

MONOCLONAL ANTIBODIES TO THE MAJOR
LOLIUM PERENNE (RYE GRASS) POLLEN
ALLERGEN *Lol p* I (Rye I)*†

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Abstract—Thirteen monoclonal antibodies (MAbs) were produced against *Lol p* I (Rye I), the major *Lolium perenne* (rye grass) pollen allergen. Spleen cells from A/J and SJL mice immunized with highly purified *Lol p* I (*Lol* I) were allowed to fuse with cells from the non-secreting Sp2/0-Ag14 myeloma cell line. Each MAb was analyzed for antigenic specificity by radioimmunoassay (RIA) using ¹²⁵I-*Lol* I. The epitope specificities of seven of the MAbs were examined by competitive binding against a labelled standard MAb for the *Lol* I antigen (Ag). The dissociation constant, K_d , of one MAb (No. 3.2) that was studied most extensively was determined by double Ab RIA to be 3.5×10^{-6} L/M. This MAb recognized the related 27,000–30,000 Group I glycoproteins found in the pollens of nine other species of grass pollens tested, including weak binding to Bermuda grass Group I (*Cyn d* I), which by conventional analysis using polyclonal anti-*Lol* I serum shows no detectable binding. Monoclonal antibody No. 3.2 was coupled covalently to Sepharose 4B and used to prepare highly purified *Lol* I from a partially purified rye pollen extract. Finally, an RIA was developed which permitted the analysis of the Group I components in rye grass and nine other grass pollen species. The latter assay is likely to prove useful in the standardization of grass pollen extracts according to their Group I contents.

INTRODUCTION

Polyclonal antibodies have permitted the immunologist to investigate the heterogeneity of the different antigenic components contained within allergenic extracts, but have not allowed the resolution of individual antigenic determinants on allergen molecules. The advent of the monoclonal antibody

(MAb)‡§ technique (Kohler and Milstein, 1975) has allowed more precise information to be obtained concerning antigenic structure and allergic function. With this principle in mind, we generated and analyzed a group of MAbs with specificities for *Lol p* I (Rye I), the 27,000 glycoprotein which is the major allergenic constituent in rye grass (*Lolium perenne*) (Johnson and Marsh, 1965; Marsh, 1975). Homologues of this molecule are also found throughout much of grass family (Gramineae) as indicated by cross-reactivity studies (Marsh *et al.*, 1970; Baer *et al.*, 1974).

The generation of 13 different MAbs specific for *Lol p* I (*Lol* I) and partial characterization of the specificities of seven of the MAbs will be described. One MAb (No. 3.2) was biochemically characterized and used in an affinity purification scheme, which allows rapid purification of *Lol* I. This particular Ab binds to the Group I homologues of nine other members of the grass family, including Bermuda grass, which shows no cross-reactivity with *Lol* I by conventional immunodiffusion techniques.

MATERIALS AND METHODS

Antigens

A dialyzed extract of defatted rye grass (*Lolium perenne*) pollen was prepared as previously described (Johnson and Marsh, 1965). In addition, non-dialyzed 1/10 (w/v) extracts (24 hr at 4°C) of the

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‡Abbreviations: Ab, antibody; Ag, antigen; BBS, borate-buffered saline (0.08 M boric acid, 0.15 M NaCl, pH 8.0); BSA, bovine serum albumin; CIE, crossed immunoelectrophoresis; DEAE, diethylaminoethyl; DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethylsulfoxide; G α M Fab, goat anti-mouse Fab (of IgG); HAT, hypoxanthine-aminopterin-thymidine; IEF, isoelectric focusing; IEP, immunoelectrophoresis; *Lol p* I and *Lol* I, the major allergen from *Lolium perenne* (rye) pollen; MAb, monoclonal antibody; PBS, phosphate-buffered saline (0.05 M potassium phosphate, 0.15 M NaCl, pH 7.0); PEG, polyethylene glycol; G α M IgG, goat anti-mouse IgG; RIA, radioimmunoassay; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; Sp2/0, Sp2/0-Ag14 non-Ig-secreting cell line.

§The nomenclature system that has been developed by the IUIS Allergen Nomenclature Sub-Committee (Marsh *et al.*, WHO Bulletin, submitted for publication) will be used throughout this paper.

following species of grass pollens were prepared by extraction of non-defatted pollen for 18 hr at 4°C in deionized water: rye (*L. perenne*), timothy (*Phleum pratense*), meadow velvet (*Holcus lanatus*), orchard (*Dactylis glomerata*), red top (*Agrostis alba*), meadow fescue (*Festuca elatior*), sweet vernal (*Anthoxanthum odoratum*), Kentucky blue (*Poa pratensis*), reed canary (*Phalaris canariensis*) and Bermuda (*Cynodon dactylon*). In each case, the raw materials were recent pollen collections from Greer Laboratories (Lenoir, N.C.) that had been stored at -20°C in sealed containers until used.

