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EPITOPE MAPPING OF TWO MAJOR INHALANT ALLERGENS, *DER P* I AND *DER F* I, FROM MITES OF THE GENUS *DERMATOPHAGOIDES*[1]

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The repertoire of antigenic sites on two major dust mite allergens, *Der p* I of *Dermatophagoides pteronyssinus* and *Der f* I of *D. farinae*, was studied using murine (BALB/c) monoclonal antibodies (Mab), polyclonal rabbit IgG antibodies, and human IgE antibodies. Fifty-three IgG Mab were analyzed from six different fusions (five vs *Der p* I, one vs *Der f* I). By antigen binding radioimmunoassay (RIA), most Mab were either *Der p* I or *Der f* I specific, and only 2/53 bound to both allergens. Epitope mapping studies using cold Mab to inhibit the binding of six ^{125}I labeled Mab to solid phase allergen defined four nonrepeated, nonoverlapping epitopes on *Der p* I, a single species-specific epitope on *Der f* I and a cross-reacting epitope present on each allergen. All but one of the 53 Mab bound to one of these six epitopes. Seventy percent (25/35) of anti-*Der p* I Mab were directed to the same epitope, suggesting that this epitope is immunodominant for BALB/c mice. Similarly, 88% (16/18) of anti-*Der f* I Mab bound to the same epitope on *Der f* I. Parallel cross-inhibition curves were obtained using the species-specific Mab, 10B9, and the cross-reacting Mab, 4C1, to compete for binding to *Der p* I, suggesting that the epitopes defined by these two Mab on *Der p* I are adjacent to one another. Both murine Mab and polyclonal rabbit IgG antibodies to cross-reacting sites on both allergens were used to inhibit binding of human IgE antibodies to *Der p* I by using 19 sera from mite allergic patients. Cross-reacting rabbit IgG antibodies strongly inhibited all sera tested (mean 79.5% ± 7.7) and two Mab, 10B9 and 4C1, partially inhibited (38% ± 12). However, the four Mab directed against separate species-specific epitopes (including murine immunodominant sites) showed little or no inhibition (≤20%). Our results suggest that most of the epitopes defined by Mab are not the same as, or close to, those defined by human IgE antibody. The striking differences in the repertoires of murine IgG and human IgE antibody responses to *Der p* I and *Der f* I could be explained by genetic differences or by altered antigen processing and presentation occurring as a result of different modes of immunization in mice and in mite allergic humans.

Inhalant allergens induce antibody responses of the IgE isotype that are clinically important in diseases associated with immediate hypersensitivity, e.g., hayfever, asthma, and atopic dermatitis. These allergens also constitute a group of low m.w. (10,000 to 50,000) protein antigens that have several advantages for studies on antigen structure and immune recognition. They are foreign, environmental proteins that sensitize 10 to 20% of the population and elicit both humoral and cellular responses. Studies with several highly purified major allergens (e.g., *Amb a* I, *Lol p* I, and *Der p* I) (1–4) have established that the human immune response includes multiple isotypes (IgG and IgA as well as IgE) (5–12), proliferative T cell responses (13–18), and complex delayed type hypersensitivity reactions involving basophils and eosinophils (19, 20). In addition, there is good evidence that allergen-specific human antibody responses, particularly to low m.w. allergens (<10,000), are genetically restricted (21–23). In spite of these studies, little is known of the structural features of allergen molecules that are important in B and T cell recognition.

Two major dust mite allergens, *Der p* I[4] from *Dermatophagoides pteronyssinus* and *Der f* I from *D. farinae*, have been used extensively in immunologic studies (3, 10, 11, 16–20, 24–28). These mite allergens both have m.w. 24,000 and appear to be structural homologues (28); however, there are significant differences between antibody responses to *Der p* I and *Der f* I in experimental animals (mice and rabbits) and in mite allergic humans. Human IgE and IgG antibodies cross-react extensively (80 to 95%) (28) and polyclonal rabbit IgG antibody directed against common epitopes on both allergens has been purified by affinity chromatography (anti-P_1 equivalent, Eq) (29). In contrast, both polyclonal and monoclonal murine IgG antibodies appear to be predominantly directed against species-specific epitopes on either allergen (28, 30).

