

3527 34220 32020

ISOLATION AND CHARACTERIZATION OF THE MAIN ALLERGEN OF *DERMATOPHAGOIDES* *FARINAE* BY MONOCLONAL ANTIBODIES THAT RECOGNIZE IgE RELATED EPITOPES

VICTORIA LEY,* JUAN C. SÁIZ and JOSÉ CARREIRA

Departamento de Investigación, Alergia e Inmunología, Abelló, Madrid, Spain

(Received 6 February 1986; accepted in revised form 20 May 1986)

Abstract—Mouse monoclonal antibodies (MAbs) with different specificities against *Dermatophagoides farinae* (*D. farinae*) extract have been obtained. Fifteen of these antibodies reacted with allergen molecules contained in *D. farinae* and *D. pteronyssinus* extracts, immunoprecipitating the main allergen of *D. farinae* (DF29) and homologous allergen of *D. pteronyssinus* (DP28). In addition, the monoclonal antibody MADF2 immunoprecipitated DF29 together with two other polypeptides (mol. wt 20,000 and 40,000) from *D. farinae* extracts. Five monoclonal antibodies (MADF2, MADF5, MADF9, MADF10 and MADF13) were selected to study their epitope specificity and the relationship of the epitope location on the allergen with the IgE binding site. By cross-inhibition studies two different epitopes and two partly overlapping determinants were found. In addition, two of these epitopes, those defined by MADF13 and MADF5, are close to, or overlapping, IgE binding site(s) on the allergen molecule. DF29 allergen from *D. farinae* extract was purified by affinity chromatography using MADF5 coupled to Sepharose. The purified allergen had capacity to bind mite specific human IgE and demonstrated an allergenic activity of up to 70% of total extract of *D. farinae*. These results indicate that DF29 molecule is the main allergen from *D. farinae* extracts.

INTRODUCTION

Dermatophagoides genus is the primary inducer of hypersensitivity to house dust (Voorhorst *et al.*, 1964) and one of the most important causes of allergy all over the world. During the last years, several mite allergens have been described, mainly from *Dermatophagoides farinae* (*D. farinae*) and *Dermatophagoides pteronyssinus* (*D. pteronyssinus*) species. Special attention has been focused on two of these allergens. One from *D. farinae* of mol. wt 29,000 (DF29) which bears charge heterogeneity and has been described as the most relevant allergen of this mite (Le Mao *et al.*, 1981; Dandeu *et al.*, 1982), and a second one from *D. pteronyssinus* with an apparent mol. wt of 28,000 (DP28) that seems to correspond to those previously identified as P1 (Chapman and Platts-Mills, 1980) D.pt. 12 (Stewart and Turner, 1980) and D.pt. 42 (Lind *et al.*, 1979) allergen. Both allergens, DF29 and DP28, recognized by most sera from mite sensitive patients, have been already purified by classical procedures (Dandeu *et al.*, 1982; Chapman and Platts-Mills, 1980; Stewart, 1982).

The availability of monoclonal antibodies (MAbs) to allergen components looks very promising for the

understanding of the molecular aspects of allergens. Preparation of monoclonal antibodies to mites and pollen extract components has already been described (Krilis *et al.*, 1981; Ley *et al.*, 1985b; Smart *et al.*, 1983; Corbi *et al.*, 1985b), and their application in allergen purification and standardization has been substantiated in the case of *Parietaria judaica* (Corbi *et al.*, 1985a) and *Ambrosia* pollens (Krilis *et al.*, 1983).

Recently, several MAbs to the major allergen of *D. pteronyssinus* have been produced (Chapman *et al.*, 1984). None of these MAbs reacted with the homologous allergen from *D. farinae*, in spite of the fact that mouse, rabbit and human antisera show a strong cross-reactivity between antigens from both species (Le Mao *et al.*, 1983; Chapman and Platts-Mills, 1980; Carreira *et al.*, 1984).

This paper describes the production of MAbs against the main allergen of *D. farinae* (DF29) and the purification of this allergen in native conditions. These MAbs define antigenic determinants on the allergen molecule shared with the main allergen of *D. pteronyssinus* (DP28). The cross-reactive epitopes are also recognized by antibodies present in the sera of allergic patients. Furthermore, two of the MAbs, significantly inhibited the binding of specific human IgE to mite components covalently bound to paper discs, indicating they recognize epitope(s) very close to, or overlapping, an allergenic determinant.

*Author to whom correspondence should be addressed at:
Departamento de Investigación, Alergia e Inmunología
Abelló, Julián Camarillo, 8, 28037 Madrid, Spain.

Preparation of antigens

Dermatophagoides mites were grown in our laboratory in medium containing 70% desiccated *Saccharomyces cerevisiae* and 30% human dander. Cultures were maintained at 24°C and 76% r.h. for 3–4 months and then the mites were killed by heating. Proteins were extracted from the whole culture with 50 mM ammonium bicarbonate after homogenization with a Potter–Elvehjem device and stirring for 1 hr at room temp. The soluble fraction was separated by centrifugation at 22,000 g for 1 hr at 4°C. The supernatant was extensively dialyzed against distilled water and filtered through a membrane with 0.2 µm pore (Sartorius, type SM).

