

ISOLATION OF THE MAJOR IgE-BINDING PROTEIN FROM *PARIETARIA JUDAICA* POLLEN USING MONOCLONAL ANTIBODIES

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Abstract—Allergen molecules from *Parietaria judaica* pollen, a widely distributed allergy inducer in Southern and Western Europe, have been studied using specific monoclonal antibodies (MAbs). MAbs against IgE-binding components were selected in a 4-step radioimmunoassay. Three different MAbs (AC/1.1, AC/7.1 and AC/15.1) were obtained which recognized epitope(s) located on a polypeptide of 10 Kd (Pj10). This polypeptide displayed the highest IgE-binding ability under either native or SDS-denatured conditions, as determined by immunoadsorption and immunodetection after SDS-PAGE, respectively. The Pj10-containing allergen, purified on an AC/1.1 MAbs-Sepharose column, was able to inhibit most of the binding of specific IgE to the pollen extract coupled to paper discs in an inhibition radioallergosorbent test (RAST). The affinity-purified allergen exhibited the same immunoelectrophoretic behaviour as the native allergen.

INTRODUCTION

Identification and isolation of molecules displaying allergenic activity in pollen extracts has become a very important goal in allergy, because of their potential utilization for diagnosis and therapy of allergic patients. So far, only a few allergens have been isolated and characterized (Elsayed and Aas, 1971; Lapkoff and Goodfriend, 1974; Elsayed and Bennich, 1975; Mole *et al.*, 1975; Klapper *et al.*, 1980; Florvaag *et al.*, 1982; Ekramoddoullah *et al.*, 1983) [reviewed by Løwenstein (1983)].

Parietaria judaica pollen is responsible for a high percentage of allergic diseases in Southern and Western Europe (Towsend, 1968). Recently, the isolation of an allergenic polypeptide from *P. judaica* pollen, originated by proteolytic degradation, has been reported (Feo *et al.*, 1984). Partial purification of allergenic fractions from the pollen of *P. officinalis*, a closely related species, has also been reported (Menegozzo *et al.*, 1976). We have previously identified a polypeptide of 10 Kd (Pj10), with the highest IgE-binding ability in *P. judaica* pollen by immunodetection after SDS treatment of the pollen extract. This polypeptide has been found to be linked to itself or to other polypeptides by disulphide bridge(s) (Corbí and Carreira, 1984). Moreover, its glycoprotein nature and certain degree of heterogeneity in the sugar moiety of Pj10 have been demonstrated by adsorption on a column of concanavalin A (Con A)-Sepharose (Corbí and Carreira, 1985).

Antibodies to individual allergens are known to be useful in the characterization and purification of these molecules (Krilis *et al.*, 1981; Smart *et al.*, 1983). Rabbit antisera against *P. judaica* pollen were

able to precipitate the Pj10-containing molecules, but attempts to obtain antibodies to the purified Pj10 glycopeptide were unsuccessful (Corbí and Carreira, 1984).

The hybridoma technology has already been applied to the study of allergens from *Lolium perenne* pollen (Smart *et al.*, 1983) and house dust mite (Krilis *et al.*, 1981) and we have used it for the preparation of monoclonal antibodies to native molecules of *P. judaica* pollen extract. In the present report, monoclonal antibodies (MAbs) that recognize the Pj10 glycopeptide are described. They were identified by using a screening method which specifically selects hybridomas secreting antibodies with allergen-binding ability. These MAbs have been used for isolation of the Pj10-containing protein in an allergenically active form. In addition, this IgE-binding glycoprotein has been proven to be the molecule bearing most of the allergenic activity of this pollen.

MATERIALS AND METHODS

Immunization, fusion and screening

P. judaica pollen was purchased from Allergon AB (Valinge, Sweden). The extract was prepared as previously described (Corbí and Carreira, 1984). Briefly, 10 g of pollen were dissolved in 100 ml of a buffered ammonium bicarbonate solution (pH 8) for 1 hr at 4°C, with shaking. After centrifugation at 39,100 g for 15 min, the extract was dialyzed, recentrifuged, filtered and freeze-dried. The protein concn was determined by the method of Lowry *et al.* (1951).

Once the pollen extract was prepared, BALB/c male mice (Iffa Credo, Lyon, France) were primed intraperitoneally on days -45 and -15 with 500 µg

of *P. judaica* pollen extract protein. Three days before fusion a mouse was boosted intravenously with the same amount of pollen protein in phosphate-buffered saline (50 mM sodium phosphate buffer, 0.9% NaCl, pH 7.4) (PBS). After killing, immune splenocytes were removed and fused with SP2 mouse myeloma cells using 50% polyethyleneglycol (Galfre *et al.*, 1977; Springer, 1980). Spleen cells fused with myeloma cells were distributed in 4 96-well culture plates and grown as described (Sanchez-Madrid *et al.*, 1983). After 2 weeks, hybridoma culture supernatants were harvested for screening.