To assess the diversity of antigenic sites on *Der p* I and *Der f* I, we compared the epitope specificity of 53 murine (BALB/c) monoclonal antibodies (Mab) raised from six different fusions and investigated whether murine Mab or anti-P_1 Eq antibodies recognize the same epitopes as

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[4] Abbreviations used in this paper: *Der p* I, antigen *Der p* I of *D. pteronyssinus*; *Der f* I, antigen *Der f* I of *D. farinae*; Mab, monoclonal antibody; anti-P_1 Eq, polyclonal rabbit IgG antibodies to cross-reacting epitopes on *Der p* I and *Der f* I.

human IgE antibodies. The results show that the Mab define six different epitopes, four on *Der p* I and two on *Der f* I, and an immunodominant site recognized by the majority of Mab can be defined on each allergen. They also show that the species-specific and immunodominant epitopes recognized by mice are distinct from the epitopes recognized by human IgE antibody.

MATERIALS AND METHODS

Monoclonal antibodies (Mab). Detailed protocols for the production of murine anti *Der p* I and anti *Der f* I Mab were reported previously (28, 30). The Mab used here were IgG antibodies produced from six fusions (five vs *Der p* I; one vs *Der f* I) using spleen cells obtained from BALB/c mice that had been immunized two to four times with 5 to 10 μg allergen in complete Freund's adjuvant. Thirty-eight Mab were produced from two fusions in our laboratory (one against each allergen) (26, 28). The five Mab selected for use in the present studies were subcloned from the original primary clones: three were *Der p* I specific, 10B9 F6 A12 (10B9), 5H8 C12 D9 (5H8 C12), and 5H8 D8 B4 (5H8 D8); one was *Der f* I specific, 6A8 B10 D12 (6A8); and one reacted with a cross-reacting epitope on both *Der p* I and *Der f* I, 4C1 B8 3F8 (4C1). Fifteen Mab raised from four fusions against *Der p* I were provided by other investigators: Dr. P. Lind, Protein Laboratory, Copenhagen (Nhp-2, Nhp-3, Nhp-4, Nhp-4, and Nhp-6, from two fusions) (31); Dr. G. Delespesse, Hospital Notre Dame, Montreal (C4.1/102, C4.1/96, C4.1/40, C3.6/104, C3.6/62, B2.1/81, B2.1/35, A2.2/79, and HY3/90); and Dr. R. Aalberse, Central Laboratory of the Bloodtransfusion Service, Amsterdam (CLB-P_1). Dr. Aalberse also provided monoclonal anti-DpX (CLB-DpX).

Selected Mab were purified from 50% ammonium sulfate fractions of ascites by preparative IEF in flat beds of Ultrodex gel using a pH 5 to 9 gradient (24, 30). Fractions were pooled on the basis of protein content (absorbance at 280 nm) and antigen binding RIA results using ^{125}I labeled allergens. The Mab peak was purified from residual ampholines (Pharmalyte; Pharmacia, Piscataway, NJ) by precipitation with 80% ammonium sulfate. Each Mab was >90% homogeneous on sodium dodecyl sulfate polyacrylamide gel electrophoresis (32). The IEF purified Mab (20 μg) were labeled with ^{125}I using the chloramine T technique (33) to a specific activity of 20 to 40 μCi/μg.

Polyclonal rabbit antibodies. Rabbit antibodies to cross-reacting epitopes on *Der p* I and *Der f* I (anti-P_1 Eq) were purified from hyperimmune anti-*D. farinae* antiserum by affinity chromatography over a *Der p* I immunosorbent. The anti-P_1 Eq ab was the same preparation as reported (29) and showed a single line of identity on immunodiffusion against *Der p* I and *Der f* I.

Sera from mite allergic patients. Sera were obtained from 17 patients who had clinical symptoms of rhinitis, asthma, and/or atopic dermatitis and gave positive immediate skin tests to mite extract (either *D. pteronyssinus* Bencard, UK, or *D. farinae*, Hollister-Stier, Spokane, WA). Four of these sera were included in two mite allergic serum pools which were also tested: NIBSC 82/518, prepared from 10 sera drawn in London, UK, (34) and UVA 85, prepared from 10 sera drawn in Charlottesville, VA. All sera contained IgE anti-mite antibody by RAST (129-9366 RAST U/ml, 1 U = approximately 0.2 ng), and the levels of IgE anti-*Der p* I determined by antigen binding RIA ranged from 47 to 562 U IgE binding activity/ml (10, 11, 24, 25).

