previous data obtained with polyclonal antibodies and mAbs from BALB/c mice.[23, 24, 26-28] AKR mice failed to make IgG antibodies to *Der p* I, even after prolonged immunization (five injections of *Der p* I in

TABLE I. Specificity of polyclonal murine IgG antibody responses to *Der p* I or *Der p* II

Strain	H-2 type	Allergen bound (μg/ml serum)*			
		Der p I	*Der f* I	*Der p* II	*Der f* II
BALB/c	d	27.2	1.5	<0.2	<0.2
C57BL6	b	15.0	0.5	11.1	7.7
C3H	k	3.3	0.4	2.9	5.0
CBA	k	10.9	2.1	12.7	16.2
A/J	a	3.5	2.0	3.1	5.0
AKR	k	<0.2	<0.2	ND	ND

Groups of five mice were immunized three times with 10 μg allergen in CFA. *ND,* Not done.
*Determined by antigen-binding RIA with ^{125}I-allergens. Values represent the geometric mean of assay results on five serum samples.

TABLE II. Polyclonal IgG anti-Group II antibody responses in congenic BALB strains

Strain	H-2 haplotype	Adjuvant	Allergen bound (μg/ml serum)	
			Der p II	*Der f* II
BALB/c	d	CFA	0.23 ± 0.17	<0.2
		Alum	3.1 ± 0.7	0.5 ± 0.1
BALB.B	b	CFA	12.7 ± 2.4	3.2 ± 0.9
		Alum	18.9 ± 1.7	4.2 ± 0.6
BALB.K	k	CFA	17.7 ± 1.3	9.2 ± 3.5
		Alum	17.4 ± 1.6	4.0 ± 0.3
BALB.A10	a.10	CFAC	15.7 ± 1.5	2.7 ± 0.5
		Alum	12.6 ± 1.7	3.2 ± 0.5
BALB.BD(2R)	g	CFA	<0.2	<0.2

Groups of three to five mice were immunized three times with 5 μg *Der p* II in either CFA or alum. Serum samples were assayed for IgG antibody by antigen-binding assay and the results are expressed as the geometric mean ± standard error of the mean.

CFA). In contrast to the response to *Der p* I, IgG antibody responses to *Der p* II were strongly cross-reactive with *Der f* II in all responding strains (Table I). BALB/c mice showed weak or undetectable responses to *Der p* II, which was unexpected because the original anti-*Der p* II mAb, anti-DpX, was raised from a BALB.B mouse.[20] Antibody responses in congenic BALB strains were compared by immunizing mice with *Der p* II in either CFA or alum (Table II). The results showed that BALB.B, BALB.K, and BALB.A10 mice produced high levels of IgG anti-group II antibody when immunized in CFA or alum. BALB/c mice had weak antibody responses after immunization with CFA and significantly lower titers than the other strains when immunized with *Der p* II in alum. Four BALB/c crossed with BALB.B hybrid mice (BALB.BD.2R) were also immunized with *Der p* II in CFA according to the same immunization protocol; these mice also had undetectable IgG antibodies to *Der p* II.

Specificity of mAb

A/J mice were used for anti-*Der p* II mAb production because a BALB/c crossed with A/J hybrids (CAF1) can be used for ascites production. Nine mAbs were derived from a single fusion of spleen cells from an A/J mouse that had been immunized with *Der p* II in CFA. Binding of these mAbs to radiolabeled *Der p* II or *Der f* II was compared with that of seven other anti-group II mAbs derived from three previous fusions from BALB/B, BALB/c, or A/J mice (Table III). The RIA results showed that most mAbs (11 of 16) bound to cross-reactive epitopes on both *Der p* II and *Der f* II. Polyclonal mouse IgG anti-*Der p* II antibodies also bound equally well to both allergens. Three clones showed five to 10 times greater binding to *Der p* II than to *Der f* II (3G5-A2, 3G5-H11, and 2B12), and two clones showed significantly higher binding to *Der f* II (15E11 and 4E5). None of the mAbs reacted with group I allergens (data not shown).