Two different indirect binding assays were performed for selection of hybridoma clones producing antibodies to *P. judaica* pollen. Firstly, 50 μ l of hybridoma culture supernatant were added to polyvinylchloride 96-well plates coated with pollen extract, and incubated for 1 hr at 37°C. Then wells were washed and 50 μ l of ^{125} I-187-1 MAb dispensed into each well and incubated for 30 min at room temp. After extensive washing, wells were gamma counted. In order to detect MABs directed against pollen components with poor adsorption to plastic surfaces, the same procedure was also performed with pollen proteins covalently linked to CNBr-activated paper discs. The coupling procedure is detailed below.

Those hybridomas selected in the first assay were subsequently screened for allergen-binding ability. For this second assay, polyvinylchloride 96-well plates were coated with 50 μ l of solution of purified anti-mouse kappa chain MAb (0.1 mg/ml). After saturation with 1% bovine serum albumin (BSA) in PBS, wells were successively incubated with: (1) supernatant of hybridoma cells (50 μ l—1 hr—37°C), (2) pollen extract (50 μ l—5 mg/ml in PBS—1 hr—37°C), (3) undiluted serum pool from *P. judaica* sensitive patients (50 μ l—1 hr—37°C), and (4) a final incubation to detect the MABs—allergen—IgE complexes, using ^{125} I-anti-IgE antiserum (50 μ l—30 min at room temp) from Pharmacia. Three washing steps with PBS containing 0.25% BSA were performed between incubations. Finally, wells were cut and gamma counted. Positive hybridomas were then cloned and subcloned in soft agar (Galfre and Milstein, 1981).

The 3 MABs which exhibited the highest binding percentage to allergens (AC/1.1, AC/7.1 and AC/15.1) were purified by immunoadsorption on protein A-Sepharose (Ey *et al.*, 1978). In addition, AC/9.1 MAB was also purified for it showed the highest binding to pollen protein coated wells and did not display any binding to allergens. The purified MABs coated to polyvinylchloride wells were again tested for allergen recognition by a radioimmunoassay similar to the one used for the second screening system. Purified AC/1.1, AC/7.1 and AC/15.1, anti-human IgE MABs and normal rabbit IgG were coupled to CNBr-activated Sepharose at 1 mg/ml of wet gel, as described by Cuatrecasas (1970).

Other MABs and sera

Anti-IgE MAB was produced in our laboratory (Sanchez-Madrid *et al.*, 1984). The 187-1 anti-mouse kappa chain MAB was the kind gift of Dr M. Scharff (Albert Einstein Medical School, N.Y.). Both MABs were purified on protein A-Sepharose and radio-labeled with ^{125} I using chloroglycoluril (Fraker and Speck, 1978).

Rabbit antiserum against *P. judaica* pollen extract was obtained in our laboratory (Corbí and Carreira, 1984). Normal rabbit IgG was purchased from Miles Laboratories (U.S.A.) and did not contain antibodies against the pollen proteins, as determined by radioimmunoassay, using the anti-*P. judaica* rabbit antiserum as the positive control.

Human sera used all through the research were obtained from 20 *P. judaica* sensitive patients. Individual serum specific IgE was measured by a radioallergosorbent test (RAST), and only those which were RAST class 3 or 4 (Pharmacia, Sweden) were pooled and aliquoted. The pool was maintained at -80°C until use.

Immunoprecipitation and immunoadsorption

The pollen proteins, iodinated by the chloramine-T method (Hunter and Greenwood, 1962) were pre-cleared with normal rabbit IgG-Sepharose to eliminate components with non-specific binding to the antibody-Sepharose complex. Then, ^{125} I-labeled pollen proteins were incubated by shaking for 1 hr with each MAB coupled to Sepharose. After washing 3 times with washing buffer (0.1% bovine hemoglobin and 0.1% Triton X-100 in PBS), once with PBS and once with 50 mM Tris-HCl, pH 6.8, samples were subjected to SDS-PAGE (Laemmli, 1970) under reducing conditions, unless otherwise indicated. Finally, gels were dried and autoradiographed with intensifying screens (Laskey and Mills, 1977).

Immunoadsorption assay was performed on a column of 1 ml of anti-human IgE MAB-Sepharose. Ten millilitres of *P. judaica* sensitive serum pool was passed through the column and after washing with PBS, the radiolabeled pollen proteins, previously pre-cleared on rabbit IgG-Sepharose, were applied on top of the column. The column was then extensively washed and the proteins retained were eluted with 1 ml of 100 mM citrate buffer, pH 3, and subjected to SDS-PAGE and autoradiography.