Production of monoclonal antibodies

Mouse monoclonal antibodies to *D. farinae* extract were raised following the technique described by Sánchez-Madrid *et al.* (1983). Briefly, BALB/c mice were immunized with an i.p. injection of 100 µg of extract protein in complete Freund's adjuvant, and boosted 20 days later with identical dose in incomplete Freund's adjuvant. After 22 days, a mouse was injected i.v. with the same amount of antigen in phosphate-buffered saline (PBS), pH 7.4, and 3 days later spleen cells from the immunized mouse were fused with P3X63Ag8.6.5.3 myeloma cells using 50% polyethyleneglycol, and distributed in 96-well culture plates. Cells were grown in HAT medium for the selection of hybrids, and after 2 weeks, hybridoma culture supernatants were harvested for screening of anti-mite antibodies.

Positive hybrids were cloned and subcloned in soft agar (Galfre and Milstein, 1981), and then reassayed for their antigen and allergen binding abilities.

The isotype of the monoclonal antibodies was determined by immunodiffusion using rabbit anti-mouse subclass antisera (Nordic, El Toro, CA).

Screening of monoclonal antibodies

Two different radioimmunoassays were performed. In the first one, 50 µl of hybridoma culture supernatant was incubated for 1 hr at room temp with *Dermatophagoides* extract covalently coupled to paper discs. Then, paper discs were washed three times with PBS containing 0.25% of bovine serum albumin (BSA) and incubated for 1 hr with 50 µl of ¹²⁵I-labeled 187-1, a rat anti-mouse kappa chain MAb (Yelton *et al.*, 1981). Discs were washed five times with PBS-0.25% BSA, and the bound radioactivity was estimated in a gamma-counter. Hybridomas selected in this assay were screened for their allergen binding ability using a four-step radioimmunoassay previously described (Ley *et al.*, 1985a). Briefly: microtiter plates (Dynatech, Alexandria, VA) were coated with 0.5 µg of 187-1 MAb in 50 µl of PBS by 14 hr incubation at 4°C or 1 hr at 37°C. Non-specific binding sites were saturated with 150 µl of 1% BSA

in PBS, then, wells were successively incubated with 50 µl of culture supernatants, 50 µl of *D. farinae* or *D. pteronyssinus* extract at a protein concn of 1 mg/ml, 50 µl of serum pool from *Dermatophagoides* sensitive patients and 1 × 10⁵ cpm (10 ng) of ¹²⁵I-labeled anti-IgE (Pharmacia, Sweden) in 50 µl of PBS with 0.25% BSA. Each incubation was carried out for 1 hr at 37°C and followed by washing twice with PBS-0.25% BSA. Finally, wells were cut out and the radioactivity estimated.

After purification of MAb, the same assay was performed adsorbing 1 µg of each MAb to the wells, and substituting the ¹²⁵I-anti-IgE polyclonal antiserum by ¹²⁵I-HE2/1 MAb (a mouse anti-human IgE MAb) (Sánchez-Madrid *et al.*, 1984).

Radiolabeling and immunoprecipitation

Dermatophagoides extract proteins were radioiodinated by the chloramine T procedure (Hunter and Greenwood, 1962) and immunoprecipitation carried out as follows: 1 × 10⁶ cpm of ¹²⁵I-*Dermatophagoides* extract (sp. act. 50 µCi/µg) was precleared on normal rabbit IgG-Sepharose in PBS-containing 0.1% bovine hemoglobin and 0.1% Triton-X-100 in order to eliminate components with non-specific binding to the antibody-Sepharose complex, and incubated with 40 µl of each MAb coupled to Sepharose (1 mg/ml of wet gel).

After 2 hr incubation with shaking, the immuno-adsorbent was extensively washed with the PBS-hemoglobin-Triton washing solution and finally with 50 mM Tris-HCl, pH 6.8. Specifically bound proteins were eluted by boiling for 2 min in the presence of 20 µl of Laemmli's sample buffer, and analyzed by polyacrylamide gel electrophoresis in presence of sodium dodecyl sulphate (SDS-PAGE) under reducing and non-reducing conditions. The gel was dried and autoradiographed with intensifying screens (Laskey and Mills, 1977).

Purification of MAb and human IgE

IgG MAbs were purified by affinity chromatography on a protein A-Sepharose column (Boehringer Mannheim) as described previously (Ey *et al.*, 1978). IgM MAbs were purified by hydroxylapatite chromatography (Stanker *et al.*, 1985). When necessary, the purified MAbs were radiolabeled with ¹²⁵I by the Iodo-Gen method (Fraker and Speck, 1978).

Human IgE was purified from mite sensitive patients serum pool by adsorption to a monoclonal anti-human IgE antibody coupled to Sepharose. The column was packed with 50 µl bed of 1 mg/ml coupled anti-human IgE monoclonal antibody per ml Sepharose. After being washed with 2 ml of 1 M MgCl₂-0.1 M sodium citrate, pH 3, the column was equilibrated with PBS, and 4 ml of the human serum pool from mite sensitive patients was passed through. Then, the column was washed with 5 ml of PBS and a solution containing 1 mg/ml Chloramine T and 1 mCi of ¹²⁵I in 20 µl of PBS was added to the column

and mixed with the Sepharose for 1 min. The column was washed with 2 ml of PBS and the radioiodinated IgE was eluted with 2 ml of 1 M MgCl₂-0.1 M sodium citrate, pH 3. The IgE purity was assessed by SDS-PAGE under reducing conditions.

