

## IDENTIFICATION OF A NOVEL ALLERGEN MOLECULE FROM *DERMATOPHAGOIDES* BY MONOCLONAL ANTIBODIES

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(Received 17 May 1985)

(Accepted 11 July 1985)

### 1. Summary

Six different monoclonal antibodies (MAb) have been obtained which bind to components contained in extracts from *Dermatophagoides*. One out of the six MAb recognized molecules displaying IgE binding ability. This MAb (MADP-1) immunoprecipitated allergenic polypeptides of 42 kDa and 30 kDa from <sup>125</sup>I-labeled extracts of *D. pteronyssinus* and *D. farinae* respectively. Purified allergen preparations from both extracts have been obtained by affinity chromatography using a column of purified MADP-1 coupled to Sepharose. The purified fractions partially compete the binding of specific IgE contained in sera from sensitized patients to the whole extracts, in a radioallergosorbent inhibition test.

### 2. Introduction

The role of allergens in the induction and production of IgE in allergic patients is being intensively studied [1]. The biochemical and structural basis of specific features which confer allergenicity to molecules, still remain to be

elucidated. Therefore, purified allergens will be required for those studies.

Purification of allergens would be also very useful for diagnosis and therapy of allergic patients as well as for standardization of allergenic extracts. Many attempts have been made to find a simple and reproducible method of general application for allergen purification. Some allergens have been isolated by conventional techniques but generally the allergen molecules obtained are contaminated with other constituents unrelated to allergic processes. Recently, some studies using hybridoma derived monoclonal antibodies have provided a suitable method for characterization and purification of some allergenic components of rye-grass pollen [5] and house dust mite [6, 7].

*Dermatophagoides* mites are the source of many allergens in house dust and cause a high percentage of allergenic diseases [8, 9]. The two more representative species of *Dermatophagoides* are *D. pteronyssinus*, dominant in Europe, and *D. farinae*, dominant in North America [10]. These two species are closely related by immunological and biochemical criteria [11, 12]. A protein of 24–30 kDa has been reported by several groups to be the molecular structure that binds the majority of specific IgE from sera of sensitized patients [2, 12].

In this report, we describe the isolation and characterization of a monoclonal antibody to an allergen molecule of *D. pteronyssinus*, which also recognizes an allergen molecule of *D. farinae*.

**Key words:** *Dermatophagoides* – allergen – monoclonal antibodies

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Both allergens have been purified and their allergenic activity proved.

### 3. Materials and Methods

#### 3.1. Antigens

*Dermatophagoides* extracts were prepared from the whole culture of mites. Proteins were solubilized in 50 mM ammonium bicarbonate and the suspension was clarified by centrifugation at  $22,000 \times g$  for 1 h at 4 °C. The supernatant was dialyzed against distilled water and filtered through a membrane filter with 0.2  $\mu$ m pore (Sartorius, type SM).

#### 3.2. Production and screening of monoclonal antibodies (MAb)

Six-week-old female BALB/c mice were immunized with an intraperitoneal injection of 100  $\mu$ g of *D. pteronyssinus* extract on 100  $\mu$ l of phosphate buffered saline (PBS), emulsified with 100  $\mu$ l of Freund's complete adjuvant. Twenty days later, mice were boosted with identical dose in Freund's incomplete adjuvant. Three days before fusion a mouse was injected intravenously with the same amount of *D. pteronyssinus* extract in PBS. The spleen cells were fused with SP2 myeloma cells as described [13].

