

IDENTIFICATION OF ALLERGENIC EPITOPES ON *Der f I*, A MAJOR ALLERGEN OF *DERMATOPHAGOIDES FARINAE*, USING MONOCLONAL ANTIBODIES

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Abstract—The antigenic and allergenic structure of *Der f I*, a major allergen of the house dust mite *Dermatophagoides farinae* (Df) was investigated by means of a panel of 11 selected monoclonal antibodies (mAb) obtained from BALB/c mice immunized with purified *Der f I*.

The species specificity of these mAb, tested with *Der f I* and *Der p I*—the homologous allergen from *Dermatophagoides pteronyssinus*—was generally restricted to *Der f I* since 10 out of 11 mAb reacted only with this allergen. Epitope specificity of the mAb was determined by both competitive inhibition and sandwich ELISA experiments. The results indicated the presence of at least four non-overlapping, non-repeated antigenic sites on *Der f I*, which were recognized by one or several mAb (sites A, B, C and D).

Comparative epitope specificity studies between human IgE antibodies and mice mAb were performed, on sera and basophils of Df sensitive patients, using different inhibition assays (ELISA and histamine release experiments). The degree of inhibition varied between the patients and upon the assay design. Most of the mAb tested were found to significantly inhibit the binding of human IgE to *Der f I* ($p < 0.01$) when compared with *Der p I* specific mAb as a control. The mAb reacting with site A was found to be the most potent inhibitor, presenting a mean inhibition of up to 56% in ELISA as well as in histamine release experiments.

The results show that both human IgE antibodies and mAb can be directed against identical or closely related epitopes of *Der f I*. Therefore anti-*Der f I* mAb constitute immunologic probes in further allergenic epitope and peptide analysis of this major mite allergen.

INTRODUCTION

Nowadays many allergens or IgE-binding antigens, are well defined molecules. Allergens from mites of the *Dermatophagoides* genus, especially *Dermatophagoides farinae* (Df) and *Dermatophagoides pteronyssinus* (Dp) house dust mites, have been extensively studied because they frequently induce IgE mediated hypersensitivity in individuals allergic to house dust (Voorhorst *et al.*, 1964; Maunsell *et al.*, 1968; Pauli *et al.*, 1972). Major and minor allergens have been identified from complex allergenic extracts of Dp and Df (Le Mao *et al.*, 1983; Lind and Lowenstein, 1983; Baldo *et al.*, 1989). Some of them have been purified and characterized, such as two major allergens, *Der f I* from Df (Dandeu *et al.*, 1982; Lind, 1986; Heymann *et al.*, 1986) and *Der p I* from Dp (Chapman and Platts-Mills, 1980; Stewart, 1982; Lind, 1985).

These major allergenic molecules are similar monomeric glycoproteins, as judged by their mol. wt (24,000–27,000) and their amino-acid composition. Their *N*-terminal amino-acid sequences show 70–77% homology (Lind *et al.*, 1988) and immunochemical studies, based on rabbit or man polyclonal antibodies,

suggest the presence of common and specific epitopes on these allergenic molecules (Le Mao *et al.*, 1983; Heymann *et al.*, 1986; Lind *et al.*, 1987).

Further studies on the antigenic and allergenic structure of *Der f I* and *Der p I* were undertaken using murine monoclonal antibodies (mAb), and more recently by recombinant DNA technology and overlapping peptides strategy. Five non-overlapping antigenic sites have been defined on *Der p I* using mAb (Chapman *et al.*, 1987; Horn and Lind, 1987). The availability of the complete amino-acid sequence of *Der p I* (Chua *et al.*, 1988) and the synthesis of a complete set of overlapping octapeptides from this molecule gave preliminary data suggesting that the epitopes are surface located and are recognized by both IgG and IgE isotypes from mite allergic individuals, although the existence of isotype-specific epitopes has also been demonstrated (Stewart *et al.*, 1989). Less information is available on *Der f I*. Using mAb against *Der f I* (Chapman *et al.*, 1987) or against Df extract (Ley *et al.*, 1986), only one species-specific antigenic epitope and one allergenic epitope cross reacting with *Der p I* have been identified on the *Der f I* molecule.

