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Isolation and Characterization of *Poa p* I Allergens of Kentucky Bluegrass Pollen with a Murine Monoclonal Anti-*Lol p* I Antibody¹

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Abstract. The *Poa p* I allergens were isolated from the retentate fraction of a dialyzed preparation of an aqueous extract of Kentucky bluegrass pollen by means of a reverse immunosorbent prepared with a murine anti-*Lol p* I monoclonal antibody, Mab 290-A-167. By sodium dodecyl sulphate-polyacrylamide gel electrophoresis and preparative isoelectrofocusing, *Poa p* I was found to consist of a 35.8-kD component with an isoelectric point of 6.4 and a 33-kD component with one of 9.1 and designated as *Poa p* Ia (acidic) and *Poa p* Ib (basic), respectively. The relative protein content of these components was estimated from the intensity of the stained bands following sodium dodecyl sulphate-polyacrylamide gel electrophoresis. Thus, *Poa p* Ia appeared to be the major protein constituent, and on Western immunoblot it also bound the monoclonal antibody to a greater extent than *Poa p* Ib. On the other hand, *Poa p* Ib was shown by Western immunoblot and autoradiographic analysis, to bind to a greater extent the IgE antibodies present in a pool of sera from grass-allergic individuals. Therefore, *Poa p* Ib was considered as the major allergenic component of *Poa p* I. By competitive inhibition of the radioallergosorbent test, it was demonstrated that the Mab inhibited the binding of *Poa p* I allergens to human IgE antibodies to the extent of 70%. Hence, it is suggested that Mab and human IgE antibodies recognize identical or closely related determinants of *Poa p* I allergens.

Introduction

Crude pollen extracts commonly used in immunotherapy are heterogeneous mixtures of allergenic and non-allergenic components. Thus, treatment with a crude extract can induce, in some patients, sensitivity to some pollen constituents to which they were not originally sensitive [Richter et al., 1957; Marsh et al., 1972; Umetsu et al., 1985]. Purified and well-characterized allergens, therefore, are essential for producing modified allergens or developing other rational approaches for immunotherapy. However, the conventional physicochemical techniques which are used for the isolation and purification of allergens from a complex mixture of components usually involve a combination of several methods and may still be unable to separate components with similar physico-

chemical properties. On the other hand, with the availability of monoclonal antibodies (Mabs) to a wide variety of allergens [Kisil et al., 1980; Kahn and Marsh, 1983; Ekramoddoullah et al., 1986], individual allergenic constituents can be readily isolated in a single step using reverse immunosorbents prepared with monoclonal antibodies [Ekramoddoullah et al., 1986a; Olson and Klapper, 1986; Kahn and Marsh, 1982].

Kentucky bluegrass pollen is one of the major hay fever causing agents in the prairies and western regions of North America and also in various areas of Europe. Studies in our laboratory [Chakrabarty et al., 1981; Ekramoddoullah et al., 1982, 1986a, b] and those of others [Kahn and Marsh, 1983; Weber, 1981; Mourad et al., 1986] have demonstrated extensive antigenic and allergenic cross-reactivities among Kentucky bluegrass, ryegrass, timothy, false oat, orchard meadow, and velvet grass pollens. Antigen *Lol p* I (Rye gr-I) is a major allergen of ryegrass pollen.

¹ This work was supported by grants from the Medical Research Council of Canada.

Based on their similar physical and immunochemical properties to *Lol p I*, gr-I-type antigens have been found in other grass pollens [Kahn and Marsh, 1983; Mourad et al., 1986]. The present study reports the utilization of the murine Mab 290-A-167 produced against *Lol p I* antigen [Mourad et al., 1986] for the isolation and characterization of the cross-reactive *Poa p I* antigens from Kentucky bluegrass pollen.

Materials and Methods

Preparation of KBG-R

The non-dialyzable components of Kentucky bluegrass (*Poa pratensis*; Hollister-Stier Laboratory, Mississauga, Ont., Canada) were prepared by extracting ether-defatted pollen with H₂O followed by dialysis through Spectrapor membrane tubing (molecular weight cutoff 6–8 kD; Spectrum Medical Industries, Los Angeles, Calif., USA). The retentate fraction was designated as KBG-R.