Immunodetection, crossed immunoelectrophoresis (CIE) and crossed radioimmuno-electrophoresis (CRIE)

Pollen extract proteins were separated by SDS-PAGE and transferred onto nitrocellulose membranes (Towbin *et al.*, 1979). After blocking the remaining adsorptive sites with 1% BSA in PBS, blots were incubated with culture supernatants diluted 1:5 in PBS with 0.1% BSA. It was followed by a final incubation with ^{125} I-anti-mouse kappa chain (187-1

MAB) in the same buffer. Then, blots were dried and autoradiographed. Immunodetection of IgE-binding polypeptides was performed as described (Corbi and Carreira, 1984). For CIE and CRIE of the pollen extract, the procedures reported by Weeke and Löwenstein (1973) and Weeke (1973) were followed. For detection of the precipitation bands recognized by the MAB, the CIE plates were incubated as for the immunodetection assay.

Purification of the Pj10-containing allergen and RAST inhibition

An excess of *P. judaica* pollen extract was passed through a column made of AC/1.1 MAB–Sephacrose previously washed with 3 M KSCN. After extensive washing with PBS to eliminate the non-retained components, the bound protein was eluted using 3 M KSCN and then dialyzed extensively against PBS. Analysis of the bound protein was done by SDS–PAGE followed by Coomassie blue staining. RAST inhibition was carried out to determine the ability of the allergen to inhibit the binding of specific IgE to the pollen proteins covalently linked to CNBr-activated paper discs. These were activated by incubation in a solution of CNBr in distilled water [5% (w/v)] for 5 min at pH 10.0–10.5, as reported by Ceska *et al.* (1972). For coupling, pollen extract was dissolved in 0.1 M PBS, pH 8, to a concn of 500 µg/ml, and incubated with the paper discs (45 ml of pollen extract solution/1 g of paper discs) for 12 hr at 4°C with shaking. They were then washed with the same buffer and 1 M Tris–HCl, pH 8, was added to block the remaining adsorptive sites. Once washed again, paper discs were used for RAST inhibition as described by Yman *et al.* (1975).

RESULTS

Selection and purification of MABs against IgE-binding molecules

Fourteen days after the fusion, 350 out of 400 culture wells presented hybrid growth and were screened for recognition of *P. judaica* pollen components using an indirect binding assay. Forty-five hybridoma supernatants yielded more than 5-fold above background binding to pollen extract and were considered positive. The binding ratios of 9 of them are shown in Table 1. Most of the MABs gave higher binding to pollen proteins linked to paper discs than to pollen proteins coated wells. These differences might be due to dissimilar presence of the bound antigens to both solid phases. In fact, this has been already reported for other proteins (Walsh *et al.*, 1984).

Twenty out of the 45 hybridoma supernatants were shown to display allergen binding ability by the four-step sandwich screening procedure. Those MABs which exhibited the highest binding percentage to allergens (AC/1.1, AC/7.1 and AC/15.1) are shown

Table 1. Binding of different MABs to *P. judaica* pollen proteins

MAB	Radioactivity bound ^a	
	Coated wells	Paper discs
	% (cpm)	% (cpm)
AC/1.1	3.3 (2000)	39 (15,600)
AC/4.1	13.2 (8000)	6 (2400)
AC/6.1	10.3 (6200)	18 (7340)
AC/7.1	6.1 (3700)	26 (10,500)
AC/8.1	8.3 (5000)	38 (15,440)
AC/9.1	15.0 (9000)	27 (10,770)
AC/10.1	11.6 (7000)	29 (11,500)
AC/12.1	3.3 (2000)	15 (6010)
AC/15.1	3.3 (2000)	25 (10,000)
P3X63	0.4 (250)	1.3 (540)

^aThe percentage of input radiolabeled rat anti-mouse kappa chain MAB (187-1) bound to the MAB which recognized pollen proteins on the solid phase. The P3X63 mouse myeloma supernatant was used to determine the non-specific binding.

in Table 2, together with the control AC/9.1 MAB that displayed the highest binding percentage to pollen protein-coated wells. The selected MABs had rates of binding more than 8-fold above background, indicating their allergen recognition capability. After purification of these 3 MABs, they were directly tested for allergen recognition by a sandwich radio-immunoassay similar to the one above described. They gave lower binding percentages than the corresponding hybridoma supernatants, although a similar amount of protein was used in both assays. It was probably due to a loss of activity in the elution process from the protein A–Sephacrose. In all cases, the percentage of bound ¹²⁵I-anti-IgE was rather low because of the very limited amount of specific IgE in serum. Nevertheless, comparison with background binding clearly stated the allergen-binding ability of AC/1.1, AC/7.1 and AC/15.1 MABs.