Supernatants were screened after 2 wk for anti-*Dermatophagoides* extract binding ability by an indirect radioimmunoassay: 50  $\mu$ l of hybridoma culture supernatant was added to *Dermatophagoides* extract covalently linked to paper discs, and incubated for 1 h at room temperature, then paper discs were washed and 50  $\mu$ l of  $^{125}$ I-labeled 187-1 MAb (a rat anti-mouse kappa chain MAb) dispensed on paper discs and incubated for 1 h at room temperature. After extensive washing, paper discs were gamma counted. The positive hybridomas in this first assay were then tested for their ability to bind allergenic proteins in a four-step radioimmunoassay as follows: polyvinylchloride wells were coated with 50  $\mu$ l of a solution of 20  $\mu$ g/ml of 187-1 MAb in PBS. Then, wells were saturated with 1% bovine serum albumin (BSA) in PBS and sequentially incubated for 1 h at 37 °C with

50  $\mu$ l of: (a) hybridoma supernatants, (b) *D. pteronyssinus* or *D. farinae* extract at 5 mg/ml, (c) human serum pool from *Dermatophagoides* allergic patients and (d)  $^{125}$ I-labeled anti-IgE polyclonal antiserum (Pharmacia). After each incubation, wells were washed three times with 0.25% BSA in PBS. Those hybrids which secrete antibodies with ability to bind allergenic proteins were cloned and subcloned in soft-agar [13].

Purification of MADP-1, containing  $\gamma$ 1,  $\kappa$  chains, was carried out by affinity chromatography on protein A-Sepharose [14].

#### 3.3. Immunoprecipitation

*Dermatophagoides* extracts were radioiodinated by the chloroglycoluril method [15]. The labeled extract was incubated with 25  $\mu$ l of MADP-1 coupled to Sepharose for 1 h at 4 °C with shaking. After washing, samples were subjected to SDS-PAGE under reducing conditions [16] and autoradiographed with intensifying screens.

#### 3.4. Purification of allergenic proteins recognized by MADP-1

Purified MADP-1 was coupled to CNBr-activated Sepharose CL4B at a ratio 1 mg/ml wet gel [17]. *Dermatophagoides* extracts were loaded into the immunoabsorbent column in the presence of 0.02 Triton X-100. The column was washed with 10 vol. of PBS, 0.02% Triton X-100 and then, the bound material was eluted with 3 M KSCN. The eluate was dialyzed against three changes of PBS for 24 h at 4 °C.

#### 3.5. Radioallergosorbent test (RAST) inhibition

Allergenic activity of purified proteins from *Dermatophagoides* extracts in a MADP-1-Sepharose column was examined by their ability to inhibit the binding of specific human IgE from sensitized patients to paper discs-bound extract in a radioallergosorbent test as described [18].

### 4. Results

#### 4.1. Selection and characterization of the specificity of anti-*Dermatophagoides* MAb

The hybridoma culture supernatants with abili-

Table 1  
Binding of MAb to *Dermatophagoides* extracts

| MAb    | Radioactivity bound (cpm) <sup>a</sup> |                   |
|--------|----------------------------------------|-------------------|
|        | <i>D. pteronyssinus</i>                | <i>D. farinae</i> |
| MADP-1 | 10,600                                 | 3,300             |
| MADP-2 | 10,500                                 | 3,000             |
| MADP-3 | 7,100                                  | 11,000            |
| MADP-4 | 4,129                                  | 8,020             |
| MADP-5 | 7,000                                  | 1,220             |
| MADP-6 | 6,400                                  | 2,200             |
| P3X63  | 200                                    | 210               |

<sup>a</sup> The <sup>125</sup>I-labeled anti-mouse kappa chain MAb (input 60,000 cpm), second antibody bound to the mouse MAb specifically complexed to *Dermatophagoides* extracts (50 µl/well of 1 mg/ml solution) coated polyvinyl-microtiter wells was determined in the indirect binding assay. The P3X63 mouse myeloma culture supernatant containing standard concentration of mouse γ, κ chains was used to determine the non-specific binding.

ty to bind *D. pteronyssinus* components were selected by an indirect radioimmunoassay. Six different hybridoma culture supernatants (MADP-1 to MADP-6) were able to bind to *D. pteronyssinus* extract components coated to polyvinylchloride wells (Table 1).