TABLE III. List of mAbs to *Dermatophagoides* group II allergens

mAb*	Immunogen	Mouse strain	^{125}I-allergen bound (cpm)†	
			Der p II	*Der f* II
αDpX‡	*Der p* II	BALB.B	18,029	11,143
6D6§	*Der f* II	A/J	5,104	12,772
7A1§	*Der f* II	A/J	9,028	11,756
4E5§	*Der f* II	A/J	1,617	22,659
4G7-F1	*Der p* II	A/J	10,738	13,778
4G7-F2	*Der p* II	A/J	12,678	6,336
1D8-C4	*Der p* II	A/J	10,775	16,796
1D8-A6	*Der p* II	A/J	10,423	14,924
3G5-H11	*Der p* II	A/J	10,674	1,105
3G5-A2	*Der p* II	A/J	7,720	808
1F9-C6	*Der p* II	A/J	11,170	5,118
2B12-B3	*Der p* II	A/J	3,024	807
1E12-E6	*Der p* II	A/J	13,946	5,493
15E11§	*Der f* II	BALB/c	3,672	21,422
13A4§	*Der f* II	BALB/c	5,682	17,346
18G8§	*Der f* II	BALB/c	1,336	2,284
Control				
6F9‖	*Fel d* I	BALB/c	1,344	872
Normal mouse serum		–	1,317	815
Polyclonal mouse antiserum	*Der p* II	CBA	11,830	15,445

*All clones were of the IgG1 isotype with the exception of 2B12, which was IgG2b.
†By antigen-binding RIA with mAb ascites at 1/2500 dilution. The mAbs were provided by Dr. R. Aalberse‡ and Dr. H. Okudaira§ or obtained in a previous study.¶[21]
‖Control mAb raised against cat allergen *Fel d* I.[37]

Previous studies showed that mAbs, αDpX, 7A1, and 6D6 defined three nonoverlapping, cross-reactive epitopes on the group II allergens.[20, 21] The epitope specificity of the 16 mAbs used in these studies was compared by cross-inhibition RIA and by two-site binding assay, with cold mAb used to present allergen to a second ^{125}I-mAb. For the cross-inhibition studies, cold mAb was used to inhibit the binding of ^{125}I-mAb (1D8 or 4G7) to allergen "presented" by a mAb coupled to a paper disk (either 7A1 or αDpX). When allergen was presented by mAb 7A1, the 1D8 and 4G7 clones showed 70% to 98% cross inhibition, suggesting that they were directed against the same epitope (Fig. 1, *A*). In contrast, several other mAbs (e.g., 4E5, 3G5, and 2B12) showed less than 30% inhibition and appeared to be directed against different epitopes. Similar results were obtained with mAb αDpX on the solid phase, although the levels of inhibition tended to be lower with this mAb (Fig. 1, *B*). Most of the mAbs raised in this fusion appeared to have the same specificity as 1D8 or 4G7 and this epitope appeared to be distinct from the three mAb-defined sites reported previously.[21]

Two-site binding assays were carried out to confirm that the epitopes were nonoverlapping and to identify mAb combinations that could be used in immunoassays. Six ^{125}I-mAb were compared for binding to *Der p* II with each of the 16 mAbs on the solid phase. Selected results are shown in Table IV. In each case, there was little or no binding when the solid-phase mAb was the same as the iodine 125 label. The mAbs could be divided into two groups on the basis of binding of ^{125}I-mAb. Some combinations (e.g. mAb αDpX, 7A1, or 3G5) on the solid phase showed strong binding (20,000 to 50,000 cpm bound) with each ^{125}I-mAb. Other mAbs (e.g. 6D6, 1D8, 4G7, or 13A4) showed binding when used in combination with ^{125}I-αDpX but not with the other five ^{125}I-mAbs (Table IV). The mAb αDpX could be used in a two-site assay in combination with any of the other mAbs, either on the solid-phase RIA or as ^{125}I-detecting mAb, with the exception of mAb 15E11, which appeared to have similar specificity to αDpX. The two-site binding assays were repeated with *Der f* II, and similar patterns of reactivity were obtained (data not shown).