In the present report, we further investigated the epitope structure of *Der f I* using mAb as precise probes for mapping the antigenic determinants in proteins. We

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have produced and characterized a panel of monoclonal antibodies against purified *Der f I*. The epitope specificities of these mAb allowed to define four non-overlapping antigenic sites on *Der f I*. To ascertain whether these different sites were also recognized by human IgE antibodies, different inhibition assays were performed between mAb and human IgE antibodies using either sera or basophils from mite sensitive patients.

MATERIALS AND METHODS

Antigens

Der f I was isolated and purified from Df 80d, a partially purified extract of *Dermatophagoides farinae* mite cultures using a combination of ammonium sulphate precipitation and ion exchange chromatography as previously described (Dandeu *et al.*, 1982).

Der p I was obtained from Dp 80d, which was extracted as previously described from *Dermatophagoides pteronyssinus* mite cultures (Le Mao *et al.*, 1981). Dp 80d contains 5% (wt/wt) of *Der p I* as determined in comparison with the international preparation reference of Dp (standard NIBSC 82/518) (Ford *et al.*, 1985) using RAST inhibition and immunoelectrophoretic assays (Le Mao *et al.*, 1989).

Monoclonal antibodies (mAb)

Nine mAb produced in our laboratories were prepared as follows: female BALB/c mice were immunized subcutaneously and in foot pads with 10 µg of purified *Der f I* emulsified in complete Freund's adjuvant. Booster injections were administered on days 10 and 20 with the same dose of *Der f I* in incomplete Freund's adjuvant. Prior to hybridization, one of these mice was injected with 10 µg of *Der f I* in phosphate-saline buffer (PBS) and 3 days later spleen cells from the immunized mouse were fused with non-secreting Ag8X 63.653 myeloma cells in the presence of polyethylene glycol according to Köhler and Milstein (1975). Hybridomas were selected in hypoxanthine and azaserine medium. Supernatant fluids were assayed for anti-*Der f I* antibody production by direct ELISA. The hybrid cells producing antibodies were cloned by limiting dilution and selected clones were expanded. Ascitic fluids were purified by precipitation with 40% saturated ammonium sulphate and ion exchange chromatography on DEAE-Trisacryl M column (IBF, France). The IgG fraction was eluted at isocratic elution step of ionic strength.

Two mAb against *Der f I* (4C1B8, 6A8B10) and one against *Der p I* (10B9F6) were gifts from Dr M. Chapman, Division of Allergy and Clinical Immunology, University of Virginia. They were freeze-dried, and the dialysed 50% ammonium sulphate fraction of ascitic fluids was reconstituted at 10 mg/ml protein concn (as determined by absorbance, O.D. at 280 nm).

Human sera and leucocytes

Sera and the corresponding leucocytes were obtained from patients selected according to the following criteria:

allergic asthma and perennial rhinitis as clinical symptoms, positive skin tests to house dust mite extracts and specific IgE antibodies for *Der f I* as determined by RAST with *Der f I* coated discs (bound radioactivity >20% of total input). Two sera pools were prepared, one from 14 and the other from 100 sera of non-hyposensitized mite allergic patients followed at the Hospices Civils (Strasbourg, France) and at the Pasteur Institute Hospital (Paris, France).

Biotin labelling

Purified mAb and *Der f I* allergen were biotinylated with the Enzotag Biotinylation Kit according to the manufacturer's instructions (Enzo Biochem Inc., NY, U.S.A.).

Enzyme-linked immunoassays (ELISA)

Several ELISA were used and the general procedure of Voller *et al.* (1976) was followed with slight modifications concerning the saturation of binding sites, here performed with 0.5% gelatine in phosphate-buffer saline (0.01 M, pH 7.4).

(1) *Screening hybridoma cultures, determination of the subclass and species specificity of mAb using a direct ELISA.* Briefly, microplates coated with *Der f I* (2 µg/ml) were incubated with appropriate dilutions of hybridoma culture supernatants, ascitic fluids, or purified mAb. After extensive washing, peroxidase-labelled antibodies specific for mouse IgG (H + L) (Institut Pasteur Production, Marnes, France) or mouse IgG1, IgG2a, IgG2b, IgG3 and IgM (ICN Miles, U.S.A.) were added (1/1000). Bound labelled antibodies were revealed by addition of 100 µl of ortho-phenylene diamine (4 mg/ml) in citrate buffer (pH 5, 0.05 M) containing H₂O₂. After 15 min, the reaction was stopped by addition of 3 N HCl and the absorbance read at 492 nm. For the determination of species specificity of mAb, mixtures of increasing *Der f I* or *Der p I* doses (range 0.01–1000 µg/ml) and mAb were added to *Der f I* coated wells. Bound mAb were detected as described above. The concns of allergen inducing 50% of inhibition were used for the determination of species specificity.

