

Monoclonal immunoassays for 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

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Monoclonal, two-site radioimmunoassays (RIAs) were developed to measure allergen *Der p I* of *Dermatophagoides pteronyssinus* or *Der f I* of *D. farinae*. Microtiter plates coated with monoclonal antibody (Mab) were incubated with mite extract, and bound allergen was detected with a second ^{125}I -labeled Mab of different epitope specificity. The Mab RIAs were very sensitive (nanogram range) and highly specific. *D. pteronyssinus* extracts with different concentrations of *Der p I* demonstrated parallel binding curves, whereas a potent *D. farinae* extract demonstrated <5% of the *Der p I* binding in the same assay. Similar parallel curves were obtained with several *D. farinae* extracts in the *Der f I* assay, whereas *D. pteronyssinus* extract demonstrated little or no binding. The Mab RIAs were compared with an inhibition RIA that measured cross-reacting determinants on both *Der p I* and *Der f I* (antigen P, equivalent [AgP,Eq].) The results demonstrated good quantitative agreement between these assays in commercial mite and house dust extracts (mean difference 1.57 ± 0.5 -fold). Thirty house dust samples with known mite counts, *Der p I*, and AgP,Eq content were also compared. The summed Mab RIA values for *Der p I* and *Der f I* demonstrated a very good correlation with AgP,Eq values ($r = 0.86$; $p < 0.001$) and with assessments of total mite-allergen content by RAST inhibition ($n = 21$, $r = 0.77$; $p < 0.001$). Furthermore, in samples with more than 10 mites per 100 mg of dust, the *Der p I*:*Der f I* ratio closely correlated with the ratio of the two mites counted by microscopy ($n = 15$, $r = 0.89$; $p < 0.001$). The Mab RIAs can measure allergen levels in mite or dust extracts without the need for purified allergen or affinity-purified antibodies and can readily be standardized. These assays will be useful in epidemiologic studies of allergic asthma, to assess patients' exposure to mite allergens, and the effects of avoidance regimens. Because of the long-term stability and reproducibility of the reagents, Mab-based assays for specific allergens will also play an important role in the standardization of mite and other allergen extracts. (*J ALLERGY CLIN IMMUNOL* 1987;80:184-94.)

On a worldwide basis, house dust mites (*Dermatophagoides* sp.) are among the most ubiquitous inhalant allergens. *Dermatophagoides* sp. usually comprise >90% of the mites present in house dust¹; how-

ever, the geographic distribution of the two principal species, *D. pteronyssinus* and *D. farinae*, varies considerably. In England and some parts of Europe, *D. pteronyssinus* is found almost exclusively, whereas in the United States either species may predominate, and mixed populations of both species often occur.²⁻⁴ Mite-allergen exposure has traditionally been assessed by microscopic identification and counting of isolated mites. This requires considerable technical expertise, is time consuming, and may underestimate the allergen contribution of mite fecal particles.^{5,6} In vitro assays to measure mite allergens have been developed with either RAST inhibition⁷ or immunoassays for individual mite allergens. Several different techniques have been used to measure the major *D. pteronyssinus* allergen *Der p I*,⁸⁻¹⁰ including inhibition

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Abbreviations used

Mab:	Monoclonal antibody
RIA:	Radioimmunoassay
<i>Der p</i> I:	A major 24,000 MW allergen of <i>Dermatophagoides pteronyssinus</i>
<i>Der f</i> I:	A major 24,000 MW allergen of <i>D. farinae</i>
WHO/IUIS:	World Health Organization/International Union of Immunological Societies
NIBSC:	National Institute for Biological Standards and Control
UVA:	University of Virginia
SAS:	Saturated ammonium sulfate
PBS-T:	Phosphate-buffered saline, pH 7.2, containing 0.05% Tween 20
BBS:	Borate-buffered saline, pH 8.0
BSA:	Bovine serum albumin
AgP ₁ Eq:	Antigen P ₁ equivalent
10B9:	Anti- <i>Der p</i> I monoclonal antibody 10B9 F6 A12
5H8:	Anti- <i>Der p</i> I monoclonal antibody 5H8 C12 D9
6A8:	Anti- <i>Der f</i> I monoclonal antibody 6A8 B10 D12
4C1:	Monoclonal antibody 4C1 B8 3F8 that recognizes both <i>Der p</i> I and <i>Der f</i> I

RIA,^{6, 8, 9} rocket and counter-current immunoelectrophoresis,^{11, 12} and radial immunodiffusion.¹³ Recently, *Der f* I, a structural homologue of *Der p* I, was purified from *D. farinae*,¹⁴⁻¹⁶ and we have reported an inhibition RIA for cross-reacting antigenic determinants on each allergen, termed AgP₁Eq. This assay measures both *Der p* I and *Der f* I in house dust and mite extracts.¹⁷ There are several problems associated with current in vitro assays for mite allergens. RAST inhibition is dependent on the composition of the extract coupled on the solid phase and requires large quantities of pooled serum from mite-allergic humans. Changes in either of these variables can significantly affect the results obtained. Specific allergen assays require either purified allergen (usually in limited supply) or polyclonal rabbit antibodies. These antibodies are not easily reproduced because they are heterogeneous and often require immunoabsorption.