Production and Purification of Mab 290-A-167

Mab 290-A-167 was one of several anti-*Lol p I* (anti-Rye gr-I) Mabs recently described by Mourad et al. [1986]. Briefly, BALB/c female mice were immunized with purified *Lol p I* antigens (obtained from the National Institutes of Health, Bethesda, Md., USA) emulsified in Freund's complete adjuvant. The spleen cells from the immunized mice were fused with SP2/0-Ag14 myeloma cells. Monoclonal cell lines were produced by the limiting dilution method. Clones of hybridoma cells secreting antibody to *Lol p I* were expanded in vitro and then propagated in the peritoneum of BALB/c female mice. The ascitic fluid containing the Mab was collected and dialyzed through Spectrapor membrane tubing (molecular weight cutoff 6–8 kD) against buffer A (0.025 M triethanolamine-HCl, pH 7.7) at 4°C overnight. The non-dialyzable components were centrifuged at 20,200 g for 10 min, and the supernatant was filtered through a sterile Millipore membrane (0.45 µm pore size, Millex-HA; Millipore, Bedford, Mass., USA). The filtered material was then fractionated by fast-protein liquid chromatography using a Mono Q (HR 5/5) anion-exchange column (Pharmacia Fine Chemicals, Montreal, Que., Canada) equilibrated with buffer A. The proteins were eluted by applying a linear gradient of buffer B (buffer A containing 0.354 M NaCl). The fractions containing antibody activity were pooled. The isotypicity of the Mab was determined by enzyme-linked immunosorbent assay (ELISA) as outlined by Ekramoddoullah et al. [1984] and confirmed to be IgG2b (k) [Mourad et al., 1986].

Allergic Human Serum

Serum was obtained in Manitoba during 1976 from 6 patients highly allergic to grass pollen. The sera were pooled, stored at -20°C, and have been used in this laboratory for the long-term studies on the allergenic constituents of Kentucky bluegrass pollen.

Enzyme-Linked Immunosorbent Assay

ELISA was performed with the 96-well EIA plate (cat. No. 3590; Costar, Cambridge, Mass.) as described by Ekramoddoullah et al. [1986a] with the following modifications: The plates

were coated with antigen (KBG-R or *Poa p I*, 10 µg/well) at 4°C overnight and blocked with bovine serum albumin 1% in phosphate-buffered saline (PBS; 0.54 g KH₂PO₄, 7.75 g NaCl, 0.5 g NaN₃, H₂O to make 1 liter, pH 7.2). For assaying the antibody activity of the fast-protein liquid chromatography fractions, the KBG-R-coated plates were incubated with the various fractions diluted tenfold with assay buffer (AB; PBS containing 0.5% bovine serum albumin and 1% Tween 20) for 1 h. After washing the plates with PBS containing Tween 20 (1%), a conjugate of rabbit anti-mouse IgG alkaline phosphatase (Sigma, St. Louis, Mo., USA) diluted 1,000-fold with AB was added and incubated for 1 h. Following washings, the enzyme substrate, *p*-nitrophenol phosphate (1 mg/ml), was added, and the colour reaction was developed for 40 min, then stopped by adding 3 M NaOH. The optical density was measured at 410 nm using the Microplate Reader (model MR600; Dynatech Laboratories, Alexandria, Va., USA).

For assaying the antigenic activity of purified *Poa p I* antigens, the Mab 290-A-167 preparation was serially diluted with AB and then added to the *Poa p I* antigen coated plates and incubated overnight. The ELISA was continued as described above.

For determining the allergenic activity of *Poa p I* by ELISA, the allergic human serum pool was serially diluted, added to *Poa p I* coated plates, and incubated overnight. After washing, a conjugate of goat antihuman IgE alkaline phosphatase (Sigma) diluted 1,000-fold with AB was added and incubated overnight. The ELISA was then continued as described above.

Preparation of Reverse Immunosorbent

The reverse immunosorbent was prepared as described previously [Ekramoddoullah et al., 1986a] with the following modifications: CNBr-activated Sepharose 4B (8.6 g; Pharmacia) was washed with 30 ml of an ice-cold aqueous solution of HCl (10⁻³ M). Eighteen millilitres of the Mab 290-A-167 preparation at a concentration of 3.42 mg/ml was mixed with the gel in a stoppered plastic centrifuge tube. The gel suspension was rotated end-over-end for 1 h. The excess antibody was washed away with coupling buffer. From a measurement of the optical density of the combined washings, it was estimated that 2.8 mg of the Mab was coupled to 1 ml of the gel. Any remaining active groups were blocked by incubating the gel with 0.1 M Tris-HCl buffer (pH 8). The gel was alternately washed with acidic and basic buffers according to the manufacturer's directions. The reverse immunosorbent, thus obtained, was then washed with 4 M guanidine-HCl buffer (pH 3.8) followed by PBS.