(2) *Determination of mAb's epitope specificity.* (a) In the competitive inhibition ELISA, microplates were coated with *Der f I* and incubated with biotinylated mAb (8–12.5 ng/well) alone or in the presence of either homologous or heterologous unlabelled mAb used as inhibitors at final concns ranging from 0.001 to 1 mg/well. Bound biotinylated mAb were revealed by addition of alkaline phosphatase conjugated to streptavidin (1/10,000) (Amersham, U.K.). The enzyme activity was determined by adding *p*-nitrophenyl phosphate (1 mg/ml) in diethanolamine buffer. The absorbance was read at 405 nm 20–30 min later. The results were expressed as a percentage of inhibition calculated as follows: 100 × (absorbance in absence of inhibitor—absorbance in presence of inhibitor)/absorbance in absence of inhibitor.

(b) In sandwich ELISA, microtiter plate wells were first coated with the mAb and then *Der f I* (100 ng/ml) was incubated with the solid-phase mAb. In preliminary experiments, this concn of allergen was able to saturate the available antibody combining sites. After removal of unbound allergen, the second biotinylated mAb was added (8–12.5 ng/well) and its binding to *Der f I* was determined as described above. For each fixed mAb, the combination of mAb giving the highest O.D. was chosen as determining the 100% reference. The other combinations were compared to this reference and the results were expressed as percentages of the maximal response.

(3) *Comparison between human circulating IgE and mAb specificity.* (a) In the IgE competition ELISA 1, microtiter plate wells coated with *Der f I* were incubated with the appropriate dilution of either individual or pooled sera alone or in the presence of mAb (500 ng/well). Monoclonal antibodies concns were sufficient to saturate the available binding sites on *Der f I*. The sera dilutions were chosen as those eliciting 50% of maximum IgE binding to solid-phase *Der f I*. The amount of IgE antibodies bound to solid-phase *Der f I* was measured with an acetyl-cholinesterase labelled monoclonal anti-human IgE (Stallergene S.A., France) and the enzyme reaction was elicited by addition of 5-5'-di-thio-bis-2-nitro-benzoic acid in phosphate buffer (0.01 M, pH 7.4). Absorbance was read at 412 nm after 1 hr and the inhibition percentage was calculated.

(b) In the IgE competition ELISA 2, IgE antibodies from individual or pooled sera (diluted 3–7-fold) were immobilized onto microtiter plate wells precoated with 40 µg/ml of Xb616 anti-IgE mAb (Bourgeois *et al.*, 1984), specific for the Fc ϵ chain. The immunoplates were incubated with biotinylated *Der f I* alone (10 ng/well) or with a mixture of labelled *Der f I* and mAb inhibitors (500 ng/well). The 10B9F6 mAb specific of *Der p I* was used as a negative control. Bound *Der f I* was revealed by the addition of streptavidin alkaline phosphatase as described above.

Histamine release from human leucocytes

Histamine release experiments were performed on washed blood leucocytes (May *et al.*, 1970). The allergen dilutions were preincubated for 90 min at room temp with serial 10-fold dilutions (10^{-2} –1 mg/ml) of the *Der f I*-specific monoclonal antibodies, or with buffer or an unrelated serotonin-specific mAb as control experiments. The cells suspended in 0.8 ml Tris–Ca–Mg–albumin buffer were added to 3-fold dilutions of the *Der f I* allergen. These concns were selected as they induce an increasing dose–response histamine release for most of the mite sensitive patients. After a 30 min incubation period at 37°C under shaking conditions, the reaction was stopped by addition of 0.1 ml of cold EDTA 125 mM, pH 7.4. All experiments were performed in duplicate, with the total and spontaneous histamine release (SHR) incubations made in triplicate. The histamine content was quantified by an automated fluorometric method (Lebel, 1983) and the results were expressed as the percentage of the total histamine con-

tent as follows: $100(a - b)/(c - b)$, where a = mean of the fluorometric readings for experimental supernatant, b = mean of blanks (SHR) and c = mean of total histamine. The mean coefficients of variation for histamine determinations were 6.2% for SHR and 3.5% for total histamine measurements (Weyer *et al.*, 1990). Inhibition of histamine release by mAb for a given allergen concn was calculated as follows: percent inhibition = $100\% - (\% \text{ histamine release in presence of mAb} / \% \text{ histamine release in absence of mAb})$.