Cross-reactivity assay with MAbs

The competition radioimmunoassay between various MAbs was performed incubating 5–10-fold dilutions of each purified MAb (50 µg–5 ng protein) with mite extracts coupled to paper discs in PBS containing 0.2% BSA–0.2% Tween-20. After 1 hr incubation at room temp 1 × 10⁵ cpm (approx. 10 ng of purified MAb) of a ¹²⁵I-labeled MAb was added and incubation continued for a further hour. Finally, discs were washed exhaustively and gamma-counted.

Binding competition of ¹²⁵I-labeled IgE by the allergen molecule

To carry out the competitive experiments between MAbs and human IgE, 10-fold dilutions of hybridoma supernatants were incubated for 1 hr at room temp with *D. farinae* extracts coupled to paper discs. Then, 2 × 10⁵ cpm of ¹²⁵I-labeled purified human IgE in 30 µl of PBS containing 0.25% BSA–0.2% Tween-20 was added to each paper disc and incubated for an additional hour at room temp. Discs were washed with PBS-0.25% BSA–0.2% Tween-20, and the bound radioactivity was estimated in a gamma-counter.

In a complementary experiment patients human serum pool was used instead of purified IgE, and the inhibition rate demonstrated by adding ¹²⁵I-anti-human IgE MAb. MAb supernatants dilutions (150 µl) were incubated with paper discs for 1 hr and then 15 µl of human serum was added and incubated for 2 hr. Discs were washed and incubated with ¹²⁵I-anti-IgE MAb for 3 additional hr.

Other MAb and antisera

Monoclonal antibody HE2/1, a mouse anti-human IgE, was prepared in our laboratory, as described (Sánchez-Madrid *et al.*, 1984). The 187-1 anti-mouse kappa chain MAb was a gift from Dr M. Scharff (Albert Einstein Medical School).

Mouse antisera to *D. farinae* and *D. pteronyssinus* extracts were obtained in our laboratory. Rabbit anti-human IgE polyclonal antiserum was purchased from Pharmacia (Sweden). Human sera used throughout this work was a pool from 42 sensitive patients which demonstrated hypersensitivity to both mite species. All patients showed similar skin reactivity to *D. pteronyssinus* and *D. farinae* extracts, having high levels of specific IgE (RAST class 4, Pharmacia). No patient had received immunotherapy at the time of serum collection.

Purification of DF29 molecule and its allergenic activity

Extracts of *D. farinae* were ultracentrifuged at

100,000 g for 1 hr to remove debris and precleared on normal rabbit IgG-Sepharose by incubation with shaking for 30 min at 4°C in order to eliminate non-specific binding components. After centrifugation, a sample of the supernatant containing 1 mg of protein was applied to a MADF5-Sepharose column previously washed with the eluting buffer (20 mM glycine-1 M NaCl, pH 10) and neutralized with PBS. The column contained 2 mg of MAB coupled to Sepharose in a vol of 2 ml wet gel. After extensive washing with PBS, the bound protein was eluted with 15 ml of eluting buffer, dialyzed against distilled water and lyophilized until use.

The purified material was assayed for its ability to bind specific human IgE by RAST inhibition, basically as described by Yman *et al.* (1975). The capacity of the whole extract and the purified DF29 to compete for the specific IgE with the solid phase was analyzed in the same experiment. The solid phase contained *D. farinae* extract coupled to paper discs.

Affinity chromatography of molecules recognized by human IgE was performed on a column of 1 ml of anti-human IgE-Sepharose. Five milliliters of the serum pool was passed through the column and after washing, with PBS, the radioiodinated extract was applied. The column was then extensively washed and the proteins retained were eluted with 1 ml of the eluting buffer and subjected to SDS-PAGE and autoradiography.

RESULTS

Obtention and specificity of MAbs anti-allergen molecules

Monoclonal antibodies were obtained in a fusion with spleen cells from a mouse which had been previously immunized with the whole extract of *D. farinae*. As many as 300 culture wells presenting hybrid growth were screened for recognition of *D. farinae* components. Eighty-nine out of the 300 hybrids resulted positive in this screening, displaying binding ratios from 3- to 137-fold above background (400 cpm). Positive hybridoma (Table 1) were cloned, subcloned and assayed for their allergen binding ability using the four-step sandwich radioimmunoassay above described. A number of those showing positive binding to allergens were purified and assayed again by the direct adsorption of 1 µg of each MAb to polyvinyl chloride wells (see Materials and Methods). As shown in Table 1 15 MAbs recognized allergen molecules on both mites; although MAB were obtained against *D. farinae* extracts, they recognized almost equally well the allergen molecules from *D. pteronyssinus* extract.