Several investigators have recently produced Mabs to mite allergens,^{16, 18-21} and these reagents have many advantages for allergen identification and quantitation. Their specificity can be accurately defined and remains constant, and they can be produced in sufficient quantities (grams) to be made widely available. We have previously reported a series of Mab to *Der p* I and

Der f I,^{16, 18, 19} and the present article describes the use of four of these Mabs to develop solid-phase RIAs for measuring these allergens. The assays were used to compare the quantities of *Der p* I and *Der f* I in commercial mite and house dust extracts and in 30 house dust samples on which mite counts and allergen levels had previously been determined. The results demonstrate that the Mab RIAs are specific and sensitive (nanogram range) and demonstrate a very good correlation with mite counts, double antibody inhibition RIAs, and RAST inhibition.

MATERIAL AND METHODS

Mite extracts

D. farinae reference extract, UVA 86/01, was prepared by extracting 3 gm of *D. farinae* culture (Hollister-Stier Laboratories, Spokane, Wash.) from which mite bodies had been removed, in 300 ml of BBS, pH 8.0, overnight at 4° C. The extract was centrifuged (500 × g for 30 minutes), lyophilized in 2 ml ampules, and stored at -20° C. The *D. farinae* extract used as the standard in *Der f* I assays was prepared by extracting 11 gm of *D. farinae* culture from Hollister-Stier in 50 ml of BBS as above. This extract was dialyzed for 2 days against BBS and was then precipitated with 40% SAS to enrich for *Der f* I.¹⁶ The pellet was resuspended in 2 ml of BBS, dialyzed, and stored at -20° C. The WHO/IUIS international reference preparation of *D. pteronyssinus* (NIBSC code 82/518)^{12, 13} was obtained from NIBSC, London, U.K. *D. pteronyssinus* extract, *D. pter* '84, was a dialyzed aqueous extract of whole *D. pteronyssinus* culture (Bencard Ltd., Brentford, Middlesex, U.K.), similar to those used in previous studies.^{5, 6, 8} *D. pteronyssinus* extract, UVA 86/02, was a 50% SAS cut of whole *D. pteronyssinus* culture (Bencard Ltd.) that had been substandardized against the WHO/IUIS reference by AgP₁Eq assay. This extract contained 6.25 mg/ml of *Der p* I and was used as the standard for *Der p* I and AgP₁Eq assays.

House dust extracts

Thirty dust samples were obtained from 25 houses: 23 in Charlottesville, Va., and two in London, U.K. The samples included 13 living room samples (11 from sofas and two from carpets), 12 bedroom samples (nine from bedding, mattresses, and soft furnishings and three carpet/floor dusts), and five samples from household vacuum cleaner bags. The dusts were collected and extracted as described previously.⁶ Briefly, 100 mg of sieved dust was extracted overnight in 2 ml of BBS and then centrifuged (500 × g for 20 minutes), and the supernatants were stored at -20° C. Mite counts were performed on a second 100 mg portion of each dust. The mites were removed by flotation on saturated NaCl per 20% of sucrose gradients, stained with crystal violet, and counted.⁶ Mite species were identified microscopically according to morphologic criteria described by Hughes.²² Commercial house dust extracts were obtained from Bencard Ltd., Hollister-Stier Laboratories, Center Laboratories (Port Washington, N.Y.), and Greer

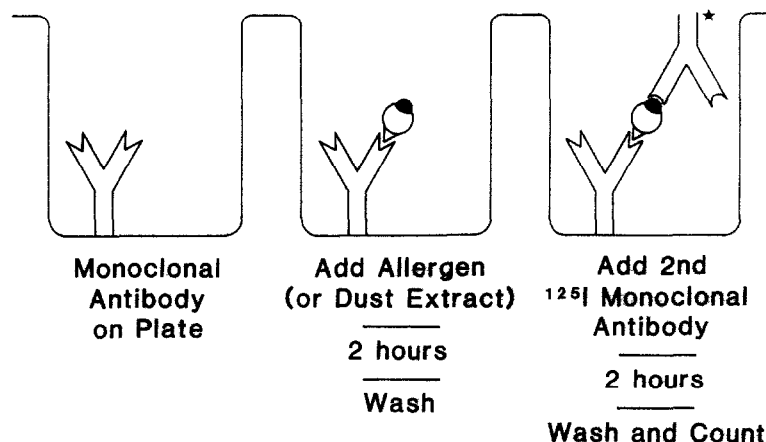


FIG. 1. Schematic illustration of the two-site monoclonal RIA for mite allergens.

Laboratories (Lenoir, N.C.) Endodust extract was also tested.

Mabs

The production and characterization of monoclonal antibodies to *Der p* I and *Der f* I have been described previously.^{16, 18, 19} The Mab used in the present studies had been subcloned from the original primary clones. Antibodies 10B9 and 5H8 were raised against *Der p* I and recognized two different epitopes on this allergen.¹⁸ Antibodies 6A8 and 4C1 were raised against *Der f* I; 6A8 binds to a *Der f* I specific epitope, whereas 4C1 binds to a cross-reacting epitope present both on *Der f* I and *Der p* I.^{16, 19}

RIAs

***Der p* I.** Flexible polyvinylchloride microtiter plates (Dynatech Laboratories, Alexandria, Va.) were coated overnight with 1 µg per well of a 50% SAS cut of 10B9 ascites in 0.1 mol/L bicarbonate buffer, pH 9.6, at 4° C. The plates were washed twice with PBS-T, incubated for 1 hour with 1% BSA/PBS-T, and then washed twice with PBS-T and dried. Diluted samples (100 µl of mite extract or house dust extract) were added to wells in the plate and incubated for 2 hours. The plates were washed five times with PBS-T and then incubated for 2 hours with 2 ng of ¹²⁵I-labeled, isoelectric focusing purified 5H8 (125,000 cpm, specific activity 20 µCi/µg). After five washes in PBS-T, the plates were air-dried, and individual wells were counted in a gamma counter (Micromedex Systems, Inc., Horsham, Pa., 4/200). The ¹²⁵I-labeled 5H8 bound (in counts per minute) was directly related to the quantity of *Der p* I in the samples. Samples were assayed in duplicate, at two different dilutions, with 1% BSA/PBS-T as diluent. Values were obtained from a control curve with doubling dilutions of *D. pteronyssinus* extract UVA 86/02 (range 250 to 0.5 ng/ml of *Der p* I).