RESULTS

Characterization of anti-*Der f I* mAb

The monoclonal antibodies were obtained using the purified *Der f I* as immunogen in two cell fusion experiments. In the fusion carried out in our laboratory, 244 wells were screened for antibody production using direct ELISA. Among 40 hybrids reacting with *Der f I*, 13 giving the most positive responses in ELISA were cloned. Nine stable clones were obtained (MAF1–MAF 9) and further characterized. Propagated in ascites, they produced antibodies of IgG 1 isotype whose specificity was restricted to *Der f I*, as tested by competitive antigen inhibition assays (Table 1). Two other mAb, 6A8B10 and 4C1B8 derived from a fusion carried out in another laboratory (gift from Dr Chapman) presented the same IgG isotype but only 4C1B8 reacted with *Der f I* and with *Der p I*, as described by Heyman *et al.* (1986).

Epitope specificity of the 11 mAb

To determine if any two mAb bind different, identical or overlapping parts of the antigen, we carried out two different ELISA techniques.

First, binding inhibition of a given biotinylated mAb to solid-phase bound *Der f I* by unlabelled mAb was

Table 1. Characteristics of anti-*Der f I* monoclonal antibodies

mAb	Isotype	Species specificity ^a	
		<i>Der f I</i>	<i>Der p I</i>
MAF 1	IgG 1	0.35 ^b	— ^c
MAF 2	IgG 1	0.32	—
MAF 3	IgG 1	0.30	—
MAF 4	IgG 1	0.42	—
MAF 5	IgG 1	0.16	—
MAF 6	IgG 1	0.21	—
MAF 7	IgG 1	0.14	—
MAF 8	IgG 1	0.15	—
MAF 9	IgG 1	0.36	—

^aBy competitive antigen inhibition ELISA: *Der f I* was immobilized on immunoplates and incubated with a mixture of increasing concn of *Der f I* or *Der p I* and mAb. Bound mAb were detected by adding labelled sheep anti-mouse IgG antibodies.

^bConcentration of allergen in µg/ml corresponding to 50% inhibition.

^cThe sign — refers to 50% inhibition doses of allergen > 1000 µg/ml.

Table 2. Competitive inhibition of biotinylated mAb to solid-phase *DerfI* by unlabelled mAb (% inhibition)^a

Inhibitors	Labelled mAb									
	MAF 1	MAF 2	MAF 3	MAF 4	MAF 5	MAF 6	MAF 7	MAF 8	MAF 9	4C1B8
MAF 1	97	98	99	95	—	—	—	—	—	—
MAF 2	92	93	92	91	—	—	—	—	—	—
MAF 3	98	91	88	95	—	—	—	—	—	—
MAF 4	92	91	88	95	—	—	—	—	—	—
MAF 5	—	—	—	—	96	100	91	—	—	—
MAF 6	—	—	—	—	83	91	99	—	—	—
MAF 7	—	—	—	—	90	95	100	—	—	—
MAF 8	—	—	—	—	—	—	—	99	34	—
MAF 9	—	—	—	—	—	—	—	90	90	—
4C1B8	—	—	—	—	—	—	—	—	—	100
6A8B10	—	—	—	—	—	—	—	—	—	—
Control 10B9F6	—	—	—	—	—	—	—	—	—	—

^aBinding of biotinylated mAb (8–12.5 ng/well) to immobilized *DerfI* (200 ng/well) was inhibited by unlabelled mAb used at 1 µg/well. The sign — indicates that the inhibition was lower than 15%.

performed. Results of mutual inhibitions are reported in Table 2. Homologous inhibitions (same labelled and unlabelled mAb) exceeded 88%, the percentage of inhibition remaining lower than 15% with the control 10B9F6 mAb (specific for anti-*DerpI*). Three groups of mAb (MAF 1–4), (MAF 5–7) and (MAF 8–9) were found to block mutual binding, indicating that within each group a mAb recognized the same or closely related epitopes. On the contrary, mAb of each group and the 6A8B10 and 4C1B8 mAb did not display any cross inhibition, suggesting binding to distinct epitopes.