Identification of allergenic polypeptides recognized by the MAbs

To identify the antigen molecules recognized by five out of the 15 selected MAbs, immunoprecipitation experiments were carried out with ¹²⁵I-labeled

Table 1. Specificity of monoclonal antibodies to *D. farinae* extracts

MAb	Dermatophagoides extracts ^a		IgE-binding components ^b	
	<i>D. farinae</i>	<i>D. pteronyssinus</i>	<i>D. farinae</i>	<i>D. pteronyssinus</i>
MADF1	4500	8000	3200 (630)	4800 (610)
MADF2	3100	2900	10,000 (705)	7000 (710)
MADF3	1900	3200	5100 (625)	3400 (840)
MADF5	40,000	10,500	16,500 (565)	14,100 (620)
MADF6	2100	1200	5400 (825)	3700 (740)
MADF7	2400	2200	4200 (475)	2800 (575)
MADF8	2500	2800	7700 (480)	6200 (595)
MADF9	13,500	15,100	8100 (520)	6200 (500)
MADF10	14,000	21,300	7300 (475)	5000 (610)
MADF11	30,800	13,700	8600 (415)	6100 (700)
MADF12	2500	2300	4900 (520)	7200 (600)
MADF13	55,000	21,000	5400 (595)	11,200 (550)
MADF14	4500	4000	11,000 (480)	13,400 (550)
MADF15	50,000	8000	7100 (600)	9300 (530)
MADF16	1200	1100	11,400 (580)	13,600 (500)
P3X63	400	400	500	550

^aValues represent binding in cpm of ¹²⁵I-anti-mouse kappa chain second antibody to the mouse MAb containing supernatants. Total radioactivity added = 1×10^5 cpm.

^bBinding in cpm of ¹²⁵I-anti-human IgE monoclonal antibody to human specific IgE against allergen molecules of mite extracts selectively retained by purified MAb (20 µg/ml) coated to polyvinyl chloride wells. Total input 1.5×10^5 cpm.

Non-specific binding was determined with P3X63 mouse myeloma culture supernatant.

Antigen specificity (data in brackets) was demonstrated by using *Parietaria judaica* extracts instead of mite extracts at the same protein concn.

extracts from both mite species. The molecular profile of polypeptides immunoprecipitated by the five MAbs is shown in Fig. 1. All of them recognized a similar polypeptide from both mites, with a mol. wt of 29,000 (DF29) and 28,000 (DP28) from *D. farinae* and *D. pteronyssinus*, respectively. These results demonstrated that the epitopes recognized by different MAbs are shared by the molecules from the two species. In addition of the 29,000 polypeptide,

MADF2 precipitated two polypeptides from *D. farinae* with apparent mol. wt of 20,000 and 40,000 (weakly visualized).

The recognized allergen of *D. farinae* (DF29) has an apparent mol. wt higher than the corresponding allergen of *D. pteronyssinus* (DP28), and both exhibited different molecular forms under reducing and non-reducing conditions (Fig. 2). The relative mobilities of DF29 and DP28 allergens were slower,

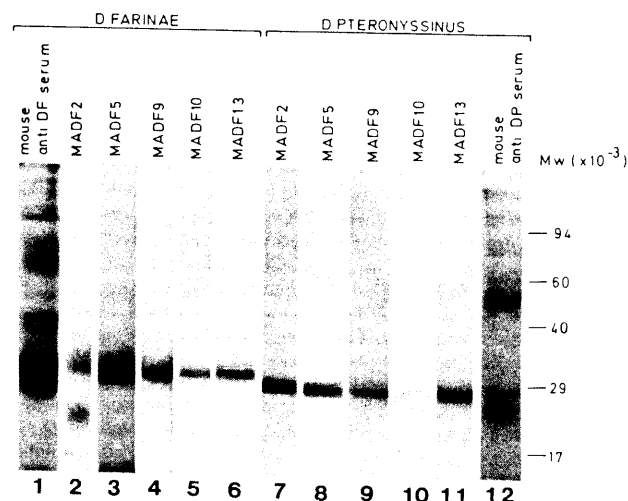


Fig. 1. SDS-PAGE analysis of the antigens recognized by the MAbs specific for *Dermatophagoides* extracts. Immunoprecipitations were carried out as described in Materials and Methods. Lane 1, proteins immunoprecipitated with mouse serum raised against *D. farinae* extract. Lanes 2-6, proteins immunoprecipitated by several MAbs, from *D. farinae* extract. Lanes 7-11, proteins immunoprecipitated by several MAbs, from extracts of *D. pteronyssinus*. Lane 12 immunoprecipitation of *D. pteronyssinus* extract proteins with mouse serum raised against the same extract.