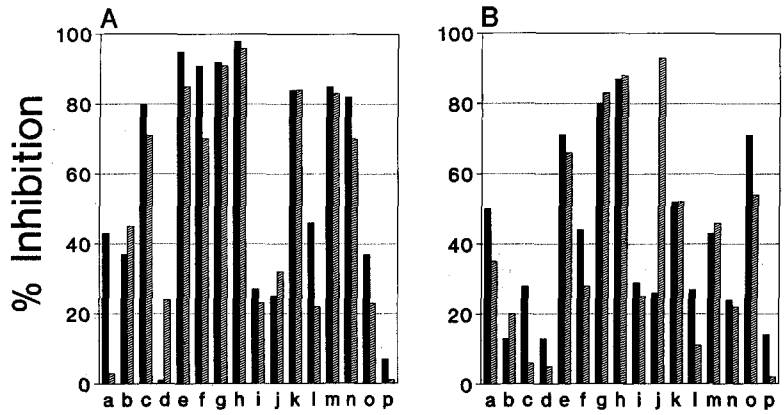

FIG. 1. Cross inhibition of the binding of ^{125}I-mAb to *Der p* II. Solid-phase mAb 7A1 **(A)** and αDpX **(B)** were used to present *Der p* II to 5 to 10 ng iodine 125–labeled 4G7 *(solid bars)* or 1D8 *(hatched bars)*. This binding was inhibited with 500 ng cold αDpX *(a)*, 6D6 *(b)*, 7A1 *(c)*, 4E5 *(d)*, 4G7-F1 *(e)*, 4G7-F2 *(f)*, 1D8-C4 *(g)*, 1D8-A6 *(h)*, 3G5-H11 *(i)*, 3G5-A2 *(j)*, 1F9-C6 *(k)*, 2B12-B3 *(l)*, 1E12-E6 *(m)*, 13A4 *(n)*, 15E11 *(o)*, *or 18G8 (p)*. Total radioactivity added (in 0.1 ml) was 108,839 cpm (4G7) or 111,840 cpm (1D8).

TABLE IV. Comparison of mAb binding to *Der p* II by two-site RIA

Solid-phase mAb	Immunogen	^{125}I-mAb added					
		αDpX	6D6	4G7	1D8	2B12	1E12
αDpX	*Der p* II	2,109	36,897	40,853*	36,572*	22,058	21,108
6D6	*Der f* II	32,320	793	1,662	2,120	1,731	3,121
7A1	*Der f* II	50,728	52,913	45,599*	41,964*	30,156	18,603
4E5	*Der f* II	11,869	20,777	23,987	26,100	20,555	9,416
1D8-C4†	*Der p* II	33,494	555	2,271	2,225	2,133	3,043
4G7-F1	*Der p* II	33,256	592	3,315	2,433	2,238	2,488
3G5-H11	*Der p* II	35,047	42,031	30,288	33,408	23,542	15,042
2B12	*Der p* II	24,748	513	1,186	1,490	1,642	2,460
15E11	*Der f* II	1,470	23,995	30,326	22,576	22,716	15,355
13A4	*Der f* II	39,552	48,713	34,774*	37,512*	22,663	2,002
Phosphate-buffered saline solution control‡		444 ± 123	408 ± 69	877 ± 145	855 ± 145	1,154 ± 201	2,268 ± 365

Solid-phase mAbs were coupled to cyanogen bromide–activated paper disks (1 μg/disk). Disks were incubated with *D. pteronyssinus* extract (1 μg/ml) overnight, washed, and incubated with a different ^{125}I-mAb (~110,000 cpm).

*These combinations of mAbs could be used in RIA on plastic microtiter wells and were used to develop an ELISA. The other combinations showed significant binding only in the paper disk assay.