In order to complete these results, a double binding solid-phase ELISA experiment was performed. *DerfI* molecules were allowed to react with polystyrene-bound mAb and then either with homologous or heterologous labelled mAb. Results are shown in Table 3. The binding of labelled mAb to *DerfI* was not significant when the allergen was presented by the homologous immobilized mAb. This observation suggests that the corresponding epitope exists only once on each *DerfI* molecule. On the other hand, the pairs of mAb (MAF 1–4), (MAF 5–7) and (MAF 8–9) were not able to react simultaneously with *DerfI* involving

binding to the same or close epitopes. The 6A8B10 mAb displayed a weak binding to either (MAF 1–4) and (MAF 8–9) suggesting that these mAb recognized close epitopes. In contrast, the other combinations of mAb were able to react simultaneously to *DerfI*, clearly indicating that the antibodies bind to the antigen at distinct sites.

In accordance with the results of both ELISA techniques, the 11 mAb could be divided into four groups which defined four non-overlapping, non-repeated antigenic sites on *DerfI*: sites A, B, C and D reacting, respectively, with 4C1B8, MAF 1–4, MAF 5–7 and MAF 8–9 mAb.

Binding inhibition of human IgE by mAb

In order to assess if the antigenic sites of *DerfI* as defined by mAb are also recognized by human IgE, we performed three different inhibition assays between six mAb representative of the different antigenic sites and human IgE from sera or basophils of mite sensitive patients.

In the IgE competition ELISA 1, we tested the capacity of mAb to inhibit the binding of circulating IgE to *DerfI* fixed on solid phase while in the IgE

Table 3. Binding combination of various anti-*DerfI* mAb in a sandwich ELISA (% binding)^a

Fixed mAb	Labelled mAb									
	MAF 1	MAF 2	MAF 3	MAF 4	MAF 5	MAF 6	MAF 7	MAF 8	MAF 9	4C1B8
MAF 1	4	4	3	3	+	+	+	+	+	+
MAF 2	5	6	7	5	+	+	+	+	+	+
MAF 3	5	4	3	6	+	+	+	+	+	+
MAF 4	6	5	5	7	+	+	+	+	+	+
MAF 5	+	+	+	+	9	9	6	+	+	+
MAF 6	+	+	+	+	7	8	5	+	+	+
MAF 7	+	+	+	+	8	5	9	+	+	+
MAF 8	+	+	+	+	+	+	+	5	15	+
MAF 9	+	+	+	+	+	+	+	3	5	+
4C1B8	+	+	+	+	+	+	+	+	+	6
6A8B10	28.5	26.4	27	31	+	+	+	18	22	+

^aBinding of biotinylated mAb (8–12.5 ng/well) to *DerfI* (10 ng/well) presented by immobilized mAb (1 µg/well) was expressed as percentage of maximal response obtained for the pair which give maximal O.D. (0.80–1.50) for a fixed mAb. The sign + indicates that the binding was superior to 80%.

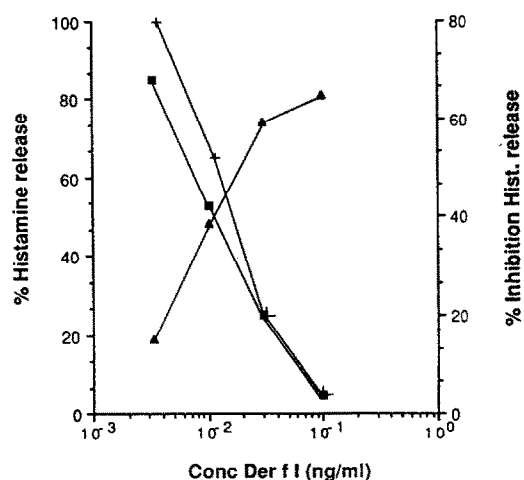


Fig. 1. Percent histamine release (▲) with increasing concns of *Der f I*. Inhibition of the antigen induced histamine release following preincubation of the allergen with the 4C1B8 (■) and MAF 6 (+) monoclonal antibodies. No inhibition was observed when preincubations were performed in the presence of buffer or an unrelated (serotonin-specific) monoclonal antibody.

competition ELISA 2, we investigated whether the mAb could block the binding of *Der f I* in its conjugated form to immobilized circulating IgE.