The six anti-*D. pteronyssinus* MAb also recognized *D. farinae* extract (Table 1). MADP-1, 2, 5 and 6, exhibited higher binding ability to *D. pteronyssinus* than to *D. farinae* extract. Conversely, MADP-3 and 4 showed a lower binding to *D. pteronyssinus* than to *D. farinae* extract. These results indicate the immunological homology between antigenic structures of the two *Dermatophagoides* species.

Then, hybridoma supernatants were assayed in a four-step radioimmunoassay in order to select those recognizing allergen molecules (Table 2). One out of the six tested MAb (MADP-1) was found to bind specifically to *D. pteronyssinus* and *D. farinae* IgE-binding components. Similar results were obtained with MADP-1 purified on protein A-Sepharose (data not shown).

Since the epitopes recognized by the IgE molecules were not blocked, the MAb so selected (MADP-1), seemed to be directed to a non-allergenic determinant.

Table 2  
Binding of MAb to allergen molecules from *Dermatophagoides* extracts

| MAb    | Radioactivity bound (cpm) <sup>a</sup> |                   |
|--------|----------------------------------------|-------------------|
|        | <i>D. pteronyssinus</i>                | <i>D. farinae</i> |
| MADP-1 | 1,330                                  | 850               |
| MADP-2 | 210                                    | 180               |
| MADP-3 | 200                                    | 190               |
| MADP-4 | 210                                    | 200               |
| MADP-5 | 240                                    | 220               |
| MADP-6 | 160                                    | 170               |
| P3X63  | 180                                    | 160               |

<sup>a</sup> Binding of <sup>125</sup>I-labeled polyclonal anti-human IgE (input 60,000 cpm) to human specific IgE bound to allergen molecules from *Dermatophagoides* extracts selectively retained by mouse MAb complexed to anti-mouse kappa chain 187-1 MAb coated to polyvinylchloride wells was determined using a four-step radioimmunoassay. Non-specific binding was determined with the P3X63 mouse myeloma culture supernatant.

#### 4.2. Identification of allergen molecules recognized by MADP-1

To determine the molecule(s) bearing the antigenic determinants recognized by MADP-1, immunoprecipitation from <sup>125</sup>I-labeled *Dermatophagoides* proteins was carried out using the purified MADP-1 coupled to Sepharose. MADP-1 immunoprecipitated a *D. pteronyssinus* polypeptide of 42 kDa, designated as DP-42 allergen (Fig. 1, lane b). MADP-1 also immunoprecipitated a *D. farinae* polypeptide of 30 kDa named DF-30 allergen (Fig. 1, lane c).

#### 4.3. Purification and allergenic activity of molecules recognized by MADP-1

The DP-42 and DF-30 molecules were purified by affinity chromatography on Sepharose—MADP-1 and assayed for human IgE binding ability by RAST inhibition (Fig. 2).

The DP-42 polypeptide was able to partly inhibit (approx. 25%) the binding of *Dermatophagoides*-specific IgE to the whole extract. The same result was reached with the purified DP-30 polypeptide. This partial inhibition suggests the existence of other allergen molecules responsible for the rest of the allergenic activity.