Two preparations of *Lol I* were used, a purified fraction (ca 95% pure) that was used for immunization purposes and a highly purified material (97–99% pure) used in radioimmunoassay (RIA) studies of anti-*Lol I* Abs, including the MABs. The former material was prepared from dialyzed rye pollen extract by a combination of DEAE-cellulose chromatography and gel filtration on Sephadex G75 and G100 columns (Marsh, 1975), followed by a second DEAE-cellulose chromatographic fractionation. The highly purified *Lol I* was prepared similarly, except that two Sephadex G75 (superfine) chromatographic columns were used in place of the Sephadex G75 and G100 columns. Also, care was taken at each chromatographic step to pool fractions of *Lol I* having the highest possible purity by immunoelectrophoresis (IEP) and agarose gel electrophoresis. This material contained a single predominant immunoprecipitate plus two minor impurities by crossed immunoelectrophoresis (CIE) against a potent goat anti-crude rye serum pool (NIAID Research Reagent).

Immunizations

In order to prepare immune spleen cells for fusion female SJL or A/J mice (Jackson Laboratory), 8 weeks of age, were immunized i.p. with 100 µg of the highly purified *Lol I* in 100 µl PBS, emulsified with an equal vol of complete Freund's adjuvant. Three weeks later animals were bled from the tail and boosted i.p. with 100 µg *Lol I* in phosphate-buffered saline (PBS).^{*} The titre of serum anti-*Lol I* Abs was assayed using the double-Ab RIA described below. Two days later (2 days prior to fusion), the mice were boosted i.p. with an additional 100 µg *Lol I* in PBS. Prior to removal of their spleens, the mice were bled from the axillary region to obtain serum for Ab determinations.

Cell lines and media

The myeloma partner used in these fusions was the

non-IgG-secreting Sp2/0-Ag14 (Sp2/0) line (Schulman *et al.*, 1978). All cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM) with 4.5 g glucose/l supplemented with 20% fetal bovine serum, 50 µg/ml gentamycin, 1×10^{-2} M Hepes, 0.2 U/ml insulin, 0.05 mg/ml pyruvate, 0.15 mg/ml oxaloacetic acid and 10% NCTC 135. Hybrids were selected in hypoxanthine-aminopterin-thymidine (HAT) medium (Littlefield, 1964) and propagated in HT medium (HAT minus aminopterin).

Somatic cell fusion

Hybridomas were generated by fusing SJL or A/J immune spleen cells with azaguanine-resistant Sp2/0 myeloma cells using polyethylene glycol (PEG-1000) and dimethylsulfoxide (DMSO) (Kennett, 1979). Briefly, spleen and myeloma cells were mixed in a ratio of 3:1 and centrifuged. The pellet was washed in PBS and centrifuged again. After removal of the supernatant fluid, the pellet was tapped to loosen the cells. One milliliter of fusion mixture (1.0 g PEG-1000 and 0.5 ml DMSO, pH 7.4) was added dropwise with constant agitation over a 1-min interval at a temp of 27–28°C. The fusion was stopped by gently adding 20 ml of PBS over a period of 4 min. The cells were centrifuged and then resuspended in DMEM. The cells were again centrifuged and very gently resuspended in HAT. Approximately 8×10^5 spleen cells were distributed into two 24-well tissue culture plates. Each 2 ml well had been seeded the day before with 2×10^4 BALB/c peritoneal exudate cells in HAT. Cultures were incubated for 7 days undisturbed, then every second day 0.5 ml of fresh HAT was exchanged with spent medium. After 2 weeks of incubation HT was substituted for HAT.

Subcloning of the hybridoma lines

Cell colonies that were secreting *Lol I*-specific Ab (as determined either by double Ab or solid-phase RIA) were subcloned on soft-agar containing 50% myeloma-conditioned medium (fresh DMEM diluted 1:1 with spent medium from Sp2/0 cultures). Individual clones were subcloned once again on agar, or by limiting dilution in 96-well plates, where a peritoneal exudate layer was used (3×10^3 exudate cells per 0.2 ml). Antibody-secreting clones were then allowed to expand in tissue culture.

Clonal amplification i.p.