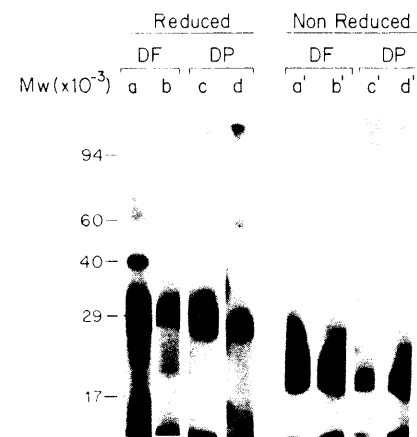


Fig. 2. SDS-PAGE analysis of ¹²⁵I-labeled DF29 and DP28 allergens. Immunoprecipitation from radiolabeled *D. farinae* (DF) and *D. pteronyssinus* (DP) extracts was carried out as described in Materials and Methods with MADF5 (lanes b, c, b', c') and mouse anti-*Dermatophagoides* serum (lanes a, d, a', d'), followed by SDS-PAGE under reducing and non-reducing conditions.

after separation by SDS-PAGE, in the presence than in absence of 2-mercaptoethanol, indicating the existence of intra-chain disulphide bonds (Allore and Barber, 1984).

Competitive binding of MAbs to *D. farinae* extracts

Epitope specificity of the five MAbs was studied by competitive radioimmunoassay with purified MAbs (Table 2). The binding of MADF2 (IgG₁) to the allergen is not inhibited by MADF5 (IgM) and MADF10 (IgG₁), and partly inhibited by MADF9 (IgG_{2a}) and MADF13 (IgM). On the contrary, MADF5 and MADF10 seem to recognize the same antigenic determinant of DF29 molecule. MADF9 and MADF13 partially compete the binding of the other MAbs. In order to further study the overlapping epitopes, we carried out competition experiments in a wide range of concns. To illustrate these experiments Fig. 3 shows the inhibition of the binding of MADF2(a) and MADF5(b) by the five cold antibodies. The results indicated that MADF2

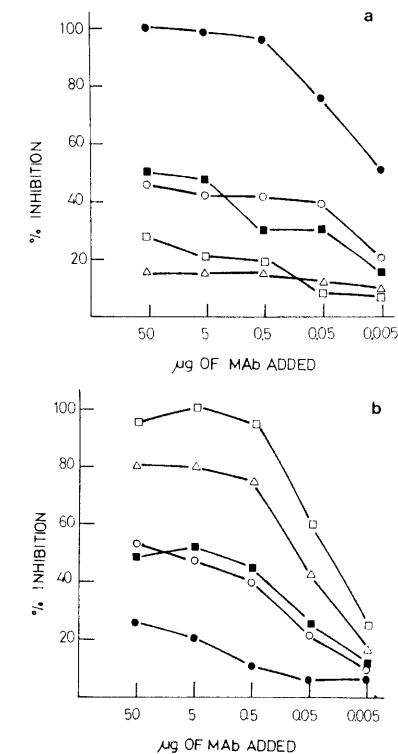


Fig. 3. Inhibition of the binding of ¹²⁵I-labeled MAbs to *D. farinae* extract coupled to paper discs. The protein concns of purified MAb used to inhibit the binding of ¹²⁵I-MADF2 (a) and ¹²⁵I-MADF5 (b) are indicated in the abscissa. Identical BSA concns were used as negative control. ●—● MADF2, ○—○ MADF9, ■—■ MADF13, △—△ MADF10, □—□ MADF5.

probably defines an epitope distinct from those defined by MADF5 and MADF10, and MADF9 and MADF13 seem to recognize other epitopes that partly overlap with those recognized by other MAbs.

Inhibition of the binding of human IgE to *D. farinae* components by the MAbs

The possibility for several MAbs to recognize antigenic determinants close to human IgE binding

Table 2. Inhibition of the binding of ¹²⁵I-labeled monoclonal antibodies to *D. farinae* extracts

MAb	¹²⁵ I-MAb ^a	MADF2	MADF9	MADF13	MADF10	MADF5
MADF2	(100)	37	45	0	0	
MADF9	50	(100)	55	73	52	
MADF13	54	51	(100)	80	52	
MADF10	7	51	43	(100)	80	
MADF5	30	52	52	80	(100)	
Human serum ^b	95	96	95	95	94	

^a¹²⁵I-labeled MAbs contained a specific activity of 25 µCi/µg of protein, and 10 ng was applied per well. Values indicate the percent inhibition at a concn of inhibitor MAb of 50 µg per well.

^bHuman serum was a 1:10 dilution of a pool from mite sensitive patients.

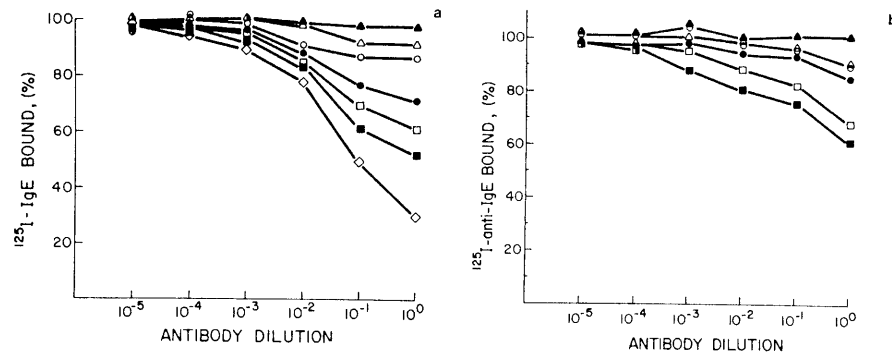


Fig. 4. Inhibition of the human IgE binding to *D. farinae* extract by MABs. Ten-fold dilutions of hybridoma supernatants were used to inhibit the binding of ^{125}I -purified human IgE (a) or IgE containing human serum pool (b) to *D. farinae* extract coupled to paper discs. Human serum was used 10 times diluted. Inhibition was estimated by using ^{125}I -anti-human IgE monoclonal antibody. $\diamond$ — $\diamond$ Human serum pool, $\blacksquare$ — $\blacksquare$ MADF13, $\square$ — $\square$ MADF5, $\bullet$ — $\bullet$ MADF2, $\circ$ — $\circ$ MADF9, $\triangle$ — $\triangle$ MADF10, $\blacktriangle$ — $\blacktriangle$ X63, a mouse myeloma supernatant, was used as negative control.