Identification of polypeptides recognized by anti-allergen MABs

Purified AC/1.1, AC/7.1 and AC/15.1 MABs coupled to Sepharose immunoprecipitated a 10-Kd polypeptide from ¹²⁵I-labeled pollen proteins (Fig. 1A, lanes 1–3). In previous studies (Corbi and Carreira, 1984), the most allergenic polypeptide of *P.*

Table 2. Specificity of anti-*P. judaica* MABs for IgE-binding components

MAB	Radioactivity bound ^a	
	Hybridoma supernatant	Purified MAB
	% (cpm)	% (cpm)
AC/1.1	8.5 (4250)	6 (3000)
AC/7.1	7.7 (3870)	3 (1500)
AC/15.1	6.6 (3300)	4 (2000)
AC/9.1	0.8 (400)	0.9 (450)
P3X63	0.8 (400)	0.8 (400)

^aThe percentage of input radiolabeled anti-human IgE antiserum bound to the specific IgE complexed to the allergens recognized by the MAB. Non-specific binding was determined using the P3X63 mouse myeloma culture supernatants. The AC/9.1 MAB was also included as control because it did not recognize any allergenic molecule.

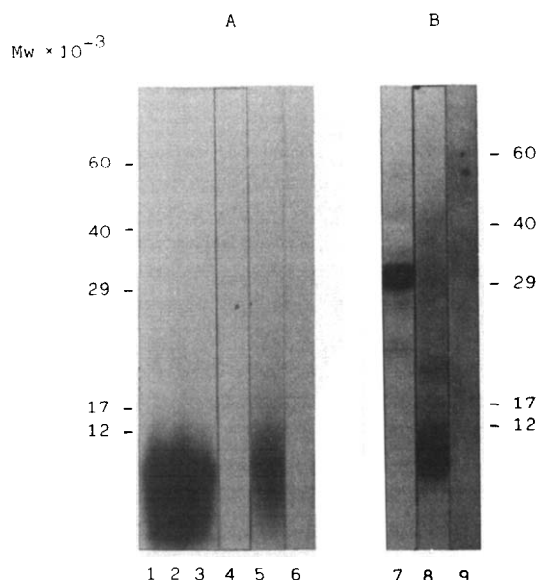


Fig. 1. (A) Immunoprecipitation of the 10-Kd polypeptide by the selected anti-allergen MABs, and identification of the main allergenic polypeptide under native conditions. Radioiodinated pollen proteins were immunoprecipitated with 25 μ l of AC/1.1 MAB- (lane 1), AC/7.1 MAB- (lane 2), AC/15.1 MAB- (lane 3) and rabbit IgG-Sepharose (lane 4), or immunoabsorbed on anti-human IgE MAB-Sepharose previously incubated with *P. judaica* (lane 5) or *Dermatophagoides pteronyssinus* (lane 6) sensitive patients serum pool. Reduced samples were subjected to SDS-PAGE (12%) and autoradiography with intensifying screens. Molecular wt markers were, from top to bottom: catalase (60 Kd), aldolase (40 Kd), carbonic anhydrase (29 Kd), myoglobin (17 Kd) and cytochrome *c* (12.5 Kd). (B) Immunodetection of polypeptides recognized by AC/9.1 MAB and *P. judaica* specific serum IgE, after blotting from a SDS-12% PAGE of the whole extract under reducing conditions. Sample in lane 7: AC/9.1 MAB (diluted 1:5) followed by ^{125} I-labeled 187-1 MAB; lane 8: *P. judaica* sensitive serum pool (diluted 1:5) followed by ^{125} I-labeled anti-human IgE MAB; lane 9: *P. judaica* non-sensitive serum (diluted 1:5) followed by ^{125} I-labeled anti-human IgE MAB, as control.

judaica pollen under SDS-denaturing conditions has been shown to have a mol. wt of 10,000 (Pj10) (Fig. 1B, lane 8). This coincidence suggested that the Pj10 glycopeptide could bear the antigenic determinant(s) recognized by the 3 selected MABs, a fact that was later confirmed. On the other hand, taking into account the IgE-binding ability of the molecule bound by the MAB in the 4-step radioimmunoassay, an immunoabsorption was carried out to identify the allergenic native structures of the pollen extract. For this purpose, ^{125} I-labeled pollen proteins were passed through a column of anti-human IgE MAB-Sepharose previously incubated with a *P. judaica* sensitive serum pool. SDS-PAGE and autoradiography of the retained components (Fig. 1A, lane 5) revealed Pj10 as the main bound molecule. This result indicated that most of the specific IgE contained in sera from patients sensitive to this pollen is directed against determinants located on the Pj10 glycopeptide.

Molecular wts of the components immuno-precipitated by the MABs (Fig. 1A, lanes 1–3) and *P. judaica* specific IgE (Fig. 1A, lane 5), and the one recognized by IgE under SDS-denaturing conditions (Fig. 1B, lane 8) were found to be identical, as determined by the method of Weber and Osborn (1969). To determine whether the Pj10 glycopeptide was the structure recognized by the MAB, and as Pj10 had been previously detected in the two main IgE-binding CIE precipitation bands (A1 and A2), CIE plates of the pollen extract were incubated with AC/1.1 MAB followed by ^{125} I-anti-mouse kappa chain MAB (Fig. 2A). AC/7.1 and AC/15.1 MABs yielded similar CRIE patterns to AC/1.1 MAB (data not shown). The 3 MABs were able to bind to A1 and A2 Pj10-containing bands, while AC/9.1, used as control, bound to an anodic band (Fig. 2B), which did not bind *P. judaica* specific IgE (Fig. 2C) (Corbí and Carreira, 1984). All these results demonstrated that AC/1.1, AC/7.1 and AC/15.1 Mabs were directed against antigenic determinant(s) located on Pj10.