In the third inhibition assay, the residual histamine releasing activity of *Der f I* on basophils from mite sensitive patients was studied after preincubation of the allergen with the mAb.

Figure 1 shows the inhibition of the histamine release after preincubation of the *Der f I* allergen with the MAF 6 or 4C1B8 monoclonal antibodies. These mAb alone did not induce any histamine release. A preincubation in the presence of unrelated mAb did not inhibit the extent of the mediator release. The inhibition was highly dependent on the concn of *Der f I* used in the incubation. For the allergen dose inducing less than 50% of the maximal value of histamine release in a given patient, the inhibition was highly significant, whereas at concentrations eliciting a histamine release close to maximum no inhibition was observed.

Representative results of the comparative assays are shown in Fig. 2. For a given mAb, the degree of inhibition varied between the patients and upon the assay design [Figs 2(A) and 2(B)]. The mean inhibition for all patients tested is illustrated in Fig. 2(C). Except for 6A8B10, the mAb were found to inhibit significantly the binding of human IgE to *Der f I*. The significance of the mean inhibition ($p < 0.01$) was analysed using Student's *t*-test as compared to 10B9F6 specific anti-*Der p I* mAb as a negative control. Higher inhibitions were observed in IgE competition ELISA 2 and in histamine release experiments, than in IgE competition ELISA 1.

DISCUSSION

This work presents results on the epitope mapping of *Der f I*, identified as a major allergen of the *Dermato-*

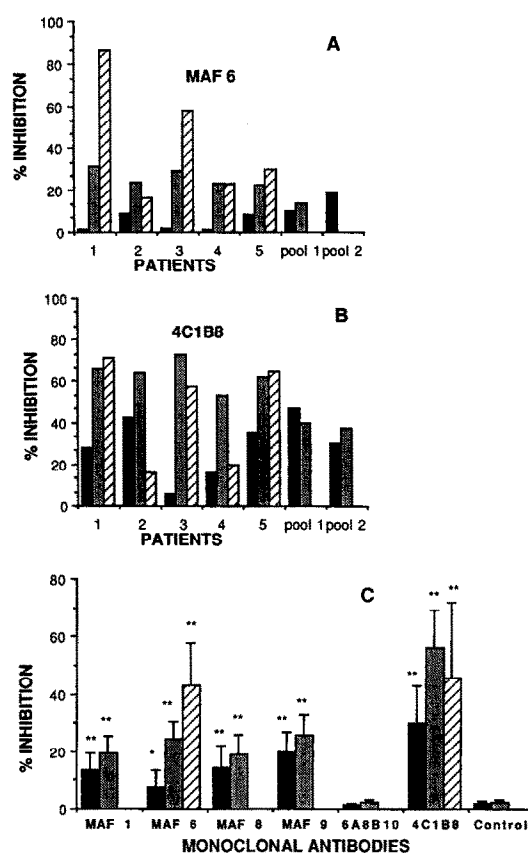


Fig. 2. Binding inhibition of human IgE antibodies to *Der f I* by mAb using three different assays: inhibition of IgE antibodies binding to *Der f I* coated by mAb (black bars), inhibition of labelled *Der f I* binding to immobilized IgE antibodies by mAb (stippled bars), inhibition of histamine releasing activity of *Der f I* after preincubation of the allergen with mAb (hatched bars). (A) Results with the MAF 6 mAb. (B) Results with the 4C1B8 mAb. (C) Mean inhibitions, SEM and significance of mean inhibition by Student's *t*-test. * $p < 0.01$, ** $p < 0.001$ (black bars $n = 8$, stippled bars $n = 7$, hatched bars $n = 5$).

phagoides species (Le Mao *et al.*, 1983), and also on the inhibition of the IgE-dependent biological activity of these epitopes by monoclonal antibodies.

Epitope mapping

Among the 11 murine monoclonal antibodies tested for species specificity with *Der p I* and *Der f I*, only the 4C1B8 mAb recognized both allergenic molecules, indicating an antigenic cross-reactivity between *Der p I* and *Der f I* allergens. Chapman *et al.* (1987) also reported that after immunization of mice with each of the allergenic molecules mainly species-specific monoclonal antibodies occurred when positive cell lines were screened with *Der p I* and *Der f I* allergens.