Affinity Chromatography

A solution of KBG-R (50 mg dissolved in 30 ml of PBS) was applied onto the reverse immunosorbent column. The column was then thoroughly washed with PBS. The material which had bound to the immunosorbent column was eluted with 4 M guanidine-HCl containing 0.25 M sodium acetate (pH 3.8). The eluate was immediately neutralized with 2 M Tris-HCl (pH 8.5). The eluted material, designated as *Poa p I*, was dialyzed against an aqueous solution of 10⁻³ M ammonium bicarbonate at 4°C overnight.

Measurement of Protein Content

The protein content of *Poa p I* was determined by the method of Lowry et al. [1951]. The protein content of the purified antibody was determined by its optical density at 280 nm (using $E_{280}^{1\%} = 13.8$ for mouse IgG).

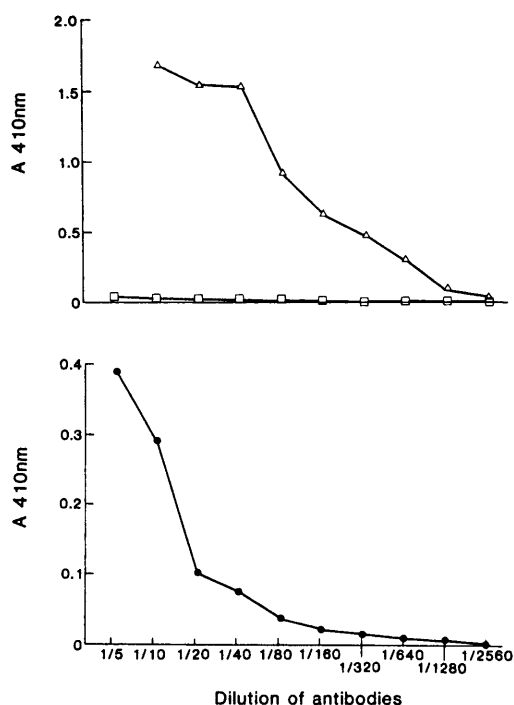


Fig. 1. Antigenic and allergenic activities of *Poa p I* antigens as demonstrated by ELISA. Plates were coated with *Poa p I* (10 µg/well). Top panel: Serial dilutions of purified Mab 290-A-167 (0.85 mg/ml; Δ) or Mab 27 in ascites form ($\square$) were added to the antigen-coated wells. A conjugate of rabbit antimouse IgG and alkaline phosphatase was used as the second antibody. Bottom panel: Serial dilutions of an allergic serum pool were added to antigen-coated wells ($\bullet$). Goat antihuman IgE conjugated to alkaline phosphatase was used as the second antibody.

Polyacrylamide Gel Electrophoresis

Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE). This was performed in the protein slab cell apparatus (Bio-Rad; Richmond, Calif.) with 1.5 mm thick polyacrylamide gels (12%) utilizing the buffer system of Laemmli [1970]. The sample was incubated in sample buffer overnight at room temperature prior to electrophoresis. The gel was stained with Coomassie brilliant blue R250 (Eastman Kodak, Rochester, N.Y., USA) according to the method of Fairbanks et al. [1971]. Low molecular weight protein standards (Bio-Rad) were used to calibrate the gels for the determination of the relative molecular weights (M_r) of the electrophoresed components.

Western Blot. Proteins separated by SDS-PAGE were electrophoretically transferred onto nitrocellulose membrane [Towbin et al., 1979]. To block its ability to further bind protein, the membrane was incubated overnight with gelatin (3%) in Tris-buffered saline (TBS) (25 mM Tris-HCl, 0.5 M NaCl, pH 8.3).