***Der f* I.** This assay was modified from that described above. Plates were coated with 1 µg per well of a 50% SAS cut of 6A8 ascites, and the *Der f* I that bound to this Mab

was detected with 2 ng ¹²⁵I-labeled, isoelectric focusing-purified 4C1. The assay was quantitated with a 40% SAS fraction of *D. farinae* extract, which contained 1.4 mg/ml of AgP₁Eq to form a control curve.

The double antibody-inhibition RIA for *Der p* I was as described previously.^{6, 8} In the inhibition RIA for AgP₁Eq,¹⁷ 0.1 ml of a 1/15,000 dilution of affinity-purified, rabbit AgP₁Eq antibody was incubated for 2 hours with diluted mite or dust extract; 2 ng of ¹²⁵I-labeled *Der p* I was added. After a further 2-hour incubation, antigen-antibody complexes were precipitated overnight with 0.1 ml of 1/11 dilution of goat antirabbit IgG. Precipitates were washed three times with BBS, and the residual radioactivity was then counted.

Rast inhibition

Diluted dust samples (50 µl) were incubated with 50 µl of a serum pool from mite-allergic subjects (UVA 85) for 6 hours in the wells of a microtiter plate. A *D. pteronyssinus*-coupled paper disc was added to each well, incubated overnight, and then washed five times with BBS/0.1% Tween. The discs were incubated overnight with 2 ng of ¹²⁵I-labeled affinity-purified goat anti-IgE (specific activity 20 µCi/µg), washed five times, and counted in a gamma counter. House dust extracts were assayed in duplicate in serial fourfold dilutions, i.e., undiluted to 1/4096. The assay was quantitated by reference to a control curve of the *D. pteronyssinus* International Reference, NIBSC code 82/518, which has an assigned potency of 1.0 × 10⁵ U/ml.^{12, 13} The reference (diluted 1/4 to 1/1024) was run in parallel with each assay, and values for the dust extracts were extrapolated from the dilutions at which they elicited 50% inhibition of the binding of ¹²⁵I-anti-IgE. All samples used for RAST inhibition were diluted in BBS containing 50% horse serum.

Statistical methods

The results of the monoclonal RIAs, inhibition RIAs, and RAST-inhibition assays were compared by linear regression analysis.

RESULTS

Mab-based, allergen-specific RIAs

The principle of the Mab RIAs is outlined in Fig. 1. Allergen (either *Der p* I or *Der f* I) binds to a Mab coupled to a microtiter plate, and a second ^{125}I -labeled Mab, directed against a different nonoverlapping epitope, is used to detect the bound allergen. The assay for *Der p* I uses two *Der p* I-specific Mabs, 10B9 and 5H8.¹⁸ With these Mabs as either first or second antibody, i.e., solid-phase 10B9 with ^{125}I -labeled 5H8 or vice versa, almost identical dose-response curves were obtained (Fig. 2). There was no binding when the radiolabeled Mab was the same as that on the solid phase. The *Der p* I assay was routinely carried out with 10B9 on the solid phase with ^{125}I -labeled 5H8 as second antibody. Parallel binding curves were obtained with purified *Der p* I, whole *D. pteronyssinus* culture extract, and *D. pteronyssinus* International Reference preparation (NIBSC code 82/518). In contrast, a 40% SAS cut *D. farinae* extract elicited no significant binding (Fig. 3, A).

The 16 *Der f* I-specific Mabs raised previously were directed against a single epitope^{16, 19} and could only be used in a two-site assay in conjunction with a second Mab, 4C1, which recognized a cross-reacting determinant on *Der p* I and *Der f* I. The binding curves for two different *D. farinae* extracts and a house dust extract (from dust containing 84% *D. farinae*) were parallel, and there was an approximately 700-fold difference in the *Der f* I content of these extracts (Fig. 3, B). This assay was *Der f* I specific and did not detect any allergen in a potent *D. pteronyssinus* extract (compare *D. pter* 84, Fig. 3, A and B).

Mite-allergen content of commercial mite and house dust extracts

The quantity of *Der p* I, *Der f* I, and AgP₁Eq was compared in 13 mite extracts and five commercial house dust extracts (Table I). Nine of the mite extracts (A1 to C2, Table IA) were prepared by commercial manufacturers, and three mite extracts were marketed as prick test reagents. All the house dust extracts (except H, Table IB) were marketed prick test solutions. The results confirmed that the Mab RIAs for *Der p* I and *Der f* I were essentially species specific. None of the *D. farinae* extracts reacted in the Mab RIA for *Der p* I, and although the *D. pteronyssinus* extracts elicited positive results in the *Der f* I assay, the quantities of *Der f* I detected were small, i.e., <0.2% of the *Der p* I levels. An extract of *D. microceras*, a "sibling" species of *D. farinae*,²³ contained more *Der f* I than *Der p* I by Mab RIA; however, both *D. microceras* extracts contained more than a hundredfold more AgP₁Eq than the sum of *Der p* I and