Antigen binding radioimmunoassays (RIA). These assays for measuring murine IgG and human IgG and IgE antibody to mite allergens have been described in detail (10, 11, 24, 25, 28). Briefly, diluted serum or Mab was incubated with ^{125}I allergen for 4 hr and was immunoprecipitated with monospecific antiserum (rabbit anti-mouse IgG, goat anti-human IgG, or goat anti-human IgE). After 16 hr, the precipitates were washed 3× in borate-buffered saline, pH 8.0, and were counted in a gamma counter.

Epitope analysis using Mab. The epitope specificity of murine Mab was compared by cross-inhibition RIA using ^{125}I labeled Mab and solid phase allergen in a modification of described techniques (28, 30). Polyvinyl chloride microtiter plates (Dynatech, Alexandria, VA) were coated with 1 μg/well of Mab 5H8 C12 in 0.1 M bicarbonate buffer, pH 9.6, overnight at 4°C, and residual binding sites were "blocked" with 0.1 ml 1% bovine serum albumin in phosphate-buffered saline, pH 7.2, containing 0.05% Tween 20 (1% BSA PBS-T). Each well was incubated with 0.1 ml of a 50% SAS fraction of *D. pteronyssinus* extract (Bencard, UK) containing 1 μg/ml *Der p* I for 2 hr, was washed 5× with PBS-T, and then was incubated with 500 ng cold Mab together with 2–5 ng ^{125}I labeled Mab for 4 hr. After further washing, the plates were dried, and individual wells were counted in a gamma counter (Micromedic 4/200; Horsham, PA). For assays using ^{125}I 5H8 C12, *Der p* I was coupled to the solid phase using 1 μg/well 10B9. All assays were carried out in duplicate, and the sample diluent was 1% BSA PBS-T. Uninhibited binding of ^{125}I Mab to allergen was calculated from the mean cpm of four wells incubated with PBS-T. The percent inhibition of each of six ^{125}I Mab by different cold Mab was calculated as:

$$\frac{\text{mean cpm of PBS control} - \text{mean cpm of cold Mab}}{\text{mean cpm of PBS control}} \times 100\%$$

In experiments using *Der f* I, 4C1 was coated on the plate and the allergen preparation was a 50% SAS cut of *D. farinae* extract (Hollister-Stier) (28) containing 1 μg/ml *Der f* I.

Cross-inhibition studies using human sera. Microtiter plates were coated with Mab followed by either *Der p* I or *Der f* I as described above, and were incubated overnight with either 50 μg cold Mab or 50 μg rabbit anti-P_1 Eq together with 0.05 ml serum from mite allergic patients. A single dilution of serum was used in the range 1/2 to 1/10. The plates were washed 5× with PBS-T, were incubated with 2 ng ^{125}I affinity-purified goat anti-IgE for 6 hr, and then were washed and were counted. Percent inhibition of human IgE binding relative to four uninhibited PBS-T controls was calculated as described above.

Statistical analysis. The percent inhibition of binding of human IgE to *Der p* I by murine Mab or rabbit anti-P_1 Eq was compared using Student's *t*-test for paired samples on the statistics program Tadpole (Elsevier-Biosoft, Cambridge, U.K.).

RESULTS

Specificity of Mab. The results of antigen binding RIA for 21 Mab from six fusions are shown in Table I. Six representative Mab raised previously in our laboratory against either *Der p* I or *Der f* I were compared with 15 Mab derived from four fusions against *Der p* I carried out in other laboratories. These 15 Mab bound a similar

TABLE I

Allergen specificity of Mab determined by antigen binding RIA[a]

Mab[b]	Isotype	pI	*Der p* I bound (cpm)	*Der f* I bound (cpm)
10B9*	IgG1	6.25	15,983	275
10H2 C6*	IgG1	—	19,594	302
5H8 C12*	IgG2a	7.40	14,846	309
5H8 D8*	IgG1	6.30	11,001	264
4C1*	IgG1	6.90	8,806	10,954
6A8*	IgG1	6.20	247	12,100
Nhp-2	IgG1	—	5,406	329
Nhp-3	IgG1	—	16,300	272
Nhp-4	IgG1	—	15,750	344
Nhp-5	IgG1	—	12,150	285
Nhp-6	IgG1	—	2,488	578[c]
C4.1	IgG1	6.45	11,147	370
C4.1/96	IgG1	—	12,000	392
C4.1/40	IgG1	—	10,513	342
C3.6/104	IgG1	—	16,320	307
C3.6/62	IgG1	—	16,236	300
B2.1/81	IgG1	—	10,443	301
B2.1/35	IgG1	—	11,812	355
A2.2/79	IgG1	—	16,055	383
HY3/90	IgG1	—	13,287	306
CLB-P1	IgG1	—	9,413	353
CLB-DpX	IgG1	—	259	337
Normal mouse serum		—	254	318
Total ^{125}I added			44,589	43,311

[a] Diluted Mab (0.1 ml, 1/10,000 ascites or 10 μg/ml 50% SAS cut ascites) was incubated with 2 ng ^{125}I labeled allergen for 4 hr and then was immunoprecipitated with rabbit anti-mouse IgG. Precipitates were washed 3× with BBS and were counted in a gamma counter. The results are the mean of duplicate assays.