Immunoblot. Detection of proteins on the nitrocellulose was achieved by immunoblot as follows: After blocking, the membrane was incubated with Mab 290-A-167 (8.5 µg/ml in TBS containing 1% gelatin) at room temperature overnight. The membrane was washed with TBS, then incubated with goat antimouse IgG horse-

radish peroxidase conjugate (Bio-Rad; diluted 3,000-fold with 1% gelatin in TBS) at room temperature for 1 h. After washing the membrane with TBS, the colour reaction was developed by adding a freshly prepared mixture of horseradish peroxidase colour development reagent: 4-chloro-1-naphthol (Bio-Rad) and 30% H_2O_2 .

Autoradiography. Following the separation of *Poa p I* antigens by SDS-PAGE and transfer to nitrocellulose, the membrane was immersed in a solution of the human allergic serum pool (diluted fivefold with TBS containing 1% gelatin) at room temperature for 40 h. The membrane was washed in TBS and then incubated with ^{125}I -labelled murine monoclonal antihuman IgE antibody (6×10^6 cpm in 60 ml of TBS containing 1% gelatin) overnight. (This antibody, clone No. CIA/E/4.15, specific for human IgE, was generously supplied by Dr. A. Saxon, UCLA, Los Angeles, Calif., USA). The membrane was washed with PBS containing Tween 20 (1%) and then air dried and exposed to X-ray film (Kodak) at $-70^\circ C$ for 4 h. The X-ray film was developed and fixed according to the manufacturer's directions.

Preparative Isoelectrofocusing (IEF)

One hundred milligrams of KBG-R was fractionated by preparative IEF as described by Ekramoddoullah et al. [1986a]. Briefly, Ultrodex (6 g) (LKB Products, Stockholm-Bromma, Sweden) was swollen in a solution (150 ml) of KBG-R (100 mg) and the ampholyte buffer (7.5 ml, Ampholine, pH range 3.5–10; LKB) and poured onto a horizontal glass tray (11 $\times$ 30 $\times$ 0.8 cm; Bio-Rad).

The IEF was carried out at $4^\circ C$ for 96 h. The gel was then divided into 38 equal sections with the aid of a gel divider (Bio-Rad). Each gel section was eluted with water (5 ml). The absorbance, at 280 nm, and the pH of each eluted fraction were measured. The ability of each fraction to bind the anti-*Poa p I* Mab 61 and Mab 62 was examined by ELISA. The specificity of these Mabs is described in the Results section.

Competitive Inhibition of Radioallergosorbent Test (RAST)

This was carried out as described previously [Ekramoddoullah et al., 1987] with the following modifications. Disposable flexible polyvinylchloride plates were coated with *Poa p I* (2 µg/well) as described for the ELISA. The plates were blocked with incubation buffer (IB; PBS containing 0.3% human serum albumin). A pool of six sera obtained from grass-allergic patients was diluted 2.5-fold with IB and mixed with an equal volume of Mab 290-A-167 in varying dilutions. As a control, Mab 12, which is specific to a KBG pollen antigen unrelated to *Poa p I*, was diluted and used in the same way as Mab 290-A-167. One hundred microliters of the mixtures was added to each well. The plates were incubated at $4^\circ C$ overnight. The extent to which the IgE antibodies bound to the allergosorbent was evaluated by the addition of ^{125}I -labelled mouse monoclonal antihuman IgE antibody. The degree of the inhibition of the binding of the IgE antibodies to the allergosorbent was assessed in terms of the corresponding decrease in the uptake of the ^{125}I -anti-IgE antibody. This was determined in relation to the amount of radioactivity bound to the allergosorbent in absence of the inhibitor, Mab 290-A-167, and was expressed as the percent of inhibition of RAST by the formula

$$\% \text{ inhibition} = (A - B) / A \times 100$$

where A = cpm in the absence of inhibitor, and B = cpm in the presence of inhibitor.

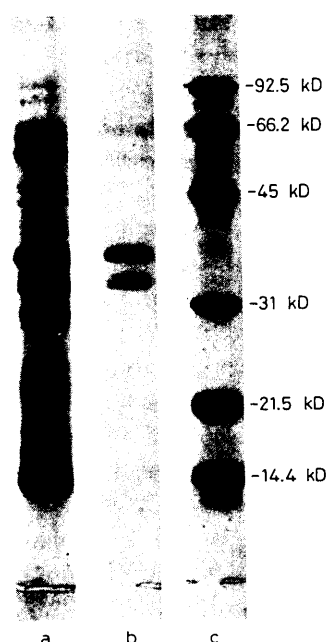


Fig. 2. SDS-PAGE. Column a: KBG-R (100 µg); column b: *Poa p I* (40 µg); column c: protein markers (2.5 µg each). All samples were reduced with 2-mercaptoethanol prior to electrophoresis.