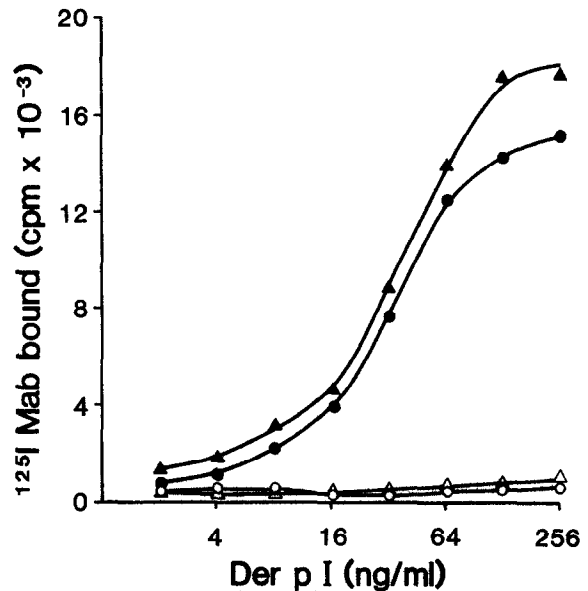


FIG. 2. Dose-response curves with different combinations of Mabs in the *Der p* I assay. Plates were coated with either 10B9 (●, Δ) or 5H8 (○, ▲), incubated with doubling dilutions of *Der p* I, and then with either ^{125}I -labeled 10B9 (Δ, ▲) or 5H8 (○, ●) as second antibody.

Der f I (Table IA), suggesting that the Mabs to *Der p* I and *Der f* I do not recognize the homologous allergen produced by this species. Extracts of *Tyrophagus putrescentiae* and *Acarus siro* demonstrated no significant binding above background in any of the RIAs (Table IA), neither did cricket, tick (*Dermacentor variabilis*), cat epithelium, nor *D. farinae* culture-medium extracts (data not presented).

There was good agreement between the quantities of AgP₁Eq and the *Der p* I and *Der f* I concentrations in *D. pteronyssinus* and *D. farinae* extracts, respectively (Table IA). The mean difference between the results of the RIAs for each allergen in the *D. pteronyssinus* and *D. farinae* extracts was 1.57 ± 0.5 -fold, and only two extracts demonstrated more than twofold differences between assays. It was evident that the house dust extracts (Table IB) contained different proportions of *Der p* I and *Der f* I, and this appeared to reflect the geographic areas in which the extracts were produced. Thus, dust extract E, manufactured in England, contained 98% *Der p* I, whereas those manufactured in the United States (D, F, and G) contained between twofold and twentyfold more *Der f* I than *Der p* I (Table IB).

Mite-allergen content of dust samples from houses

The Mab RIAs were used to assay 30 house dust extracts for which total and differential mite counts

TABLE IA. Comparison of RIAs for *Der f* I, *Der p* I, and AgP₁Eq in mite extracts

Mite extracts*	Assigned† concentration	Mab RIAs		
		<i>Der p</i> I (μg/ml)	<i>Der f</i> I (μg/ml)	AgP ₁ Eq (μg/ml)
<i>D. pteronyssinus</i>				
A1	41,000 PNU	64.0	0.075	88.0
B	1.2%	58.4	0.020	158.0
C1	2.5 × 10 ⁶ SQU	20.5	0.015	25.3
UVA 86/02	—	5100.0	nd	6250.0
<i>D. farinae</i>				
A2	29,000 PNU	<0.005	79.0	123.0
A3	1:50 w/v	<0.005	32.0	66.0
A4	1:50 w/v	<0.005	32.0	61.0
C2	2.5 × 10 ⁶ SQU	<0.005	60.8	68.0
UVA 86/01	—	<0.005	16.0	20.0
<i>D. microceras</i> (a)	—	0.010	0.58	78.0
<i>D. microceras</i> (b)	—	0.032	2.96	320.0
<i>T. putrescentiae</i>	—	<0.005	<0.005	<0.005
<i>A. siro</i>	—	<0.005	<0.005	<0.005

nd = Not done.

*Mite extracts A1 to C2 were commercially prepared by three different manufacturers (Bencard Ltd., Hollister-Stier Laboratories, and ALK, Copenhagen, Denmark). *D. microceras* extracts were prepared by extracting 100 mg of culture (kindly provided by Dr. Fritz Spijksma, Academisch Ziekenhuis, Leiden, The Netherlands) in 2 ml of either BBS (a) or BBS/50% glycerol (b). Extracts of *T. putrescentiae* and *A. siro* were prepared from freeze-dried culture material (kindly provided by Dr. W. Rolfsen, Pharmacia, Uppsala, Sweden) that was resuspended at 10 mg/ml in BBS.

†Concentration of "strength" of extract as reported by the manufacturer.

TABLE IB. Comparison of mite-allergen levels in commercial house dust extracts

Commercial house dust extracts*	Origin	Assigned† concentration	<i>Der p</i> I (ng/ml)	<i>Der f</i> I (ng/ml)	AgP ₁ Eq (ng/ml)
D	USA	20,000 PNU	5	125	50
E	UK	150%	738	10	942
F	USA	40,000 PNU	200	692	881
G	USA	10,000 PNU	155	300	600
H	—	—	<5	<5	<5

H = Endodust.

*Dust extracts D to G were commercial prick test solutions obtained from four different manufacturers.