In addition, the common immunoprecipitation and CRIE pattern rendered by the 3 MABs pointed out the possibility that these anti-allergen MABs were recognizing the same molecular structure. To further confirm this fact, a sequential immunoprecipitation was performed (Fig. 3). Preclearing with each anti-

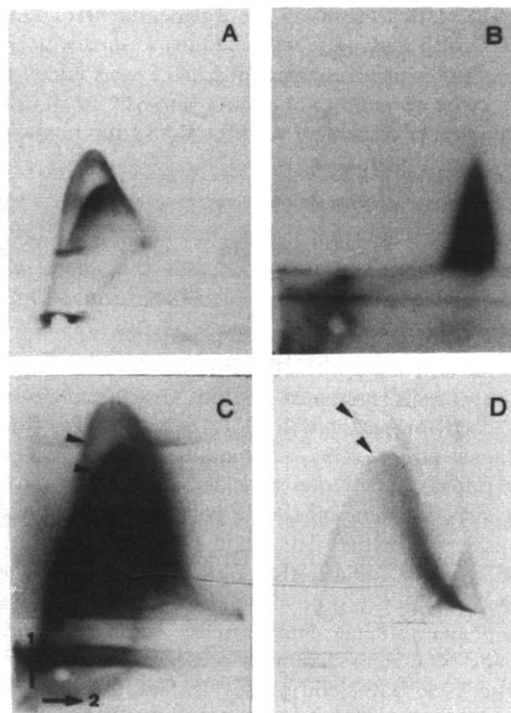


Fig. 2. Crossed-radioimmuno-electrophoretic patterns of *P. judaica* pollen components developed with AC/1.1 MAB (A), AC/9.1 MAB (B) and *P. judaica* sensitive serum (C), at a dilution ratio of 1:5. Further incubation steps were as in Fig. 1B. A typical crossed-immuno-electrophoretic pattern of the whole extract is shown in D. All the antigens shown were those displaying anodic mobility, as indicated in C.

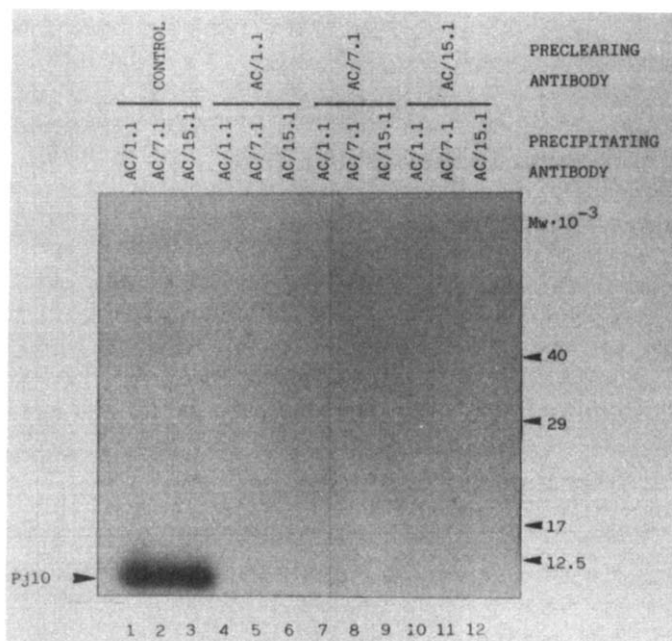


Fig. 3. Sequential immunoprecipitation of the Pj10 polypeptide. Radioiodinated pollen proteins were precleared with rabbit IgG- (lanes 1-3), AC/1.1 MAb- (lanes 4-6), AC/7.1 MAb- (lanes 7-9) and AC/15.1 MAb-Sepharose (lanes 10-12), with shaking for 1 hr at 4°C. The resulting supernatants were then immunoprecipitated with AC/1.1 MAb- (lanes 1, 4, 7 and 10), AC/7.1 MAb- (lanes 2, 5, 8 and 11) and AC/15.1 MAb-Sepharose (lanes 3, 6, 9 and 12). The reduced samples were subjected to SDS-12% PAGE and autoradiography. Molecular wt markers are the same as in Fig. 1.

allergen MAb coupled to Sepharose depleted the immunoprecipitated Pj10 glycopeptide. This result clearly showed that AC/1.1, AC/7.1 and AC/15.1 MAbs recognize epitope(s) on the same allergenic structure.