†The mAbs with the same epitope specificity to *Der p* II were grouped and clones with the similar specificity as the 1D8-C4 were as follows: 1D8-A6, 1E12-E6, 1F9-C6, 4G7-F1, 4G7-F2.

‡Phosphate-buffered saline solution control results are expressed as the mean counts per minute ± standard deviation for each group of mAb tested.

ELISA for group II allergens

The original mAb RIA for group II was carried out with anti-*Der f* II mAb 7A1 coupled to CNBr-activated cellulose disks and ^{125}I-labeled anti-*Der p* II mAb αDpX for detection.[21] Although several combinations of solid-phase mAbs coupled to CNBr-activated disks were effective in two-site RIA for the detection *Der p* II and *Der f* II, only three of 16 mAbs (7A1, αDpX, 13A4) bound to plastic microtiter wells and could be used to develop an ELISA (Table IV). These mAbs were used in combination with biotinylated mAbs 1D8 or 4G7, and ELISA control curves for either *Der p* II or *Der f* II were compared (Fig. 2). On the basis of these curves, the combination of mAb 7A1 on the solid phase and biotinylated 1D8 was selected for use. This assay produces parallel control curves for both group II allergens and is approximately two times more sensitive to *Der f* II than to *Der p* II (limits of sensitivity 2 to 4 ng/ml) (Fig. 3).

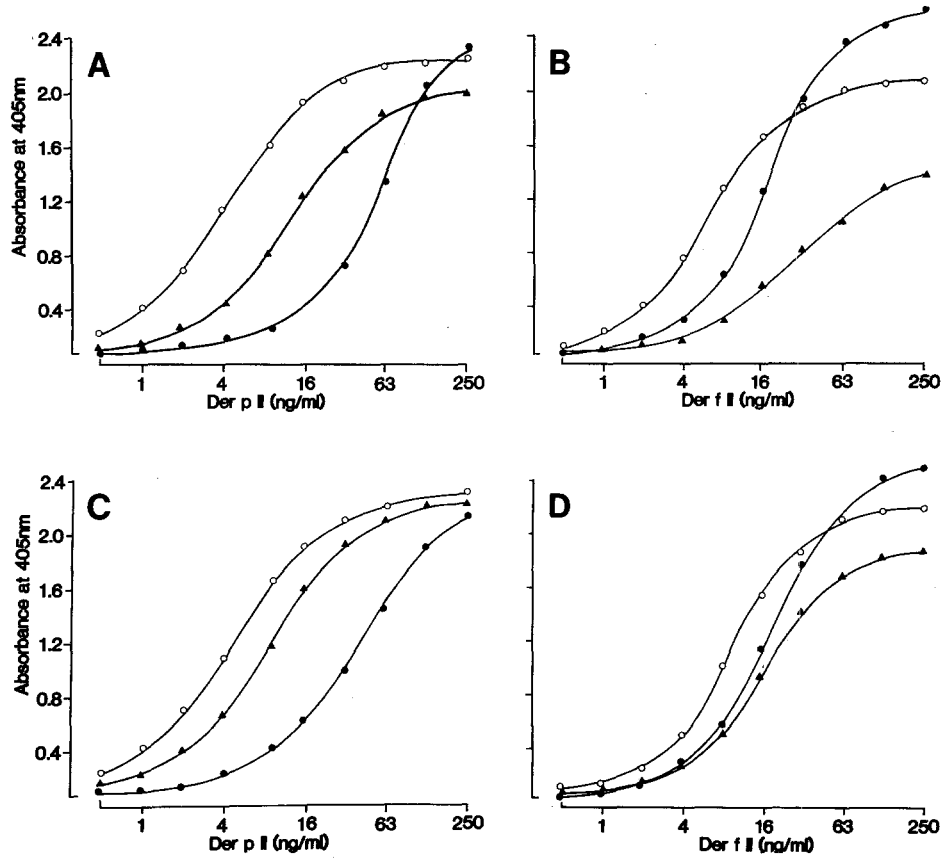

FIG. 2. ELISA curves for group II mite allergens with different combinations of mAb. The curves compare binding of *Der p* II (**A** and **C**) or *Der f* II (**B** and **D**) with mAb 13A4 (○), 7A1 (●), or αDpX (▲) as capture mAbs and biotinylated mAb 1D8 (**A** and **B**) or 4G7 (**C** and **D**) for detection.