In order to generate ascites fluid 1×10^7 Ab-secreting hybridoma cells were injected i.p. into either (SJL $\times$ BALB/c)F1 or (A/J $\times$ BALB/c)F1 mice which had been previously primed with pristane (0.5 ml/mouse) (Potter *et al.*, 1972). After a 9–14 day interval the mice were cervically dislocated, bled from the axillary region and ascites fluid tapped from the distended peritoneum. One wash of the peritoneal cavity with 5 ml PBS was used to increase the recovery of the ascites fluid. Cells and debris were removed

by centrifugation at 1400 g for 10 min.^{*} The supernatants were stored frozen (-20°C) until used for assays or for purification of the MAB.

Purification of MABs

The IgG fraction of the ascites fluid was partially purified by elution from a DEAE-cellulose column with 0.01 M phosphate (Ey *et al.*, 1978). It was further purified by Sepharose 4B-Protein A affinity chromatography in 0.05 M Tris, 0.15 M NaCl at pH 8.6 (Oi and Herzenberg, 1980). The IgG MAB fraction was eluted with 0.05 M citrate (pH 2.5) into tubes containing 1.0 M Tris buffer (pH 9.0). The MAB was dialyzed against borate-buffered saline (BBS), conc. by Amicon ultrafiltration and stored frozen until used. The purity of the resultant isolate in terms of its IgG content was assessed by SDS-PAGE (Laemmli, 1970). The purity and specificity of the anti-*Lol I* MAB was examined by IEF in agarose (Rosen *et al.*, 1979). The gel was fixed and then stained with Coomassie blue to reveal the protein bands (Axelson *et al.*, 1973). Alternatively, *Lol I*-specific MAB bands were revealed by allowing the gel to incubate for 18 hr at 23°C with a mixture of GxM Fab (1:10) and ^{125} I-*Lol I* (1×10^5 cpm/ml). After washing and drying the gel, the *Lol I*-specific Abs were revealed by autoradiography at -70°C using Kodak X-omat film with two X-ray intensifying screens (Swanstrom and Shank, 1978).

Solid-phase RIA

For rapid screening of culture supernatants a solid-phase RIA was developed using *Lol I*-coated Immulon flat-bottomed breakaway micro-titer wells (Dynatech). Microtiter wells were saturated with 50 µl aliquots of dialyzed rye extract (containing 2 µg *Lol I*/ml). The wells were allowed to dry overnight at 23°C. The plates were washed with BBS and then incubated with 1% bovine serum albumin (BSA) and 1% Triton in PBS (37°C, 90 min). Following aspiration of the BSA solution, 50 µl of culture medium from each hybridoma culture was added (37°C, 90 min). The plates were washed 5 times with Triton-PBS. Fifty microliters of ^{125} I-GxM Fab† (0.02 µg/ml) were added (37°C, 90 min). The plates were washed 5 times in Triton-PBS and individual wells counted to detect the presence of specific Ab.

In order to determine whether each MAB recognized a specific *Lol I* epitope, a competitive binding assay was performed on the rye-immunosorbent plates. Non-specific binding, B_0 , was determined by the binding of ^{125} I-GxM Fab to ragweed antigen-coated plates. Radiolabelled reference MAB No. 21.3

was incubated along with serial dilutions of each of the other MABs. Inhibition was calculated according to the formula:

$$\text{Inhibition} = 1 - \frac{\text{cpm bound} - B_0 \text{ cpm}}{\text{cpm total} - B_0 \text{ cpm}} \times 100\%$$

(cpm total: cpm bound by MAB No. 21.3 in absence of competitor).

To measure *Lol I* directly, the plates were coated with goat α -*Lol I* serum and bound *Lol I* was detected with ^{125} I-MAB No. 3.2.

Isotype determination

The isotypes of the mouse immunoglobulin from hybrid culture fluids and of purified mouse IgG were determined using a solid-phase RIA. Each sample was incubated on a *Lol I*-coated plate; the plate was washed and iodinated α -isotype reagents were added. After addition of appropriate dilutions of culture fluids or purified IgG, respectively, to wells coated with *Lol I* extract, 50 µl of ^{125} I-labelled goat anti-mouse IgG₁, anti-IgG_{2a,2b}, or anti-IgG₃ were added (37°C, 90 min). (These reagents were kindly provided by Dr Patricia J. Gearhart, Johns Hopkins University.)