[b] Mab (21) raised against either *Der p* I or *Der f* I were compared: six were reported previously (*) (28, 30), and the other 15 were provided by three other investigators. CLB-DpX = Mab raised against *D. pteronyssinus* allergen DpX (*Der p* II) (4).

[c] This clone produced weak ascites. Subsequent retesting at lower dilution (1/2500) showed 9390 cpm *Der p* I bound and 2605 cpm *Der f* I bound (total ^{125}I allergen added = 115,000 cpm).

percentage of ^{125}I *Der p* I to those raised previously (12 to 37%), but showed little or no binding to *Der f* I (<1%). The results confirm our previous observations that murine (BALB/c) ab responses to *Der p* I and *Der f* I are almost exclusively directed against species-specific epitopes on these allergens. Only two Mab, 4C1 B8 and 4C1 C6, bound equally to both allergens in antigen binding RIA (Table I) (28).

Epitope mapping of Der p I and Der f I. Epitope specificity was assessed by cross-inhibition RIA using a panel of ^{125}I labeled, IEF purified Mab (see Table I for pI values): four were *Der p* I specific (10B9, 5H8, C12, 5H8 D8, and C4.1); one was *Der f* I specific (6A8); and one bound to both allergens (4C1). The Mab directed against the *Der p* I specific epitopes did not bind in two-site RIA when the same Mab was used on the solid phase and as radiolabel, suggesting that these Mab defined four distinct epitopes on *Der p* I that were not repeated (data not shown). In the cross-inhibition experiments, allergen was "presented" to the ^{125}I labeled Mab by another Mab coated on plastic microtiter wells, and binding of the radiolabel to either *Der p* I or *Der p* I was competitively inhibited using cold Mab. The results (Fig. 1) showed that binding of each ^{125}I-labeled Mab was inhibited 94 to 98% by cold Mab from the same clone, whereas the other Mab generally inhibited by <20%. Thus 5H8 D8, C4.1, and 5H8 C12 each completely inhibited their own binding to *Der p* I and 6A8 inhibited its own binding to *Der f* I, but there was no significant inhibition by the other five Mab. The quantity of homologous cold Mab required to give 50% inhibition ranged from 5 to 20 ng. Binding of the species-specific Mab, 10B9, to *Der p* I was fully inhibited by the cross-reacting Mab, 4C1, and conversely, 10B9 inhibited the binding of 4C1 to *Der p* I but not to *Der f* I. Moreover, parallel cross-inhibition curves were obtained using both these Mab to cross-inhibit binding to *Der p* I (Fig. 2*A* and *B*), and the same quantity of each Mab (4 to 8 ng) was required for 50% inhibition. These results strongly suggest that the species-specific epitope recognized by 10B9 and the cross-reacting epitope recognized by 4C1 are adjacent to one another on *Der p* I.

Table II shows mean cross-inhibition data for 25 Mab, including 20 of the clones shown in Table I together with four additional *Der f* I specific Mab that were produced previously (28). Each of the Mab could be classified as binding to one of the epitopes defined by the six ^{125}I labeled Mab and those that bound to the same epitope gave mean cross-inhibitions of >94%. Interestingly, one Mab that showed weak binding to *Der p* I and *Der f* I (Nhp-6; Table 1) did not fit this classification and inhibited binding of both 5H8 C12 to *Der p* I (91%) and 6A8 to *Der f* I (81%). A summary of the epitope specificity of 53 Mab raised against either *Der p* I or *Der f* I, including, all those from previous studies (28, 30), is shown in Table III. The majority of the *Der p* I-specific Mab had the same specificity as 5H8 C12 (24/35, 70%) and all 16 of the *Der f* I-specific Mab reacted with the same epitope as 6A8.