sites, was analyzed by competitive radioimmunoassay [Fig. 4(a)]. The five MABs studied produced a variable degree of inhibition of ^{125}I -human IgE binding to *D. farinae* extracts ranging from 50 (MADF13) to 6% (MADF10). The results were confirmed in a parallel experiment, in which a serum pool and ^{125}I -labeled anti-human IgE monoclonal antibody were used instead of radiolabeled IgE [Fig. 4(b)]. The

order of inhibition was the same in both experiments and it can be indicated as follows: MADF13 > MADF5 > MADF2 > MADF9 > MADF10. The results suggest that, at least, MADF13 and MADF5 MABs recognize epitope(s) coincident or close to those recognized by human IgE.

Partial purification and allergenic activity of DF29

The main allergen from *D. farinae* extracts, DF29, was partially purified by affinity chromatography on MADF5-Sepharose. As shown by SDS-PAGE, the purified allergen contains two polypeptides with mol. wt 29,000 and 30,000, in a nearly homogeneous form (Fig. 5, lane b). The fact that DF29 appeared split into two different polypeptides will be discussed later.

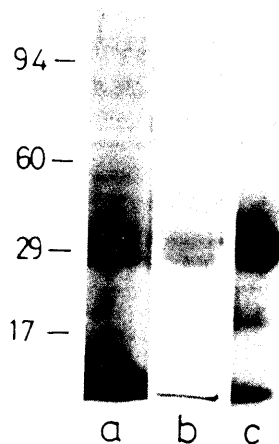


Fig. 5. SDS-PAGE analysis of purified DF29. DF29 was purified by affinity chromatography on a MADF5-Sepharose column and analyzed by SDS-PAGE followed by silver staining. (a) *D. farinae* whole extract. (b) Extract component retained by MADF5 MAB. The acrylamide concn was 10%. (c) Autoradiography of ^{125}I -labeled extract components retained by human IgE-anti-IgE-Sepharose. On the left side mol. wt markers $\times 10^{-3}$.

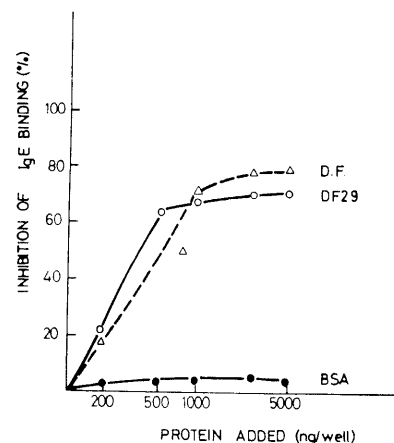


Fig. 6. Allergenic activity of purified DF29 determined by RAST inhibition. The solid phase contained the whole *D. farinae* extract coupled to paper discs. As positive and negative controls, *D. farinae* extract (DF) and BSA were used, respectively.

Identical relative mobility is shown by the molecule purified by affinity chromatography using a column of anti IgE-Sepharose which had been incubated with a serum pool from *Dermatophagoides* allergic patients (Fig. 5, lane c). This result indicated that most of the specific IgE contained in the pool of sera, is directed against DF29 molecule.

Allergenic activity of the purified DF29 was studied by RAST inhibition, in which the allergen competed for the human IgE against the whole *D. farinae* extract (Fig. 6). Purified DF29 can inhibit up to 70% the binding of human IgE to *D. farinae* extracts demonstrating this polypeptide(s) is the main allergen of the mite.

DISCUSSION

In this report, the characterization and purification of the main allergen molecule of *D. farinae* by means of MABs is described. Immunization of mice with *D. farinae* extract followed by fusion of spleen lymphocytes with mouse myeloma cells led to the production of eighty-nine antibody secreting hybridoma which recognized components from *D. farinae* extracts. The specificity of the MABs was studied by means of two different indirect binding assays. The first one allowed identification of the MABs specific for *D. farinae* components and the second assay was used for selection of the allergen-binding MABs. This four-step radioimmunoassay has proved to select MAB directed against allergens from *P. judaica* pollen and *Dermatophagoides* extracts (Ley *et al.*, 1985a,b; Corbi *et al.*, 1985a). Some of the MABs showed different relative positivities in both types of radioimmunoassay. Thus, MADF2 and MADF9 resulted more positive on the second assay, while MADF13 behaviour was opposite. The decrease in positivity of MADF13 could be explained by the competition for the same epitope with IgE antibodies. The results of the competition assays are in support of this hypothesis.