Double-Ab RIA

Selected Abs were studied by analysis of their ability to bind ^{125} I-*Lol I* over a wide range of antigen concns in a modified double-Ab RIA similar to that of Ishizaka *et al.* (1967). Purified MAB was incubated with ^{125} I-*Lol I* (highly purified) (20 µl total vol) for 5 hr at room temp. The vol was brought to 200 µl with 1/40 v/v of carrier normal mouse serum and antigen-antibody complexes were then precipitated with GxM IgG. Immunoprecipitates were washed 4 times in 3 ml aliquots of BBS and were counted in a gamma counter. The average dissociation constant (K_d) was calculated from the plot of $B/F = K_d$ ($Ab_0 - B$) (Scatchard, 1949), where B and F designate the conc. of bound and free antigen, and Ab_0 is the initial Ab-combining site concn. The slope of the regression line is $1 - K_d$.

Agarose electrophoresis and IEP

Agarose electrophoresis at pH 8.6 was performed at 10°C essentially as described by Laurell (1972). Agarose (1%) (Seakem, Type ME, FMC, Marine Colloids) was dissolved in Tris-barbital buffer (pH 8.6) and applied to plastic sheets (Gel bond, FMC) on a level table. Protein samples were electrophoresed at 10 V/cm for 34–45 min, fixed and stained with Coomassie Blue. Immunoelectrophoresis was performed by the Scheidegger (1955) procedure using 10 V/cm for 60 min at 10°C and pH 8.6. Antisera were added to troughs cut in the gels, which were then incubated overnight at 23°C. The gels were washed, pressed, dried and stained with Coomassie Blue (Axelson *et al.*, 1973).

^{*}In the early stages of the study the boost was administered intravenously into the tail vein, but this protocol frequently resulted in anaphylaxis and death, presumably due to the high titer of IgG₁, which is known to sensitize mice for passive cutaneous anaphylaxis.

^{*}All centrifugations were performed at 200 g for 10 min unless otherwise noted.

[†]The GxM Fab required for the solid-phase RIA was generated by immunizing a goat with 0.5 mg of Fab fragment of mouse IgG in saline (in equivolume CFA) every 2 weeks for 2 months.

Isoelectricfocusing (IEF) and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

Ampholine PAG plates (LKB) were used for IEF over the pH 5.5–8.5 range. The cathodic buffer was 0.1 N NaOH and anodic buffer 0.4 N HEPES. The ampholytes were pre-focused for 1 hr, the samples (2–5 μ g) were applied directly to gel and allowed to focus for 2.5 hr. The protein components were fixed for 1 hr in TCA and sulfosalicylic acid and stained for 10 min in Coomassie Blue.

SDS-PAGE was performed in slab gels according to the method of Laemmli (1970).

Immunoadsorption chromatography

Purified MAb No. 3.2 was linked covalently to Sepharose 4B-CL using a modification of the cyanogen bromide procedure of March *et al.*, 1974. Following CNBr activation 1.5 mg MAb were coupled per ml of the activated gel slurry in 0.1 M NaHCO₃, 0.5 M NaCl (pH 8.5) for 20 hr at 4°C with constant agitation. After washing on a Buchner funnel with 1 l of deionized water, any unreacted activated groups on the beads were allowed to react with 1 M ethanolamine (pH 8.0) for 4 hr at 23°C. The beads were washed with 500 ml buffer, pH 4 (0.1 M sodium chloride), followed by pH 8.5 buffer (0.1 M sodium borate, 1.0 M sodium chloride). This procedure was repeated twice more. The beads were equilibrated with PBS–0.5 M NaCl and packed into a small column (30 ml). A partially purified rye pollen extract, containing 62.5 mg of Lol I (determined by radial immunodiffusion assay), was prepared by a single DEAE-cellulose chromatographic fractionation on a Buchner funnel of dialyzed rye extract, the crude Lol I-containing material being eluted between 0.04 M and 0.25 M NH₄HCO₃ (Marsh, 1975). This fraction was applied to the column of MAb No. 3.2 immunoadsorbent which has a theoretical binding capacity of 5.0 mg of Lol I. Immunoadsorption of Lol I was carried out in PBS–0.5 M NaCl for 30 min at room temp. The column was washed extensively with the same buffer until no u.v.-absorbing material was eluted. The Lol I was recovered by elution with 0.05 M citrate, pH 4.5 into 1 M Tris buffer at pH 9.0 (to adjust the pH to near neutrality).