†Concentration or "strength" of extract as reported by the manufacturer.

and the quantities of *Der p* I and AgP₁Eq (by inhibition RIA) had previously been determined. The extracts were assayed without knowledge of the mite counts, and the results were analyzed according to whether the dust samples contained predominantly *D. pteronyssinus* (Table II) or *D. farinae* (Table III). Mean variation between two assays on the same dust samples was $14.9 \pm 10.6\%$ for the *Der p* I assay ($n = 26$) and $16.6 \pm 11.0\%$ for the *Der f* I assay ($n = 18$). Linear regression analysis demonstrated an excellent quantitative correlation between the Mab

RIA for *Der p* I and the inhibition RIAs for *Der p* I ($r = 0.96$; $p < 0.001$) and AgP₁Eq ($r = 0.95$; $p < 0.001$) in the *D. pteronyssinus*-dominant samples. By Mab RIA, three of these samples contained no detectable *Der f* I, and seven samples contained much larger quantities (fivefold to 186-fold more) of *Der p* I than *Der f* I (Table II). In samples containing $\geq 50\%$ *D. farinae* (Table III), there was also a good correlation between the quantities of *Der f* I and AgP₁Eq ($r = 0.87$; $p < 0.001$), and these samples generally contained more *Der f* I than *Der p* I. One

TABLE II. Comparison of RIAs for *Der p* I, *Der f* I, and AgP₁Eq in *D. pteronyssinus*-dominant house dust samples

Dust sample	Mite count	% <i>D. pt</i>	Inhibition RIA		Mab RIAs		RAST inhibition (IU/ml × 10 ⁵)
			<i>Der p</i> I (ng/ml)	AgP ₁ Eq (ng/ml)	<i>Der p</i> I (ng/ml)	<i>Der f</i> I (ng/ml)	
1	120	75	48	120	80	66	0.12
2	10	100	345	471	600	<5	ND
3	40	100	7	17	30	48	<0.1
4	20	100	234	417	370	66	0.22
5	210	91	2088	3024	6750	162	2.0
6	940	73	4014	11808	8550	1115	6.6
7	140	100	63	105	80	42	0.18
8	130	100	1332	1944	3450	36	0.79
9	710	89	489	372	520	<5	1.44
10	54	100	54	51	70	<5	ND
11	2940	100	3528	7848	9480	51	2.6
12	50	100	2376	5400	5330	150	2.7
13	620	100	8064	15980	13500	108	7.6

D. pt = *D. pteronyssinus*; ND = not done.

TABLE III. Quantities of *Der f* I, *Der p* I, and AgP₁Eq in house dust samples containing ≥ 50% *D. farinae*

Dust sample	Mite count	% <i>D.f.</i>	Mab RIAs		AgP ₁ Eq (ng/ml)	RAST inhibition (IU/ml × 10 ⁵)
			<i>Der f</i> I (ng/ml)	<i>Der p</i> I (ng/ml)		
14	960	89	1800	160	3024	4.20
15	330	100	108	30	73	0.25
16	220	100	462	20	480	0.57
17	780	56	1554	380	2952	2.80
18	360	50	639	700	1836	1.50
19	8480	52	975	6980	7776	7.10
20	1920	53	282	340	582	2.0
21	800	84	3600	320	10800	6.0
22	620	100	2880	80	9000	4.0
23	170	100	471	30	537	0.69

D.f. = *D. farinae*.

sample (No. 19) with the highest mite count (8480 mites per gram of dust) contained equal proportions of both mite species; however, the level of *Der f* I in this extract was sevenfold lower than *Der p* I.

A very good quantitative correlation was obtained when the combined *Der p* I and *Der f* I levels in all 30 dust extracts were compared with the values for AgP₁Eq ($r = 0.86$; $p < 0.001$, Fig. 4). Two extracts (open circles, Fig. 4) contained 10.9-fold and 8.1-fold more AgP₁Eq than the sum of the two allergens assayed separately. These extracts were prepared from two dust samples collected in London, U. K., each of which contained >90% *Euroglyphus maynei*. The

total mite-allergen content of selected *D. pteronyssinus*- and *D. farinae*-dominant house dust extracts was assessed relative to the *D. pteronyssinus* International Reference (NIBSC code 82/518) by RAST inhibition (Tables II and III). The results demonstrated a very good quantitative correlation with the summed Mab RIA values ($n = 21$, $r = 0.77$; $p < 0.001$) (Fig. 5) and with the AgP₁Eq content of the extracts ($n = 21$, $r = 0.91$; $p < 0.001$; Table II and III) irrespective of whether they contained predominantly *D. pteronyssinus* or *D. farinae*.

The ratio of *D. pteronyssinus*:*D. farinae* was compared with the *Der p* I:*Der f* I ratio to see whether