Results

The allergenic and antigenic properties of *Poa p I* isolated from KBG-R with the Mab 290-A-167 immunosorbent were evaluated by ELISA in terms of the ability of *Poa p I* to bind IgE antibodies present in the pool of human grass allergic sera and Mab 290-A-167, respectively. Figure 1 (bottom panel) clearly shows that the *Poa p I* antigens were capable of binding the human IgE antibodies. *Poa p I* also bound Mab 290-A-167 (see top panel of figure 1). The specificity of the assay with Mab 290-A-167 was demonstrated by the observation that another murine monoclonal antibody, Mab 27 (IgG1, κ), produced against KBG-R [Kisil et al., 1980; Ekramoddoullah et al., 1986a] and possessing a specificity unrelated to Mab 290-A-167, did not bind to the *Poa p I* antigens. Moreover, the antigenic components of *Poa p I* which could be detected by Western and immunoblot analysis (see figure 4) with Mab 290-A-167 failed to bind Mab 27 on the immunoblot (data not shown). By means of SDS-PAGE, using either reducing or non-reducing conditions, the affinity-purified *Poa p I* was shown to be composed of two components with M_r of 35.8 and 33 kD (fig. 2, column b), and they have been

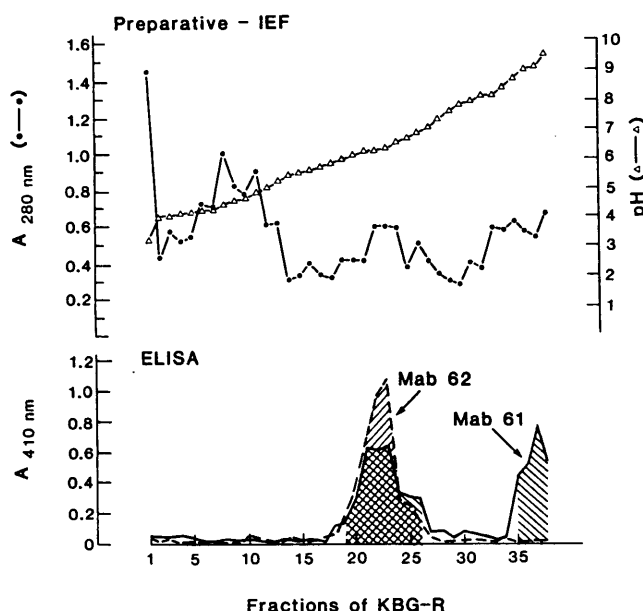


Fig. 3. Preparative IEF of KBG-R (100 mg) on Ultradex (Ampholine, pH 3.5–10, 96 h). The extent to which the fractions bound Mabs 61 and 62 on ELISA is indicated with the solid and dashed lines, respectively.

provisionally designated as *Poa p Ia* (acidic) and *Poa p Ib* (basic), respectively (see below). Based on their intensity of staining, it would appear that *Poa p Ia* was the major protein component of the *Poa p I* antigens. *Poa p Ia* was also shown to be one of the major protein components of KBG-R (fig. 2, column a).

Poa p I was used to immunize the mice to produce Mab. Based on their specificity, two groups of anti-*Poa p I* Mabs were obtained (details will be reported elsewhere). One group bound both *Poa p Ia* and *Poa p Ib*, whereas the other group bound only *Poa p Ia*. Surprisingly, none of the clones secreting antibody to *Poa p I* was directed exclusively to *Poa p Ib*. These results were interpreted to indicate that *Poa p Ia* is the major immunogenic component of *Poa p I*.

The isoelectric point (pI) values of the components of *Poa p I* were determined by preparative IEF of KBG-R. (KBG-R was used due to a lack of a sufficient amount of the purified *Poa p I*.) The separated fractions were analyzed by ELISA using Mab 61 which recognized both *Poa p Ia* and *Poa p Ib* and Mab 62 which recognized *Poa p Ia* (fig. 3). The results revealed that the components of KBG-R capable of binding Mab 61 were associated with well-separated acidic (pI ~6.4) and basic (pI ~9.1) fractions. On the



Fig. 4. SDS-PAGE and Western immunoblot analysis of KBG-R and *Poa p I*. The immunoblot was developed with Mab 290-A-167. Column a: *Poa p I* (40 μ g); column b: KBG-R (150 μ g).