Cross-inhibition experiments using human IgE antibodies. The epitope specificity of human IgE antibodies was investigated using mouse Mab and rabbit anti-P_1 Eq

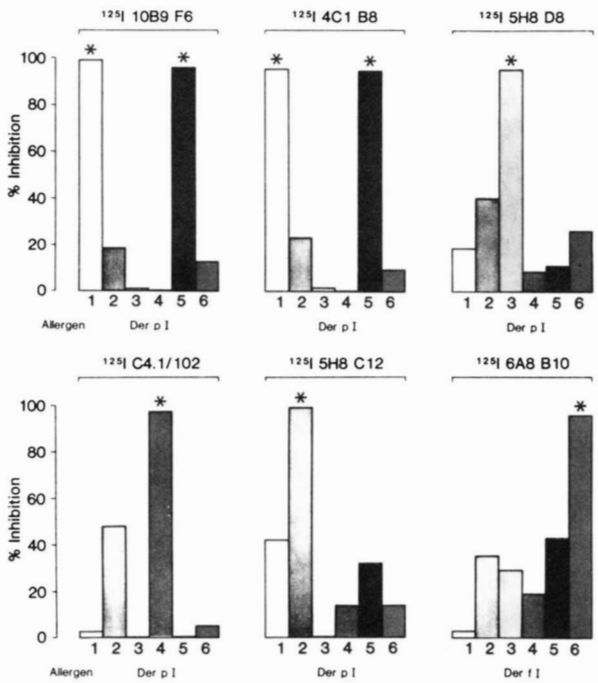

Figure 1. Cross-inhibition of the binding of ^{125}I-labeled Mab to *Der p* I or *Der f* I. Binding of 2 to 5 ng of each ^{125}I-labeled Mab was inhibited using 500 ng of 10B9 (*Bar 1*), 5H8 C12 (*Bar 2*), 5H8 D8 (*Bar 3*), C4.1 (*Bar 4*), 4C1 (*Bar 5*) or 6A8 (*Bar 6*). The results are shown as percent inhibition and Mab giving >90% inhibition are indicated (*). Nonspecific inhibition by Mab directed against different epitopes was generally <20%. However, when the cold Mab was the same as the "presenting" Mab on the solid phase (usually 5H8 C12), nonspecific inhibition was higher (suggesting limited direct competition between the free and bound Mab for the same site on the unlabeled allergen).

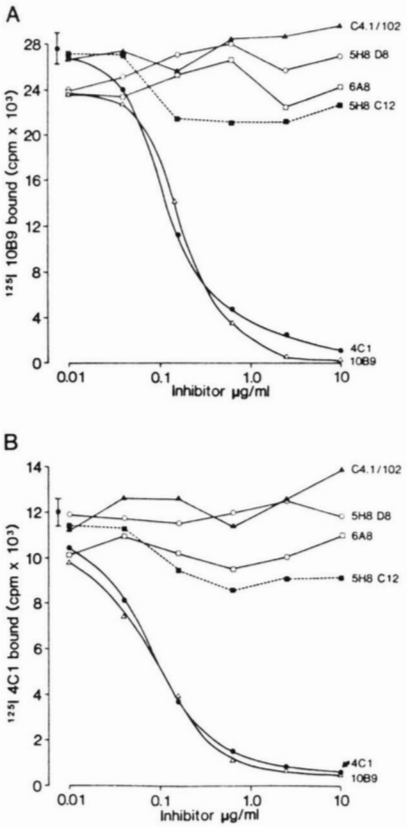

Figure 2. Comparison of cross-inhibition curves using different concentrations of cold Mab to inhibit binding of either ^{125}10B9 (*Panel A*) or ^{125}4C1 (*Panel B*) to *Der p* I. Serial fourfold dilutions (from 10 µg/ml to 0.01 µg/ml) of cold C4.1 (▲), 5H8 D8 (○), 6A8 (□), 5H8 C12 (■), 4C1 (●), or 10B9 (△) were used to competitively inhibit binding of 2 to 5 ng of each radiolabeled Mab to *Der p* I. Uninhibited binding, determined by incubating eight wells with PBS-T instead of cold Mab was 27,700 ± 1,526 for ^{125}I 10B9 and 12,019 ± 613 for ^{125}I 4C1. Total radioactivity added = 125,000 cpm (10B9) and 136,700 cpm (4C1).