Purification of Pj10 in allergen-active form

Immunoprecipitation studies indicated that these MAbs coupled to Sepharose could be used for purification of the Pj10-containing molecule. Pollen proteins specifically adsorbed to a column of AC/1.1 MAb-Sepharose were eluted with 3 M KSCN. Analysis of the eluted proteins by SDS-PAGE and Coomassie blue staining only revealed the presence of the Pj10 glycopeptide (Fig. 4).

The purified allergen was then tested for its ability to inhibit the binding of *P. judaica* specific IgE (from the patients' serum pool) to the pollen components covalently linked to paper discs. The protein was able to inhibit, at least, 70% of the specific binding (Fig. 5). On comparing the inhibitory capabilities of the purified allergen and the whole extract, it was found that, for a similar percentage of inhibition, the Pj10-containing allergen was 5 times more effective than the whole extract. This was in spite of a possible loss of IgE-binding ability during the purification. Therefore, the Pj10-containing allergen, which is recognized by the 3 MAbs, accounts for most of the allergenic activity exhibited by the *P. judaica* pollen extract.

To determine whether the affinity-purified allergen showed the same CIE distribution as the native molecule, a mixture of ^{125}I -labeled purified allergen with unlabeled pollen extract proteins was subjected to CIE (Fig. 6). Coomassie blue staining on the CIE plate revealed the whole band pattern of the extract (Fig. 6B), while autoradiography showed the ^{125}I -Pj10-containing allergen to be distributed in the 2 allergenic precipitation bands A1 and A2 (Fig. 6A and C). The similarity of the distribution of A1 and A2 and the purified allergen pointed out that the AC/1.1 MAb recognizes an epitope which is borne on all the Pj10 structures.

Disulphide bridges in the Pj10-containing allergen

In a previous report (Corbi and Carreira, 1984), the absence of reducing agents in SDS-PAGE has been shown to alter the pattern of IgE-binding polypeptides after immunodetection. To further investigate the nature of the molecule recognized by the anti-allergen MAbs, an immunoprecipitation experiment was performed, either in the presence (Fig. 7, lane 1) or in the absence (Fig. 7, lane 2) of β -mercaptoethanol. Under non-reducing conditions, the allergen was detected as a 20-Kd band. As Pj10 was the only molecule recognized by AC/1.1, AC/7.1 and AC/15.1 MAbs, this result strongly suggested that the 20-Kd band was formed by 2 Pj10 glycopeptides linked by disulphide bridge(s). Nevertheless, this Pj10 dimer might not be the *P. judaica*

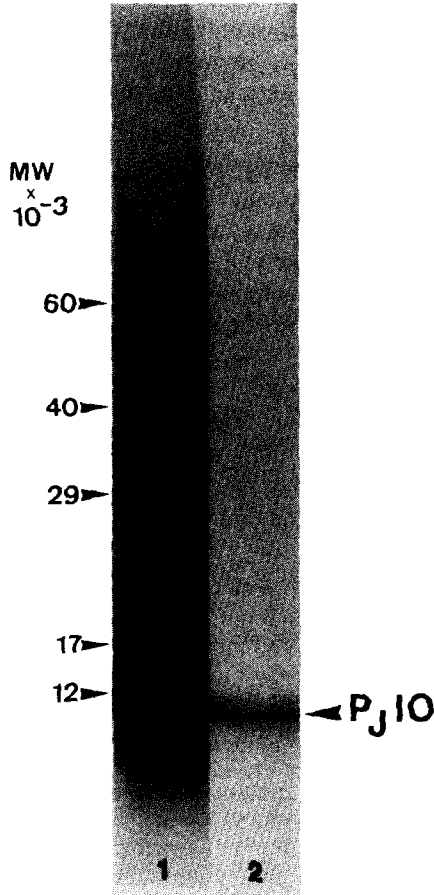


Fig. 4. Analysis of purified Pj10 polypeptide by SDS-PAGE. The Pj10 polypeptide was purified on AC/1.1 MAb-Sepharese as described in the text. Two aliquots corresponding to 500 μ g of *P. judaica* pollen protein (lane 1) and 10 μ g of purified Pj10 polypeptide (lane 2) were subjected to SDS-12% PAGE and subsequently stained with Coomassie blue. Molecular wt markers are the same as in Fig. 1.