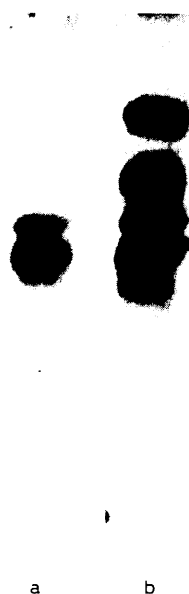


Fig. 5. Detection of allergenic components in *Poa p I* and KBG-R by SDS-PAGE. Western immunoblot and autoradiography. Components separated by SDS-PAGE were transferred onto the nitrocellulose membrane. The membrane was then incubated with a human allergic serum pool. The components which bound human IgE antibody were detected by employing ^{125} I-labelled murine monoclonal anti-human IgE antibody as described in 'Materials and Methods'. Column a: *Poa p I* (40 μ g); column b: KBG-R (100 μ g).

other hand, the components of KBG-R capable of binding Mab 62 were associated only with the acidic fractions. Since Mab 61 recognizes both *Poa p Ia* and *Poa p Ib*, and Mab 62 recognizes only *Poa p Ia*, it was concluded that *Poa p Ia* was acidic and *Poa p Ib* was basic. This conclusion was confirmed on further examination of the acidic and the basic fractions by SDS-PAGE and Western immunoblot analysis employing Mabs 61 and 62 (data not shown). Also, similar results were obtained by using anti-*Lol p I* Mabs 6, 8, 11, and 20, of which Mabs 6 and 11 bound both *Poa p Ia* and *Poa p Ib*, while Mabs 8 and 20 bound *Poa p Ia* only (data not shown). (Mabs 6, 8, 11, and 20 were produced in our Hybridoma Unit of the MRC Group for Allergy Research, using *Lol p I* antigen that was kindly supplied by Dr. D. Marsh, Department of Medicine, Johns Hopkins University, Baltimore, Md., USA.)

For further analyses of *Poa p I*, combinations of SDS-PAGE, Western immunoblot and autoradiography techniques were used. Both components of *Poa p*

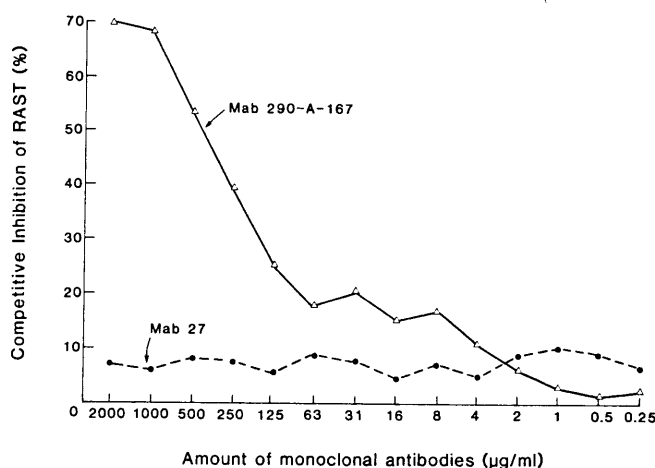


Fig. 6. Competitive inhibition of RAST. The plates were coated with *Poa p I* (2 μ g/well). One hundred microliters of the mixtures containing 50 μ l of human allergic serum (diluted 2.5-fold with IB) and 50 μ l of Mab 290-A-167 in varying amounts was added to each well. Similarly, Mab 27 in varying amounts was used for control. The extent of binding of human IgE to *Poa p I* was evaluated by the addition of ^{125} I-labelled mouse monoclonal antihuman IgE antibody (1×10^5 cpm/well).

I separated on SDS-PAGE were capable of binding Mab 290-A-167 (fig. 4, column a). As expected, these two components were also detected in KBG-R (fig. 4, column b). The major protein-staining component, *Poa p* Ia (35.8 kD, cf. fig. 2, column b) strongly bound the monoclonal antibody (fig. 4, column a). By contrast, the minor protein-staining component, *Poa p* Ib (33 kD, cf. fig. 2, column b) reacted more strongly with human IgE antibodies as revealed by autoradiography (fig. 5, column a) and is, therefore, referred to as the major allergenic component. At least four radiostaining bands were detected on the autoradiograph in the analysis of KBG-R (fig. 5, column b). These results indicated that KBG-R possessed at least four groups of allergenic components which differed in their sizes. Clearly, *Poa p* Ib is one of the major allergens of KBG-R.