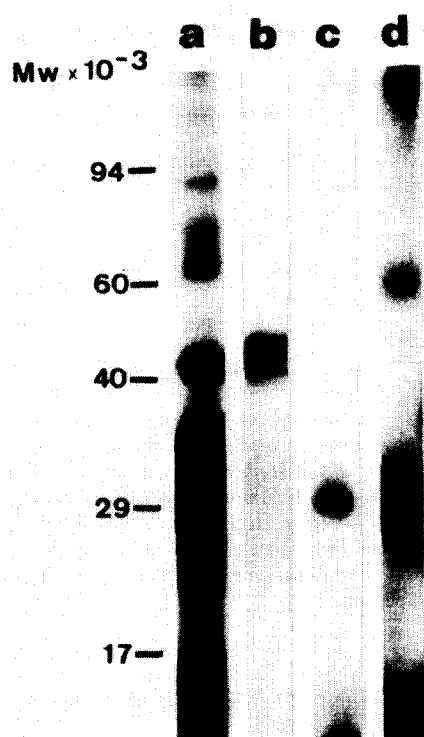


Fig. 1. Immunoprecipitation of DP-42 and DF-30 allergen molecules.  $^{125}\text{I}$ -labeled extracts from *D. pteronyssinus* (a, b) and *D. farinae* (c, d), respectively, were precipitated with purified MADP-1-Sepharose (lanes b and c) or with sera from mice immunized to *D. pteronyssinus* (a) and *D. farinae* (d) extracts. The precipitates were subjected to SDS-10%-PAGE under reducing conditions and autoradiography.

## 5. Discussion

In the present report, we describe the identification of a novel allergen in extracts of dust mites by using a MAb. The selection of MAb which bind individual allergenic proteins, was carried out using a four-step screening technique which proved to be highly selective.

The binding of the MAb to the allergen does not overlap with the binding of specific IgE. This can be explained by either recognition of different epitopes, or presence of multiple allergenic epitopes.

Production of MAb against *D. pteronyssinus* allergens has already been reported [6] although

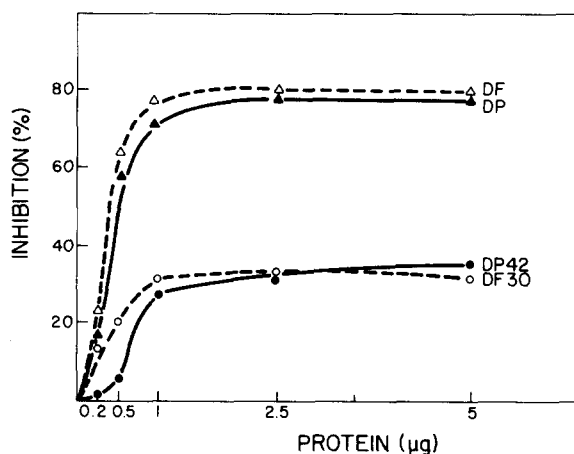


Fig. 2. Allergenic activity of purified DP-42 and DF-30 polypeptides. Different allergen protein concentrations were assayed for inhibition of the binding of specific human IgE to allergenic extracts coated to paper discs. Binding of  $^{125}\text{I}$ -labeled polyclonal anti-human IgE in the presence of equal amounts of a non-relevant protein preparation was considered 0% inhibition (3,500 cpm).

the molecules recognized by them have not been characterized. Recently, [7] MAb to *D. pteronyssinus* antigen P1 were obtained. No antigen has been reported with similar structure to the one described here.

Crossreactivity between *D. pteronyssinus* and *D. farinae* extracts has been reported in in vitro and in vivo studies [11, 12]. However, to our knowledge, this is the first study using MAb where proteins from *D. pteronyssinus* are shown to contain determinants shared with proteins from *D. farinae*. The antigenic determinant recognized by MADP-1 is localized in a 42 kDa polypeptide from *D. pteronyssinus* and in a 30 kDa polypeptide from *D. farinae*. These results can be explained by location of the epitope recognized by MADP-1 in polypeptide chains with different length, distinct carbohydrate content, etc. The nature of those allergen molecules in native form is unknown. It remains possible that the polypeptides detected by immunoprecipitation are subunits of a native allergen complex.

Allergenic activity of purified preparations of DP-42 and DF-30 allergens accounts for 25% of

total allergenic activity. The rest of allergenicity is due to the existence of other distinct allergen molecules in the extracts. Some of them have been identified using immunological techniques by several authors [2, 3].

## Acknowledgements

We thank J. C. Sáiz and F. de la Hoz for their excellent cooperation, Dr. C. Bernabéu for the helpful discussions and M. J. González for secretarial assistance.

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