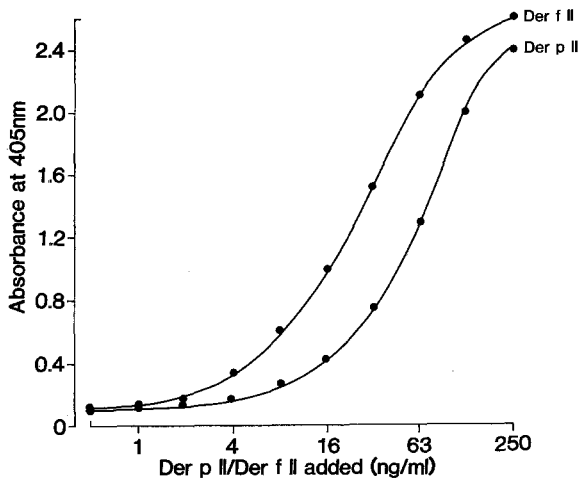

FIG. 3. ELISA control curves for *Der p* II and *Der f* II with mAb 7A1 on the solid phase and biotinylated mAb 1D8 for detection.

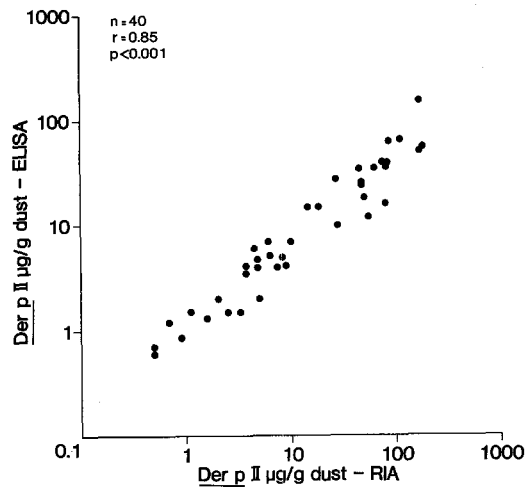

FIG. 4. Correlation between the mAb ELISA and mAb RIA for *Der p* II in 40 house dust extracts collected from 20 houses in São Paulo, Brazil.

Forty house dust extracts from São Paulo, Brazil, were analyzed for group II allergens with both the mAb ELISA and mAb RIA. The results showed good correlation between group II levels obtained by ELISA and those obtained by RIA ($n = 40$, $r = 0.85$, $p < 0.001$) (Fig. 4). The mean intraassay coefficient of variation for two separate group II assays was 12.1% ($n = 69$) and the mean

interassay coefficient of variation on two assays was 16.1% ($n = 15$). In addition, the correlation coefficient between ELISA and RIA for 11 mite extracts from different manufacturers was also good ($r = 0.95$, $p < 0.001$) (data not shown).