TABLE II

Cross-inhibition of the binding of ^{125}I-labeled Mab to either Der p I or Der f I: mean data for 25 clones[a]

Group Specificity of Mab[b]	Percent Inhibition of ^{125}I Mab binding to					
	Der p I					*Der f* I
	10B9	5H8 C12	5H8 D8	C4.1	4C1	6A8
10B9 (n = 3)	*98%*	48%	−1%	−23%	*96%*	17%
5H8 C12 (n = 10)	33%	*97%*	36%	50%	35%	26%
5H8 D8 (n = 1)	15%	25%	*94%*	19%	13%	30%
C4.1 (n = 5)	12%	26%	5%	*94%*	12%	20%
4C1 (n = 1)	*96%*	52%	10%	2%	*95%*	43%
6A8 (n = 5)	19%	25%	21%	38%	20%	*96%*
Control Tc-8G2[c]	26%	27%	28%	31%	31%	30%

[a] Binding of 2 to 5 ng ^{125}I Mab to either *Der p* I or *Der f* I was inhibited using 500 ng of different cold Mab (either IEF purified or 50% SAS cut of ascites), and the results are expressed as the mean percent inhibition for each group of Mab tested. Uninhibited PBS control values (in cpm) for each ^{125}I Mab were as follows: 10B9, 17,525; 5H8 C12, 10,397; 5H8 D8, 4,124; C4.1, 10,802; 4C1, 9580; and 6A8, 3733. Mab that gave >90% inhibition of binding of ^{125}I Mab to either *Der p* I or *Der f* I are shown in italics.

[b] Mab with the same epitope specificity were grouped and the number (n) of Mab in each group is shown in parenthesis. Clones with the same specificity as the ^{125}I Mab were as follows: *10B9* – 10B9, 10H2 C6, Nhp-4; *5H8 C12* – 5H8 C12, 3H3 G8, CLB-P$_1$, Nhp-2, Nhp-3, HY3/90, C3.6/104, A2.3/79, C3.6/62; *C4.1* – C4.1/102, Nhp-5, C4.1/96, B2.1/81, C4.1/40; *6A8* – 6A8, 3E2 F12, 5B5 D8, 5G5 A4, and 6A9 D7.

[c] Control for nonspecific inhibition, Mab directed against a *T. cruzi* antigen (35).

TABLE III

Summary of epitope specificity of 53 murine Mab raised against either Der p I or Der f I[a]

Group Specificity of Mab	Immunogen	Frequency in Mab Tested[b]	Specificity in Antigen Binding RIA
10B9		4/35	*Der p* I
5H8 C12	*Der p* I	24/35	*Der p* I
5H8 D8		1/35	*Der p* I
C4.1		5/35	*Der p* I
6A8		16/18	*Der f* I
4C1	*Der f* I	2/18	*Der p* I and *Der f* I

[a] Mab (35) were raised from five fusions against *Der p* I, and 18 Mab were obtained from a single fusion against *Der f* I. The 53 Mab include some of those previously reported (28, 30).

[b] Mab (34/35) could be assigned to one of the four *Der p* I specific epitopes; however, Nhp-6 (Table I) (31), which also showed weak binding to *Der f* I, could not be assigned to either group.

to inhibit the binding of IgE antibody to solid phase *Der p* I. These assays were carried out using 17 individual sera and two serum pools from mite allergic patients. The results for five selected sera and the mean data for the 19 sera are shown in Figure 3. Rabbit anti-P$_1$ Eq gave consistently high inhibition of human IgE binding to *Der p* I (79.5% ± 7.7) with all sera tested. Two Mab, 10B9 and 4C1, also showed significant but incomplete inhibition (38% ± 12) for both Mab), whereas Mab directed against species-specific epitopes generally showed <20% inhibition, and only one species-specific Mab (C4.1) showed significantly greater inhibition than the *Der f* I-specific Mab used as a negative control (Fig. 3). Using a paired Student *t*-test, inhibition by rabbit anti-P$_1$ Eq was significantly greater than for either 10B9 ($p < 0.001$) or 4C1 ($p < 0.001$), and inhibition by both of these Mab was significantly greater than for the other four Mab tested (Fig. 3). In these experiments, the quantities of antibody required to give maximum inhibition were very high (50 μg) when compared with the cross-inhibition experiments using murine Mab (compare Figs. 2 and 3). Three dose response curves using rabbit anti-P$_1$ Eq and 4C1 to inhibit IgE antibody binding to *Der p* I are shown in Figure 4.