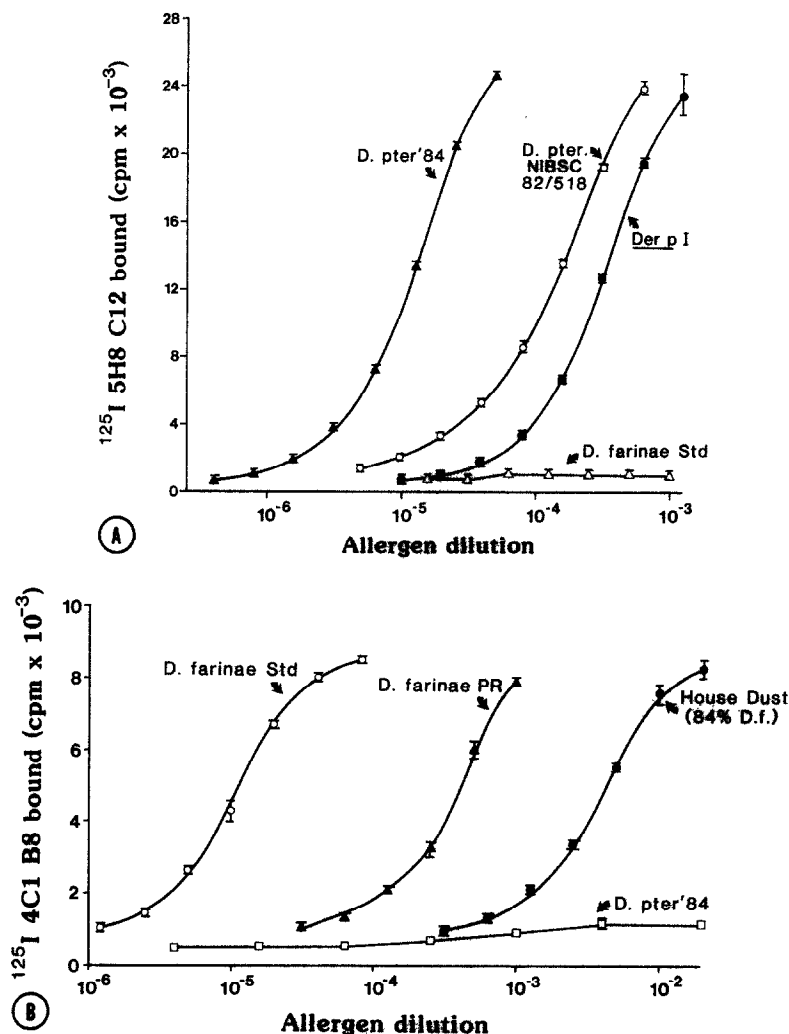


FIG. 3. Comparison of the binding curves of *D. pteronyssinus* or *D. farinae* extracts in the Mab RIA for *Der p* I (A) or *Der f* I (B). Serial twofold dilutions of each extract were tested. Extracts were as follows: *D. pter*'84, whole *D. pteronyssinus* culture (Bencard Ltd.); NIBSC code 82/518, WHO/UIS international reference preparation of *D. pteronyssinus*^{12,13}; *D. farinae* Std, a 40% SAS cut of *D. farinae* extract (see Methods); *D. farinae* PR, commercial prick test solution (Hollister-Stier); and house dust (84% *D. farinae*), a dust extract that contained predominantly (84%) *D. farinae* (sample 21, Table III). The purified *Der p* I preparation was ~90% homogeneous on sodium dodecyl sulfate-polyacrylamide gel electrophoresis, similar to preparations reported previously.⁸

the results obtained with the Mab RIAs accurately reflected the proportions of each mite species in dust samples (Fig. 6). When all 28 samples were compared, a positive correlation was obtained ($r = 0.64$; $p < 0.01$); however, in many of the dust samples (13/28) less than 10 mites were positively identified (open circles, Fig. 6). For samples in which the mite counts were more than 10 and therefore more accurate (closed circles, Fig. 6), there was a very good correlation with the Mab RIA ratio ($n = 15$, $r = 0.89$; $p < 0.001$).

DISCUSSION

The main advantages of the mite-allergen assays described in this study are that they are as sensitive as inhibition RIAs (down to 0.5 ng/ml) and do not require the use of purified allergen. They are also technically easy to perform, and results on large numbers of extracts can be obtained within a few hours. Another important advantage of the Mab RIAs is that they are highly specific. Assaying *D. pteronyssinus* extracts with the *Der f* I assay elicited values $<1\%$ of the true values for *Der p* I, whereas the *Der p* I

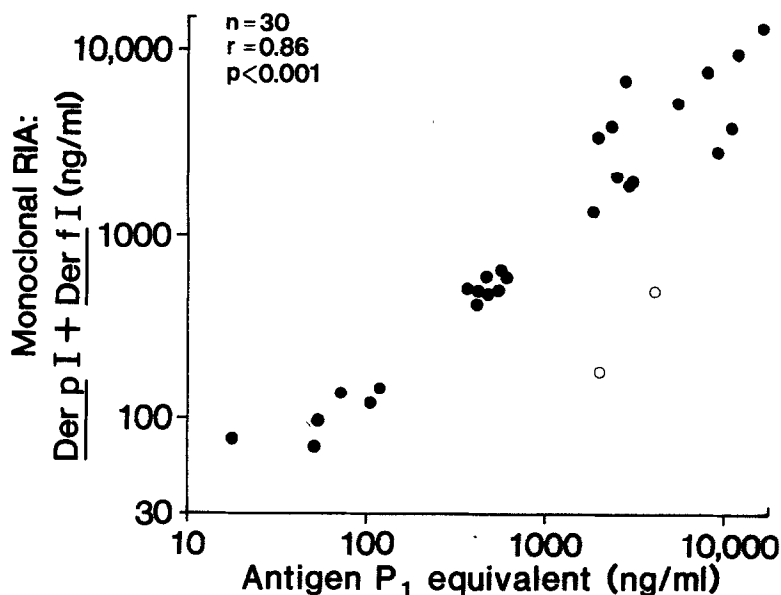


FIG. 4. Linear regression analysis of mite-allergen levels in house dust. Values for AgP₁Eq were compared with the summed values for *Der p* I and *Der f* I. Solid circles: 28 house dust samples from Charlottesville, Va., containing >95% *Dermatophagoides* sp.; open circles: two dust samples from London, U.K., containing 94% *E. maynei*.

assay was fully species specific. It has not been possible to achieve the same degree of specificity with polyclonal rabbit antibodies. Indeed, our inhibition RIA for *Der p* I could be inhibited up to 50% with *D. farinae* extracts,¹⁷ and similar cross-inhibition has also been noted by Lind²⁴ with heterologous mite allergens in ELISA.