In this study, two ELISA procedures, a direct competition assay and a sandwich assay, were used to investigate whether mAb bind to structurally distinct or closely related epitopes at the surface of *Der f I*. Both methods showed effective competition between the nine mAb obtained in our laboratory (Tables 2 and 3). Three groups of mAb can be defined within which 4, 3 and 2 mAb, respectively, cross-inhibited each other. Within

each group the mAb reacted with identical or overlapping or nearby epitopes thus delineating an antibody binding site. So, three antigenic regions, B, C and D were defined on the surface of the *DerfI* molecule. The 4C1B8 mAb undoubtedly interacted with an antigenic determinant which was structurally independent of the other epitopes. Moreover, the results of sandwich ELISA between homologous mAb (Table 3) support the hypothesis that the defined antigenic sites are not repeated on each *DerfI* molecule.

On the other hand, the 6A8B10 mAb weakly competed with MAF 1–4 and MAF 8–9 in sandwich experiments whilst it did not show any inhibition in the direct competition. This mAb recognized either an antigenic site close to sites B and D or a structurally independent site. In the latter case the weak binding can be explained if the binding of the 6A8B10 monoclonal antibody to *DerfI* induced structural changes in the molecule which decreased the accessibility for the sites B and D epitopes. Such conformational changes in epitopes induced by antibody binding to an antigen have been previously reported (Cook *et al.*, 1985; Wilhelm *et al.*, 1988). This topographical study has led to the identification of four non-repeated, non-cross-reacting antigenic sites (A, B, C and D) on *DerfI*.

IgE binding epitopes

The binding inhibition of human IgE to *DerfI* by monoclonal antibodies was performed using three different competitive assays so as to prevent limited conclusions due to a single assay. Experimental data displayed a significant inhibition of human IgE binding to *DerfI* by most of the monoclonal antibodies [Fig. 2(C)]. These results are in agreement with those of Lind *et al.* (1988) on the *DerpI* molecule. We obtained the strongest inhibition with 4C1B8 mAb and no significant inhibition with 6A8B10 mAb. The same observation was reported by Chapman *et al.* (1987).

The inhibition studies reported here indicated that both human IgE antibodies and mAb could be directed against identical or closely related epitopes of *DerfI*, as it has also been demonstrated for antigen E, *DacgI* and *LolpI* allergens (Olson and Klapper, 1986; Esch and Klapper, 1989; Mourad *et al.*, 1988). We observed that the degree of inhibition varied with the patients, as expected by the variability of the polyclonal human IgE response. The efficiency of inhibition also depended on the assay design, for example the inhibition values obtained in the IgE competition ELISA 1 are smaller than those observed in the IgE competition ELISA 2. We can assume that certain antigenic sites were not well exposed because of their attachment to the solid matrix (Olson and Klapper, 1986).

In the third method, the biological activity of *DerfI* was measured by its capacity to induce histamine release in sensitized human basophils. Experimental procedure variations can explain the discrepancy between our data and those observed by Mourad *et al.* (1989) in a similar study on histamine release inhibition after preincubation of *LolpI* with the corresponding monoclonal anti-

bodies. The histamine release experiments presented here were performed after preincubation of mAb with an antigen concentration inducing less than the maximum percentage of histamine released in the allergen dose-response curve, while Mourad *et al.* (1989) performed incubations with mAb only at allergen doses corresponding to the plateau of histamine release. Comparing the IgE-recognizing epitope mapped by immunochemical methods and by histamine release tests, it is noteworthy that the 4C1B8 mAb was the strongest inhibitor [Fig. 2(C)]. One of the explanations for high inhibition activity of 4C1B8 mAb may be that a higher proportion of human IgE antibodies was directed against the epitope defined by this mAb.

In conclusion, the anti-*DerfI* mAb enabled us to define IgE binding epitopes on *DerfI*. These epitopes induce the human allergic responses and their further analysis could provide a better understanding of the mechanisms leading to immediate hypersensitivity states. Their identification at the molecular level would open new approaches for the treatment of allergic reactions.

In addition, the anti-*DerfI* mAb are useful tools for studying the relationship between allergenic epitope structures and immunological cell activations in mite allergy.

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