By competitive inhibition of RAST, it was found that Mab 290-A-167 was able to inhibit the binding of human IgE antibodies to *Poa p* I up to the extent of 70% (fig. 6). Mab 27, used as a control, was unable to inhibit the binding of human IgE to *Poa p* I.

Discussion

A murine monoclonal antibody, Mab 290-A-167, which had been prepared against the *Lol p* I antigens of Rye grass pollen [Mourad et al., 1986], was used to identify the cross-reactive *Poa p* I antigens of Kentucky bluegrass pollen. Whereas the *Lol p* I antigens have been reported [Mourad et al., 1986] to consist of a major component with a M_r of 34 kD and a minor component of 23 kD, the analysis of the retentate fraction of the aqueous extract of KBG pollen by a combination of SDS-PAGE and Western immunoblot with Mab 290-A-167 detected two components with M_r of 35.8 and 33 kD which were designated as *Poa p* Ia and *Poa p* Ib, respectively. These two components of *Poa p* I were isolated directly from KBG-R by means of a reverse immunosorbent prepared with Mab 290-A-167. The 35.8-kD component (*Poa p* Ia) stained more heavily than the 33-kD component (*Poa p* Ib) and was, therefore, regarded as having the higher protein content. Western immunoblot analysis revealed, as expected, that both components bound Mab 290-A-167. Since *Poa p* Ia bound Mab 290-A-167 to a greater extent than *Poa p* Ib, it is suggested that the density and/or the total number of epitopes recognized by Mab 290-A-167 is greater in *Poa p* Ia

than in *Poa p* Ib. In any event, it was obvious that *Poa p* Ia and *Poa p* Ib each possessed at least one epitope that was similar, if not identical, to the other. This conclusion was substantiated by the finding that both components bound also the anti-*Poa p* I Mab 61 and the anti-*Lol p* I Mabs 6 and 11 [Bose et al., 1986]. On the other hand, evidence was obtained to indicate that *Poa p* Ia possessed at least another epitope that was not present on *Poa p* Ib. (This additional epitope on *Poa p* Ia was detected by anti-*Lol p* I Mabs 8 and 20 and anti-*Poa p* I Mab 62.) As a corollary, it follows that the specificities of Mabs 290-A-167, 6, 11, and 61 are different from Mabs 8, 20, and 62. This differential ability of the Mabs to bind to either *Poa p* Ia or both *Poa p* Ia and *Poa p* Ib was used to identify that *Poa p* Ia focused at a pH value of ~ 6.4 and *Poa p* Ib at one of ~ 9.1 .

It appeared that *Poa p* Ib had a greater capacity to bind IgE antibodies of sera from grass-allergic individuals. Therefore, it is suggested that the density and/or the total number of epitopes recognized collectively by the human IgE is greater for *Poa p* Ib than for *Poa p* Ia.

The ability of Mab 290-A-167 to inhibit the binding of human IgE to *Poa p* I suggested that this Mab and human IgE antibodies recognized identical or closely related determinants of the *Poa p* I allergens.

One of our recent findings demonstrated that murine Mab 27, produced by immunization of mice with KBG-R, had the ability to bind strongly a 30-kD component of the *Lol p* I antigen [Ekramoddoullah et al., 1986a]. Therefore, the possibility that Mab 27 could bind to *Poa p* I was considered. However, the results of the present study revealed that neither *Poa p* Ia nor *Poa p* Ib was able to bind Mab 27. It was concluded that the specificity of Mab 27 was different from Mab 290-A-167 and the other Mabs used in the present study.

According to the physical and immunochemical properties of *Lol p* I (group I) antigens, *Poa p* Ia belongs to the group I antigens. On the other hand, it may not be entirely appropriate to classify *Poa p* Ib as a group I antigen, since its pI (highly basic) is markedly different from that of the group I antigens (acidic). The use of the nomenclature *Poa p* Ia and *Poa p* Ib is provisional and is solely for convenience to distinguish the acidic component, *Poa p* Ia, from the basic component, *Poa p* Ib. Clearly, further studies are required to elucidate the nature and the extent of the relationship of these two components.

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Received: April 4, 1988

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