The measurement of mite allergens by Mab RIA has two principal applications: (1) the standardization of mite or house dust extracts²⁵ and (2) assessments of mite-allergen exposure in the houses of allergic patients. These assays can be fully standardized because the specificity of the Mabs has been well defined^{16, 18, 19} and because allergen concentration can be determined with a known reference. The *D. pteronyssinus* International Reference (NIBSC code 82/518) was used as the primary reference in these studies because it contains a known quantity of *Der p* I that has been measured by several different laboratories.^{12, 13} Although we recently purified *Der f* I,¹⁶ our quantitation of this allergen has so far been based on the AgP₁Eq assay, which elicits parallel inhibition curves with both *Der p* I and *Der f* I.¹⁷ Similarly, in the present studies, the quantity of *Der f* I was assessed relative to a *D. farinae* extract that had been substandardized against the *D. pteronyssinus* International Reference by AgP₁Eq assay. The results demonstrated good agreement between the *Der p* I, *Der f* I, and AgP₁Eq assays in commercial

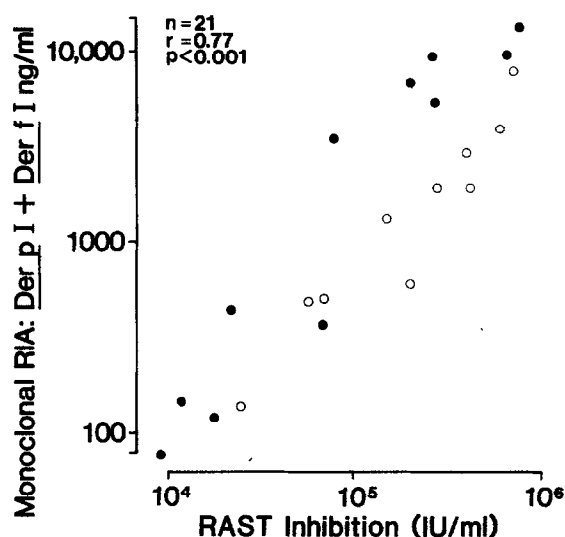


FIG. 5. Correlation between RAST inhibition and Mab RIA. Twenty-one house dust extracts containing either >75% *D. pteronyssinus* (●) (Table II) or ≥50% *D. farinae* (○) (Table III) were compared.

mite and house dust extracts and confirm that the Mab RIAs measure equivalent quantities of each allergen. Although similar amounts of *Der p* I and *Der f* I (10 to 80 µg/ml) were measured in the commercial *D. pteronyssinus* and *D. farinae* extracts, respectively, it is evident from previous studies^{12, 13} that these

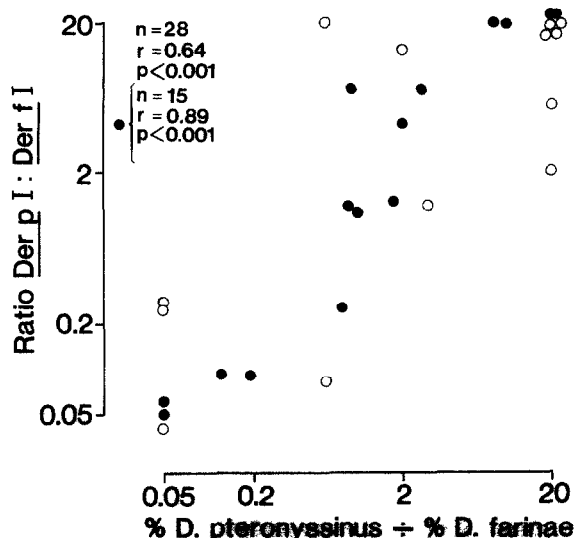


FIG. 6. Comparison of differential mite count with *Der p I* and *Der f I* allergen levels. The percent of *D. pteronyssinus* divided by percent *D. farinae* was compared with the ratio of *Der p I*:*Der f I* in 28 house dust samples, and the results were expressed as a range between 0.05 to 20. Negative samples for either mite counts or allergen levels were assigned an arbitrary value of 5%. Thus, samples that contained 100% *D. pteronyssinus* or *Der p I* were scored as 20 (100/5), and samples that contained 100% *D. farinae* were scored as 0.05 (5/100). *Open circles*: samples in which less than 10 mites of either species were counted; *solid circles*: 10 to 83 mites per 100 mg of dust were counted and identified as *Dermatophagoides*, and 6 to 44 mites could be distinguished at the species level.

values need not necessarily correlate with estimates of total potency as assessed by RAST inhibition. Other mite allergens, e.g., Dp-X and Ag 23¹² also affect total potency, and the ratio of *Der p I* of these allergens varies with different types of mite extract. Whole body extracts usually contain a higher proportion of Dp-X, whereas whole culture extracts contain relatively more *Der p I*,^{12,13} presumably because a greater proportion of the allergen in whole cultures is derived from mite feces. At the present time, absolute quantitation of Dp-X is not possible because this allergen has not been fully purified. However, an anti-Dp-X Mab has been produced²⁰ and used to quantitate Dp-X in units relative to the WHO/IUIS reference *D. pteronyssinus*.^{12,13} As more monoclonal reagents become available and the other allergens become better defined, it will be possible to accurately quantitate several mite allergens and make better assessments of total potency for standardization purposes.