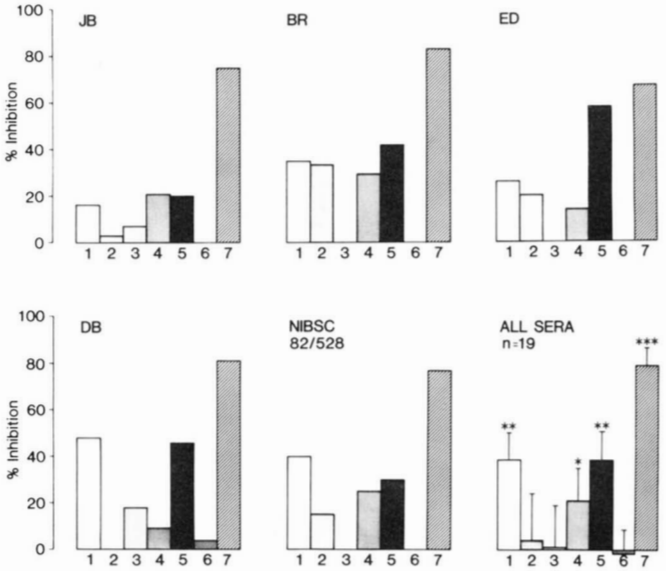

Figure 3. Inhibition of the binding of human IgE antibodies to *Der p* I by either murine Mab or rabbit anti-P$_1$ Eq. Binding of IgE antibodies in 19 sera from mite allergic patients (diluted 1/2 to 1/10) was inhibited by 50 μg 10B9 (*Bar 1*), 5H8 D8 (*Bar 2*), 5H8 C12 (*Bar 3*), C4.1 (*Bar 4*), 4C1 (*Bar 5*), 6A8 (*Bar 6*) or by 50 μg anti-P$_1$ Eq (*Bar 7*). With the exception of C4.1 and 6A8, the Mab had been purified by IEF; C4.1 and 6A8 were 50% ammonium sulfate fractions of ascites. The results are expressed as percent inhibition of ^{125}I goat anti-IgE. Representative results for five sera; JB, BR, ED, DB, and the mite allergic serum pool NIBSC 82/518 are shown. The quantity of ^{125}I anti-IgE bound by these sera in the absence of inhibitor (four PBS-T controls) was as follows: JB, 9,346 cpm; BR, 9,400 cpm; ED, 2,290 cpm; DB, 8,122 cpm, and NIBSC 82/518, 4,873 cpm (total ^{125}I anti-IgE added = 92,000 cpm). The mean data and SD for all 19 sera tested is also shown. Antibodies that gave significant inhibition (i.e., $p < 0.001$) when compared by Student's *t*-test with the *Der f* I-specific negative control, 6A8, are indicated by asterisks (•). Inhibition by both 10B9 (mean 38.7% ± 11.7) and 4C1 (38.8% ± 12.0) was significantly greater than that of C4.1 (20.8% ± 14.7) (••; $p = 0.004$); and inhibition by anti-P$_1$ Eq (79.5% ± 7.7) was significantly greater than that of any of the Mab (•••; $p < 0.001$). In contrast, affinity-purified rabbit anti-κ chain antibody showed no significant inhibition (9.3% ± 11.0).

DISCUSSION

The results confirm previous observations that BALB/c mice produce predominantly species-specific antibody responses to *Der p* I and *Der f* I (28, 30, 31). We have now analyzed 53 Mab raised from six fusions and only 2/53 bind equally to both allergens. Thus although *Der p* I and *Der f* I share physicochemical properties and show immunologic cross-reactivity in both rabbits and mite allergic humans (28, 29), they are recognized as almost completely different proteins by BALB/c mice. These results contrast with those obtained using Mab raised against the major rye grass pollen allergen, *Lol p* I, where only 12% were species specific (36, 37).

The cross-inhibition experiments show that murine Mab recognize at least four nonrepeated, nonoverlapping, species-specific epitopes on *Der p* I; a single species-specific epitope on *Der f* I; and a single common epitope on both molecules. The majority of the *Der p* I specific