The physicochemical properties of DF29 are very similar to the ones of Ag11 described by Dandeu *et al.* (1982). DP28, probably corresponds to the already described antigens P1 (Chapman and Platts-Mills, 1980), Dpt 12 (Stewart, 1982) and Dpt 42 (Lind *et al.*, 1979) from *D. pteronyssinus*. The electrophoretic mobility of DP28 (28,000) is slightly faster than that of DF29 (29,000) and they are in accordance with the mol. wts of Ag11 (28,000) and P1 (24,000). Dandeu *et al.* (1982), have shown that Ag11 contained several cystine residues. Now we can add that these residues probably form intra-chain bonds, since DF29 move faster in absence of 2-mercaptoethanol than in presence of the reducing agent (de la Hoz and Carreira, 1985). This characteristic is also applied to DP28 allergen.

With the only exception of MADF2, all the anti-allergen MABs are specific for epitopes located on DF29 and DP28. These results are not surprising

given the fact that both allergens are highly homologous and represent the most abundant proteins of the extracts (Dandeu *et al.*, 1982; Lind *et al.*, 1979; Chapman and Platts-Mills, 1980; Stewart, 1982; Le Mao *et al.*, 1983). However, specific determinants must also be present in both allergens since Chapman *et al.* (1984) obtained MABs, upon immunization with P1 antigen, unable to cross-react with *D. farinae* allergens.

Binding inhibition experiments demonstrated that MADF5 and MADF13 recognize epitopes overlapping with those recognized by the human IgE. The fact that serum pool caused a higher percentage of inhibition than MABs can be explained considering that IgE antibodies are poly-specific and probably recognize many different allergenic determinants on the same molecule. Thus, each MAB inhibits the binding to just one epitope of IgE, leaving the other epitopes free for more IgE molecules to be bound.

Five MABs specific for DF29 defined, at least, two epitopes on the allergen molecule. One of the epitopes was recognized by MADF2 while MADF10 and MADF5 reacted with a different epitope partly recognized by MADF9 and MADF13.

Purification of DF29 by affinity chromatography has facilitated the evaluation of its individual allergenic activity in native conditions. The purified allergen appeared to be composed by two very similar polypeptides. In a recent report, de la Hoz and Carreira (1985) found identical results, and showed that both bands bind specific human IgE as demonstrated by immunoblotting. The heterogeneity of DF29 molecule could be interpreted by assuming the possibility that the carbohydrate moiety (Dandeu *et al.*, 1982) varies widely specially after treatments at low pH and high ionic strength. This interpretation appears acceptable considering that DF29 is constituted by a large number of molecular species, which in analytical isoelectric focusing precipitate between pH 4.5 and 6.6 (de la Hoz and Carreira, 1985).

The availability of these MABs may be of great importance since they can facilitate the standardization of extracts from both mite species, and the study of the antigenic structure of DF29 and DP28 molecules considered the most important allergens in *Dermatophagoides* as they represent about 70% of the total allergenicity of the extracts. In addition, some of these MABs could be an important tool in elucidating the mechanisms of allergy by taking advantage of the fact that they recognize the same epitopes as the human IgE from allergic patients.

Acknowledgements—The authors would like to thank Drs C. Bernabeu, F. Sánchez-Madrid and J. Sancho for helpful discussions and I. Courinha for her excellent secretarial assistance in the preparation of the manuscript.

REFERENCES

Allore R. J. and Barber B. H. (1984) A recommendation for visualizing disulphide bonding by one-dimensional