In dust extracts from patients' houses, there was a good correlation between the Mab RIAs and both the AgP₁Eq assay and assessments of total allergenic po-

tency with mite counts and RAST inhibition. Some dust samples differed in their Mab RIA and AgP₁Eq values, but the differences were small (less than threefold, Tables II and III) compared to house-to-house differences in mite-allergen levels, which can vary by a hundredfold to a thousandfold.^{4,6,17} Although the correlation between specific allergen levels and total allergen potency was good, the relationship between absolute levels of *Der p I*/*Der f I* (in nanograms per milliliter) to that predicted from international units determined by RAST inhibition (10 IU $\approx$ 1 ng *Der p I* per milliliter)^{12,13} was not as good as expected and could vary by up to tenfold. This was surprising because both techniques were standardized relative to the WHO/IUIS reference *D. pteronyssinus* and serves to illustrate the difficulties in the use of RAST inhibition to quantitate specific allergen levels in heterogeneous allergen extracts.

The results confirm previous findings^{4,6,17,24} that the quantities of *Der p I* and *Der f I* in dust are a consistent marker of mite growth and can be reliably used to monitor exposure to *D. pteronyssinus* and *D. farinae* in houses. The allergen content of dust samples containing *D. microceras* or *E. maynei* would be underestimated with the current Mab RIAs; however, these species are not as common as the other *Dermatophagoides* sp. *E. maynei* is uncommon, and whereas *D. microceras* has recently been reported in Europe,^{15,24} it has not been found in the United States. There is good evidence that both these species produce an allergen that is antigenically related to *Der p I*/*Der f I* and can be detected with affinity-purified polyclonal rabbit antibodies.^{15,17,24} However, it has proved difficult to raise Mab to cross-reacting determinants on these allergens because the murine (BALB/c) immune response is predominantly species specific.^{16,18,19} Although our cross-reacting Mab, 4C1, does bind to *D. microceras* extracts (data not presented), other Mabs that bind equally to the homologous allergens from all three *Dermatophagoides* species have not yet been produced. Without the availability of Mab directed against a second, nonoverlapping cross-reacting site, a Mab RIA capable of measuring *Der p I*, *Der f I*, and *Der m I* (*D. microceras*) would be difficult to develop. The species-specific Mab RIAs can be used to estimate exposure to either *D. pteronyssinus* or *D. farinae* without the need to do differential mite counts (Fig. 6), and this should be a useful guide to the distribution of these species in areas where mite counts are unavailable.

In conclusion, our results demonstrate how Mabs can be used to develop specific, sensitive, and reliable immunoassays for allergen quantitation. An increas-

TABLE IV. An abridged list of allergen-specific Mabs

Source	Allergen	Reference source
House dust mite	<i>Der p</i> I	Chapman et al. ¹⁸
		Wu et al. ²¹
	<i>Der f</i> I	Heymann et al. ¹⁶
		Chapman et al. ¹⁹
Grass pollen	<i>Der p</i> II (Dp-X)	
	<i>Lol p</i> I (Rye I)	Kahn and Marsh ²⁶
		Esch and Klapper ²⁷
		Aalberse (unpublished)
Ragweed pollen	<i>Par j</i> I (P. j 10)	Corbi et al. ^{28,29}
	<i>Amb a</i> I (AgE)	Krilis et al. ³⁰
		Olsen and Klapper ³¹
		Brown et al. ³²
Cat dander	<i>Fel d</i> I (Cat-I)	
<i>Alternaria</i>	<i>Alt a</i> I (Alt-I)	Agarwal et al. ³²

Added in proof: Other groups have recently produced allergen-specific monoclonal antibodies directed against *Der p* I (Horn, Lind. Int Arch Allergy Appl Immunol 1987 [in press]), *Der f* I (Ley, Saiz, Carreira. Mol Immunol 1986;23:1311), or *Lol p* I (Bose et al. Immunology 1986;59:309). We have also recently produced monoclonal antibodies to a 29 kd mite allergen, *Der f* III (Heymann et al. J ALLERGY CLIN IMMUNOL 1987;79:192 [Abstract]), and to cat allergen *Fel d* I (Chapman MD. Unpublished).

ing number of allergen-specific Mabs have now been produced. We have recently analyzed more than 50 *Der p* I-specific Mabs produced in four different laboratories, and during the past 2 to 3 years, Mabs have been produced against several other major inhalant allergens, including *Amb a* I (ragweed antigen E), *Lol p* I (Rye I), and *Fel d* I (Cat-I) (Table IV). Because of their homogeneity and long-term stability, these reagents can serve as primary standards for allergen identification (since their specificity has already been defined), and in future most allergen quantitation could also become Mab based. An almost unlimited supply of Mabs can readily be produced for these purposes. One gram of Mab (easily obtainable from ascites or tissue culture) would suffice for 2×10^6 assays, as described here. For dust allergens, such as mite and cat, Mab-based assays should facilitate epidemiologic and individual studies of the quantities of allergens both in dust and airborne in patients' houses. In addition, by use of Mabs to pollen allergens, it should be possible to develop quantitative assays for pollen allergens, as alternatives to pollen counts, which would be less dependent on airborne sampling techniques. Thus, it would be possible to make objective measurements of pollen allergen(s) collected on a single sampling filter without the need for microscopic identification and to make assessments of pollen-allergen levels in nanograms per cubic meter. Although the assays we have described use radiolabeled antibodies, they can be readily adapted to enzyme immunoassays with either fluorescent or colorimetric detection systems. This would make these

assays more widely suitable for general use. With continued improvements in in vitro technology, it may soon be possible for doctors themselves to carry out these assays so that patients can be notified of the levels of specific dust allergens in their homes and advised of appropriate avoidance regimens.

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