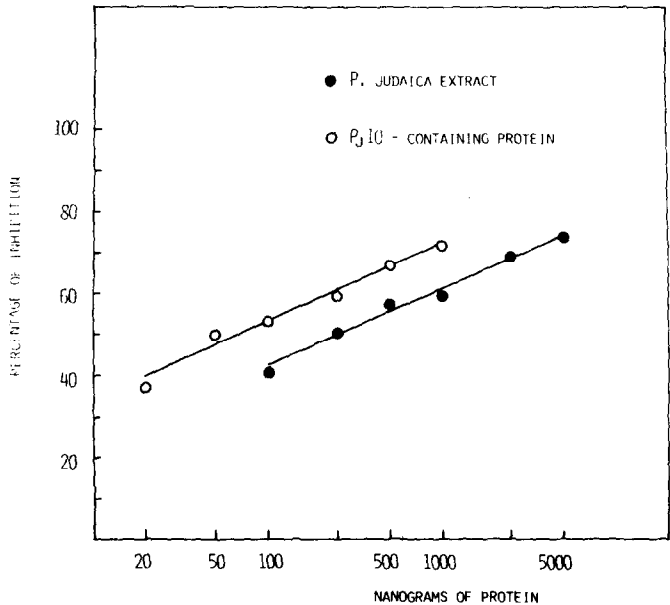


Fig. 5. Allergenic activity of the purified Pj10 polypeptide. *P. judaica* pollen extract (dark circles) and the Pj10-containing allergen (open circles) were tested for inhibition of the binding of specific human IgE from *P. judaica* sensitive serum pool to paper disc bound pollen extract. The data shown are inhibitions of ^{125} I-labeled anti-human IgE MAb binding to the paper discs at various protein concns. In both cases, when no inhibitory protein was added (0% inhibition) 10,000 cpm of binding were obtained. Background binding was 350 cpm.

pollen major native allergen because possible interactions between dimers and other molecules in a detergent-free medium cannot be ruled out.

DISCUSSION

The screening method for MABs to *P. judaica* components used 2 indirect-binding assays, with pollen proteins adsorbed to polyvinylchloride wells or covalently linked to CNBr-activated paper discs. The binding of individual MABs in the 2 different assays was quite dissimilar. The lack of correlation observed between the results obtained in both methods can be

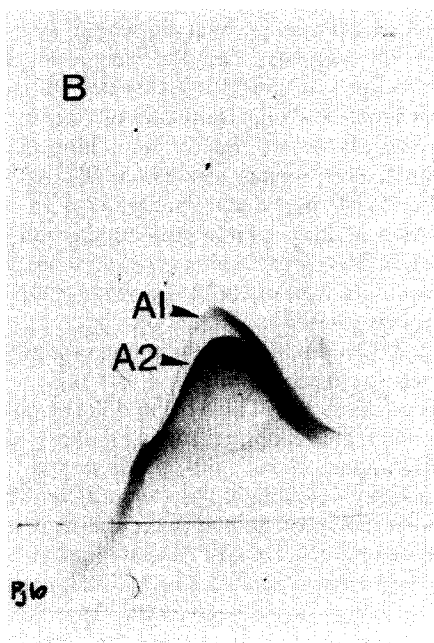


Fig. 6. Distribution of the affinity-purified Pj10-containing allergen. A mixture of the ^{125}I -labeled allergen with $200\text{ }\mu\text{g}$ of pollen extract protein was subjected to crossed immunoelectrophoresis. After drying the gel, it was developed for autoradiography (A) to detect the distribution of the allergen. For comparison with the whole-extract precipitation bands, the gel was later stained in a Coomassie blue solution (B). Detection of IgE-binding precipitation bands, shown in C, was carried out on a CIE plate run under identical conditions. The direction of each dimension is indicated in A.

explained by: (1) the higher amount of protein linked to the paper discs than adsorbed to the well, and (2) the different ability of a single protein of being physically adsorbed or covalently linked to a solid phase. This last case has already been reported by Walsh *et al.* (1984).

It is known that adsorption to plastic surfaces is mainly mediated by hydrophobic interactions with aromatic residues (Parsons, 1981). In the case of coating with pollen extract, presence of high amounts of pigments can impair the adsorption of certain pollen proteins. In addition, it has been demonstrated that, the higher the carbohydrate content, the worse the hydrophobic interaction with the plastic surface (Nieto *et al.*, 1984). On the contrary, covalent attachment to paper discs is only dependent on amino groups, thus avoiding interferences of aromatic residues. Taking all these facts together, distinct percentages of a single protein can be immobilized on both solid phases. For this reason, both indirect-binding assays were performed in the first screening, trying to avoid losses of MABs against antigens poorly represented in either of the solid supports.