DISCUSSION

There have been few previous studies on murine immune responses to mite allergens. Studies on IgE antibody responses showed that CBA and C57B1 were high responders to *Der p* I, whereas BALB/c, C3H, and AKR were poor responders.[29] Most of the studies on IgG antibody responses have been confined to BALB/c mice because this is the preferred strain for mAb production.[23, 24, 26-28] Our results show that all the mouse strains tested had high-titer, species-specific IgE antibody responses to *Der p* I, with the exception of AKR mice, which were nonresponders. Seven of the eight strains tested, including BALB congenics, also had antibody responses to *Der p* II that were cross reactive with *Der f* II. The poor antibody response of BALB/c (H-2^d) mice to group II allergens has been previously reported and was confirmed by these studies.[21] In addition, BALB.DB.2R mice, which are also H-2^d at the I-A and I-E loci, did not have detectable anti-*Der p* II antibodies. These results are in keeping with a recent study of T-cell responses to *Der p* II, in which H-2^d mice had poor T-cell responses to *Der p* II or *Der p* II peptides; whereas H-2^b mice had high responses, and H-2^k mice had intermediate responses.[30] In our studies some H-2^k mice produced high levels of IgG antibody to *Der p* II, comparable to those of H-2^b haplotype. Given both the antibody and the T-cell data, it is difficult to reconcile the production of three high-affinity anti-*Der f* II mAbs from BALB/c mice.[22] The BALB/c-derived mAbs were produced after prolonged high-dose immunization (seven doses of 30 μg *Der f* II in CFA), and in fact, the antibody and T-cell studies each used different immunization protocols, suggesting that the route and dose of immunization can significantly affect immune responsiveness to *Der p* II. Analysis of the specificity of anti-group II mAbs produced as part of this study, together with those produced previously, showed that the mAbs define four nonoverlapping epitopes present in both *Der p* II and *Der f* II. These results are consistent with the high degree of amino acid sequence homology between the two allergens (88%).[31]

The development of an ELISA for group II allergens has several potential applications in asthma and allergy research, including environmental studies, assessment of avoidance procedures, and allergen standardization. Immunoassays for the group I allergen are established as the primary method of assessing mite allergen exposure, and threshold values have been proposed as risk factors for the induction of IgE antibody responses and for the exacerbation of asthma attacks.[1-6] A good correlation between group I and group II allergen levels has been reported in house dust samples from Japan and Brazil, and an approximately 1:1 ratio of the two groups of allergens in dust extracts.[9, 12, 19] The availability of the ELISA makes it possible to compare group II allergen levels in other parts of the world to investigate the epidemiologic significance of measuring group I and group II allergens. Measurements of both allergens would provide a better measure of "total" allergen load, and the group II assay has the advantage of measuring allergen from both *D. pteronyssinus* and *D. farinae*.

The group II ELISA will be particularly useful for mite avoidance studies and for allergen standardization. In terms of avoidance, the assay will be of practical value in assessing the effects of chemical treatments to reduce mite allergen levels, particularly treatments involving allergen denaturation, because it has been established that group II allergens are more resistant to denaturation than are group I allergens.[32] Recent studies have shown that measurements of group II allergens were effective in monitoring the efficacy of benzyl benzoate and tannic acid, currently the two most commonly used acaricidal and chemical treatments.[18, 33, 34] Group II measurements should also play an important role in the standardization and quality control of mite extracts. The allergen content of mite extract varies significantly with the source material, and it is now clear that extracts prepared from whole-mite cultures usually have high ratio of group I to group II (often >10:1), whereas extract of pure mite bodies usually have ratios of less than 2:1.[21] Measurements of either group of allergens alone may not be representative of the composition or potency of the extract. Preliminary studies of mite body extracts marketed in the United States have shown that measurements of both group I and group II allergens generally correlate with potency estimates based on skin testing.[35] In those studies it was also clear that some extracts had low major allergen content or had aberrant ratios of group I to group II. Measurement of both allergens by ELISA provides a simple quantitative method of standard-

ization that will also enable companies to improve their extraction and quality control procedures.[36]

Our studies further emphasize the use and applications of mAbs in research on mite and other indoor allergens, particularly for assessing environmental exposure. Although several other mite allergens have been identified, and in some cases fully sequenced, data on sensitization and exposure to these allergens are limited because few mAbs to other allergens are available. Our studies suggest that the panel of mAbs will provide useful probes for analyzing B-cell epitopes on group II allergens. The ELISA will be a useful adjunct for further epidemiologic studies of mite allergy, for developing improved diagnostic and therapeutic allergen extracts, and for monitoring the efficacy of allergen avoidance procedures as part of the management of asthma.

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Note added in proof: An improved format for the group II ELISA has been developed since this manuscript was accepted for publication, using mAb 1D8 for allergen capture and biotinylated mAb 7A1 for detection. A detailed assay protocol is available on request.

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