- sodium dodecyl sulfate-polyacrylamide gel electrophoresis. *Analyt. Biochem.* **137**, 523-527.
- Carreira J., Rubio M., Herrero T., Horak F., Businco L., Wuthrich B., Bearzi de Prada V., Berry J. B., Lischka G. and Brighton W. D. (1984) Collaborative clinical assay of the biological potency of allergen skin-test extracts. *Clin. Allergy* **14**, 503-507.
- Chapman M. D. and Platts-Mills T. A. E. (1980) Purification and characterization of the major allergen from *Dermatophagoides pteronyssinus*-antigen P1. *J. Immun.* **125**, 587-592.
- Chapman M. D., Sutherland M. W. and Platts-Mills T. A. E. (1984) Recognition of two *Dermatophagoides pteronyssinus*-specific epitopes on antigen P1 by using monoclonal antibodies: binding to each epitope can be inhibited by serum from dust mite-allergic patients. *J. Immun.* **133**, 2488-2495.
- Corbi A. L., Ayuso R., Lombardero M., Duffort O. and Carreira J. (1985a) A competitive solid-phase radioimmunoassay for quantitation of the major allergen of *Parietaria* pollen. *J. Immun. Meth.* **83**, 83-88.
- Corbi A. L., Ley V., Sánchez-Madrid F. and Carreira J. (1985b) Isolation of the major IgE-binding protein from *Parietaria judaica* pollen using monoclonal antibodies. *Molec. Immun.* **22**, 1081-1089.
- Dandeu J. P., Le Mao J., Lux M., Rabillon J. and David B. (1982) Antigens and allergens in *Dermatophagoides farinae* mite II. Purification of Ag11, a major allergen in *Dermatophagoides farinae*. *Immunology* **46**, 679-687.
- Ey P. L., Prowse S. J. and Jenkin C. R. (1978) Isolation of pure IgG₁, IgG_{2a} and IgG_{2b} immunoglobulins from mouse serum using protein A-Sepharose. *Immunochimistry* **15**, 429-436.
- Fraker P. J. and Speck J. C. (1978) Protein and cell-membrane iodinations with a sparingly soluble chloroamide 1,3,4,6-tetrachloro α -3,6 α -diphenylglycoluril. *Biochem. biophys. Res. Commun.* **80**, 849-857.
- Galfre C. and Milstein C. (1981) Preparation of monoclonal antibodies: strategies and procedures. *Meth. Enzym.* **73**, 3-46.
- Hoz F. de la and Carreira J. (1985) Identification of the main allergens from *Dermatophagoides farinae* and their properties under native and dissociating conditions. *Int. Archs Allergy appl. Immun.* **79**, 238-245.
- Hunter M. W. and Greenwood F. C. (1962) Preparation of iodine-131 labeled human growth hormone of high specific activity. *Nature, Lond.* **194**, 495-496.
- Kriliš S., Baldo B. A. and Basten A. (1981). Standardization of allergens. Lectins and monoclonal antibodies as primary standards for house dust mite allergens. *Int. Archs Allergy appl. Immun.* **66**, 252-258.
- Kriliš S., Baldo B. A., Raison R. L., Callard R. E. and Basten A. (1983) Standardization of antigen E in ragweed pollen extracts using a monoclonal antibody based enzyme immunoassay. *J. Allergy clin. Immun.* **71**, 261-265.
- Laskey R. A. and Mills A. D. (1977) Enhanced autoradiographic detection of ³²P and ¹²⁵I using intensifying screens and hypersensitization films. *FEBS Lett.* **82**, 314-316.
- Ley V., Corbi A. L., Sánchez-Madrid F. and Carreira J. (1985a) A four-step sandwich radioimmunoassay for direct selection of monoclonal antibodies to allergen molecules. *J. Immun. Meth.* **84**, 265-270.
- Ley V., de la Hoz P., Corbi A. L., Sánchez-Madrid F. and Carreira J. (1985b) Identification of a novel allergen molecule from *Dermatophagoides* by monoclonal antibodies. *Immun. Lett.* **11**, 98-93.
- Le Mao J., Dandeu J. P., Rabillon J., Lux M. and David B. (1981) Antigens and allergens in *Dermatophagoides farinae* mite I. Immunochemical and physico chemical study of two allergenic fractions from a partially purified *Dermatophagoides farinae* mite extract. *Immunology* **44**, 239-247.
- Le Mao J., Dandeu J. P., Rabillon J., Lux M. and David B. (1983) Comparison of antigenic and allergenic composition of two partially purified extracts from *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* mite cultures. *Allergy clin. Immun.* **71**, 588-596.
- Lind P., Korsgaard J. and Lowenstein H. (1979) Detection and quantification of *Dermatophagoides* antigens in house dust by immunochemical techniques. *Allergy* **34**, 319-326.
- Sánchez-Madrid F., Morago G., Corbi A. L. and Carreira J. (1984) Monoclonal antibodies to three distinct epitopes on human IgE: their use for determination of allergen-specific IgE. *J. Immun. Meth.* **73**, 367-378.
- Sánchez-Madrid F., Szklut P. and Springer T. (1983) Stable hamster-mouse hybridomas producing IgG and IgM hamster monoclonal antibodies of defined specificity. *J. Immun.* **130**, 309-312.
- Smart I. J., Hedde R. J., Zola H. and Bradley J. (1983) Development of monoclonal antibodies specific for allergenic components in ryegrass (*Lolium perenne*) pollen. *Int. Archs Allergy appl. Immun.* **72**, 243-248.
- Stanker L. H., Vaderlaan M. and Juarez-Salinas H. (1985) One-step purification of mouse monoclonal antibodies from ascites fluid by hydroxylapatite chromatography. *J. Immun. Meth.* **76**, 157-169.
- Stewart G. A. (1982) Isolation and characterization of the allergen Dpt. 12 from *Dermatophagoides pteronyssinus* by chromatofocusing. *Int. Archs Allergy appl. Immun.* **66**, 224-230.
- Stewart G. A. and Turner K. J. (1980) Physicochemical and immunochemical characterization of the allergens from *Dermatophagoides pteronyssinus*. *Aust. J. exp. Biol. med. Sci.* **58**, 259-274.
- Voorhorst R., Spieskma-Boezeman M. I. A. and Spieskma F. th. M. (1964) Is a mite (*Dermatophagoides* sp.) the producer of house dust mite allergen. *Allergie Asthma* **10**, 329-334.
- Yelton D. E., Desaymard C. and Scharff M. D. (1981) Use of monoclonal anti-mouse immunoglobulins to detect mouse antibodies. *Hybridoma* **1**, 5-11.
- Yman L., Ponterius G. and Brandt R. (1975) RAST-based allergen assay methods. *Devl biol. Stand.* **29**, 151-165.