The second radioimmunoassay, used for selection of allergen-binding MABs, allowed us a rapid and specific identification of the hybridoma supernatants

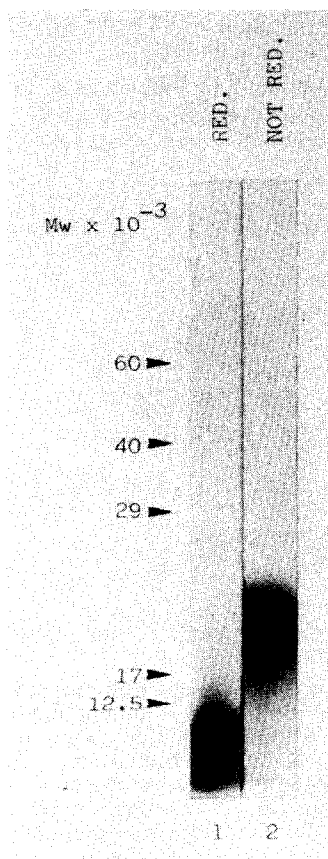


Fig. 7. Immunoprecipitation of the allergenic glycopeptide under nonreducing conditions. Radioiodinated pollen proteins were immunoprecipitated with 25 μ l of AC/1.1 MAb. Retained proteins were then eluted with Laemmli's sample buffer either with (lane 1) or without (lane 2) reducing agent (β -mercaptoethanol), and subjected to SDS-12% PAGE and further autoradiography. Molecular wt markers are the same as in Fig. 1.

that recognized IgE-binding molecules, in spite of the limited concn of specific IgE in serum which produced low binding percentages. In fact, this 4-step radioimmunoassay has been used to select MAbs directed against a minor allergen from *Dermatophagoides pteronyssinus* (Ley *et al.*, unpublished results).

The 3 selected MAbs immunoprecipitated a 10-Kd glycopeptide which was found to be the structure with the highest IgE-binding ability in *P. judaica* pollen. Analysis of the proteins immunoprecipitated by the anti-Pj10 MAb under nonreducing conditions yielded a single band corresponding to 20 Kd. It suggests that the main allergen under non-reduced/SDS-denatured conditions consists of 2 Pj10 glycopeptides linked by disulphide bridges. In a previous paper (Corbí and Carreira, 1984) we have already reported the linkage of Pj10 to other polypeptides or to itself by disulphide bridges, a feature that supports the above hypothesis. Although the possibility of further interactions between the Pj10 dimers cannot be ruled out, the attachment of 2

IgE-binding polypeptides by disulphide bridges would facilitate cross-linking of membrane-bound IgE molecules, thus leading to mast cell degranulation. Obviously, this fact does not imply the need of Pj10 dimers for histamine release to be triggered if the 10-Kd allergenic glycopeptide bears more than 1-allergenic determinant. As Pj10 has been detected in the 2 allergenic CIE precipitation bands, A1 and A2 (Corbí and Carreira, 1984), the immunoelectrophoretic heterogeneity of the ^{125} I-labeled affinity-purified allergen further confirms the nature of the molecule recognized by the 3 anti-allergen MAbs. This heterogeneity may be caused by the sugar moiety of the allergen, since 2 different subsets of Pj10 glycopeptide have already been described (Corbí and Carreira, 1985). One of the subsets was bound by a column of Con A-Sepharose, while the second was recovered in fractions which were unretained by Con A. The ability of AC/1.1 MAb to bind to an antigenic determinant shared by all the Pj10 glycopeptides is deduced from the wide distribution of the affinity-purified allergen, which overlaps with that of the A1 and A2 precipitation bands. Moreover, AC/1.1 MAb was able to recognize both fractions (bound and flowed-through) from the Con A-Sepharose column, supporting the above results. When all these facts are considered together, they suggest that the Pj10-containing allergen is composed of different sugar moieties attached to a common polypeptide.

On the other hand, the appearance of a blur in the immunoprecipitation studies was firstly thought to be due to the sugar heterogeneity in the glycopeptide. Nevertheless, analysis of the purified allergen by SDS-PAGE and Coomassie blue staining yielded no smear, indicating it was probably due to chemical modifications induced by chloramine-T used in the radiolabeling procedure. The property of Pj10 of being recognized by polyclonal specific IgE after SDS denaturation suggests that the allergenic determinants located on the Pj10 glycopeptide are either non-dependent on its conformation or recover their tertiary structure after transfer onto nitrocellulose membranes. This thought can also be inferred from a number of allergy-inducing pollens (Sutton *et al.*, 1982). As a matter of fact, immunodetection after SDS-PAGE has been used as the only assay for identification of the proteins recognized by MAbs in some reports (Smart *et al.*, 1983).

The 3 MAbs described in the present report have been used for the purification of the main native allergen protein from *P. judaica* pollen. The purified Pj10-containing allergen accounts for most of the total IgE-binding ability both in native and under SDS-denaturing conditions. Monoclonal antibodies to allergenic polypeptides will be very useful reagents not only for the biochemical characterization of the IgE-binding molecules, but also for the standardization of allergy-producing extracts. The anti-Pj10 MAbs here reported are presently used in our lab to

quantify the Pj10 polypeptide contained in different pollen extracts. In addition, the quantification of this polypeptide in *P. judaica* related pollens can be used to determine the potential allergenic activity of some other pollens. Furthermore, the use of the anti-Pj10 MAb Urticaceae for isolation of the native allergen will provide invaluable material for the therapy and diagnosis of *P. judaica* sensitive patients.

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