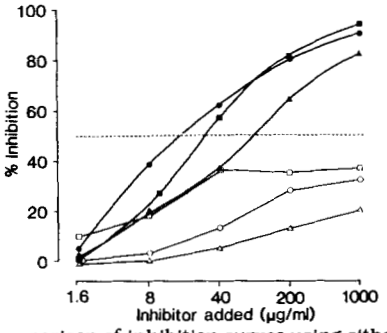

Figure 4. Comparison of inhibition curves using either 4C1 or anti-P_1 Eq to inhibit human IgE binding to *Der p* I. Serial fivefold dilutions of 4C1 (*open symbols*) or anti-P_1 Eq (*closed symbols*) from 1,000 to 1.6 μg/ml, were used to inhibit IgE antibodies in sera from three mite allergic patients: MK (*circles*), BR (*squares*), or NM (*triangles*). The results are expressed as percent inhibition of ^{125}I anti-IgE. Uninhibited binding (four PBS-T controls) was as follows: MK, 9,461 cpm; BR, 7,742 cpm, and NM, 34,002 cpm (total ^{125}I anti-IgE added = 103,500 cpm). Using these three sera, the highest concentration of anti-P_1 Eq (1,000 μg/ml or 50 μg added) was required to give maximum inhibition (>80%) of IgE antibody binding to *Der p* I. Similar high quantities of IEF-purified 4C1 gave <40% inhibition.

Mab (70%) had the same epitope specificity, and because these Mab were derived from five separate fusions, they appear to define an immunodominant epitope for BALB/c mice. These results are analagous to those of Olsen and Klapper (38) who reported that 66% (19/29) of BALB/c anti-*Amb a* I Mab recognized the same epitope (38). The 16 *Der f* I-specific Mab also appear to define an immunodominant site on *Der f* I, and we have recently produced four more Mab directed to this site from A/J mice. Binding to the immunodominant sites on both allergens could be inhibited >80% by one Mab (Nhp-6) (31), suggesting that there may be some structural similarities between these two species-specific sites or a common epitope adjacent to them. A striking feature of the epitope mapping studies was the complete cross-inhibition shown by the *Der p* I-specific Mab, 10B9, and the cross-reacting Mab, 4C1. The only way to distinguish these two Mab is to compare them for binding to *Der f* I. The epitopes recognized by these Mab on *Der p* I either partially overlap, such that binding of one Mab completely prevents binding of the other, or lie sufficiently close to one another that binding of either Mab induces conformational changes that make the other site inaccessible.

Previous immunoabsorption experiments showed that 80 to 95% of human IgE ab was directed against cross-reacting sites on *Der p* I and *Der f* I (28). Given the species specificity of the murine antibody response, it is not surprising that most Mab did not significantly inhibit human IgE antibody binding to *Der p* I. We have shown a consistently high level of inhibition of IgE antibody binding using anti-P_1 Eq, partial inhibition using Mab which bind at or close to a cross-reacting site (i.e., 10B9 and 4C1), and no consistent inhibition by the species-specific Mab. Thus human IgE (and presumably IgG) antibodies to *Der p* I and *Der f* I appear to be directed against different epitopes to those defined by mouse Mab. These results differ from those obtained with the ragweed pollen allergen, *Amb a* I, in which the murine immunodominant and human IgE binding sites appear to be same (50 to 80% cross-inhibition) (38), but may be comparable with those recently obtained using Mab to *Lol p* I, which partially inhibited (up to 35%) human IgE binding (39). Although our results suggest that IgE antibodies recognize several different cross-reacting epitopes on *Der p* I/*Der f* I, they do not formally exclude the possibility that the IgE binding epitopes may be repeated. The fact that at least two murine Mab can bind to *Der p* I and not inhibit IgE binding suggests that special constraints would only allow for a limited number of IgE binding sites.

The profound differences between the specificity of murine IgG and human IgE antibody responses to *Der p* I and *Der f* I would conventionally be attributed to structural differences between the two molecules and genetic or immunoregulatory differences between the two hosts (40, 41). However, another factor that could influence the epitope specificity of the responses is the mode of immunization. The Mab were all derived from mice that had been immunized two to four times with 5 to 10 μg allergen in complete Freund's adjuvant. In contrast, humans inhale only nanogram quantities of mite allergen/year without adjuvant (42). It is also well established that repeated low dose (nanograms) immunization in alum is critical for the development of IgE antibody responses in mice (43). Our current hypothesis is that these different modes of immunization affect the way in which *Der p* I and *Der f* I are processed and are presented in mice and in humans. In mice immunized with complete Freund's adjuvant, extensive processing is assumed to occur, resulting in proteolytic digestion of structurally homologous regions of both allergens, whereas in humans either limited processing or a different form of processing occurs so that a conserved region is presented to T cells. This hypothesis clearly needs to be tested to see whether changes in immunization regimes and adjuvants that are known to affect isotype expression can also influence the epitope specificity of murine antibody responses.

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