RESULTS

Production of anti-Lol I MAbs

Thirteen hybridoma clones were produced from the fusion of six individual spleens from A/J or SJL mice (Table 1). Single hybridoma cells were isolated from Ab-containing tissue culture wells by cloning on agar. To insure that the progeny of a single cell were obtained, each agar clone was either subcloned on agar at least once again or subcloned by limiting dilution in microtitre wells. Individual hybridoma lines secreting anti-Lol I MAb were allowed to expand in culture and were injected into pristane-

Table 1. Characterization of anti-Lol I MAbs

Monoclonal antibody	Heavy chain isotype	pI ^a	Mouse strain
Clone 3.1	IgG ₁	6.25, 6.15	SJL
Clone 3.2 ^b	IgG ₂	7.80, 7.50	SJL
Clone 8.2	IgG ₁	6.00, 5.90	SJL
Clone 10.0 ^b	IgG ₁	6.30	A/J
Clone 11.0 ^b	IgG ₁	6.60, 6.55	A/J
Clone 12.0 ^b	IgG ₁	6.25	A/J
Clone 13.2 ^b	IgG ₂	8.35, 8.20	A/J
Clone 13.6	IgG ₂	8.45, 8.35	A/J
Clone 14.1	IgG ₁	5.40, 5.35	SJL
Clone 16.0	IgG ₁	8.00	SJL
Clone 19.0 ^b	IgG ₁	6.30, 6.20	A/J
Clone 21.3 ^b	IgG ₂	7.60, 7.55	SJL
Clone 21.8	IgG ₂	7.55, 7.50	SJL

^aThe pI values of the major band(s) observed on IEF.

^bMAbs selected for further study.

primed (A/J $\times$ BALB/c)F1 or (SJL $\times$ BALB/c)F1 mice in order to generate ascites fluids with high MAb titers. A typical culture supernatant contained 4 μ g MAb/ml, while the corresponding ascites fluid contained 400 μ g MAb/ml, as determined by purification of the Ab and spectrophotometric measurement at O.D._{280 nm}.

Purification of MAb from ascites fluid

Anti-Lol I MAb obtained from clone No. 3.2 was isolated from ascites fluid by ion-exchange chromatography on DEAE-cellulose, followed by affinity chromatography on protein A-Sepharose. The homogeneity of the Ab preparation was demonstrated by: (a) a single band on agarose electrophoresis; (b) single heavy and light chain bands on SDS-PAGE electrophoresis; and (c) a restricted banding pattern on IEF after staining with Coomassie Blue. To investigate whether the observed IEF banding patterns resulted from minor charge variations rather than contamination with non-specific IgG, the antigenic specificity of each variant was examined by overlaying the IEF gel with ¹²⁵I-Lol I and G α M Fab. It

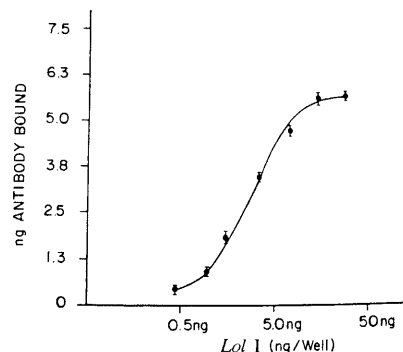


Fig. 1. Binding curve for the reaction of ¹²⁵I-MAB No. 3.2 with varying amounts of Lol I allergen absorbed to microtitre wells. Ten nanograms of ¹²⁵I-MAB were added to each well.

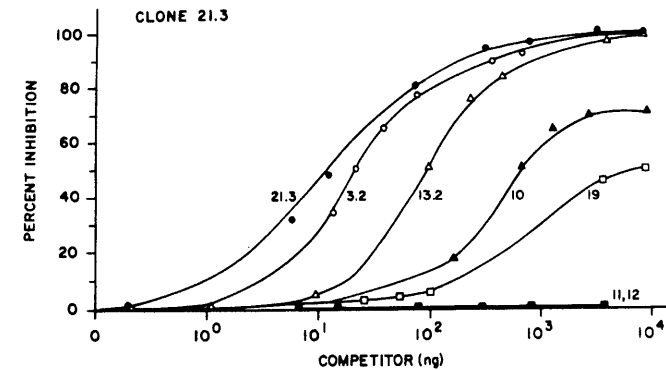


Fig. 2. Solid-phase inhibition RIA examining the epitope specificity of several MAbs relative to MAB No. 21.3. ¹²⁵I-MAB No. 21.3 (10 ng) was allowed to incubate with unlabelled "competitors", either MAB No. 21.3 or one of six other MAbs, in varying concns.

was found that each of the Coomassie-stained bands bound radiolabelled Lol I, demonstrating that each was immunoreactive. Similar results were obtained with the other MAbs. The isoelectric points of the major components of each MAb are recorded in Table 1. The seven MAbs indicated in the table were selected for further study.

Monoclonal Ab No. 3.2 was radiolabelled with ¹²⁵I and used in a solid-phase RIA to investigate the relationship between the amounts of MAB bound for different amounts of Lol I in the wells of the microtitre plates. There were sigmoidal relationships between Ab bound and the log [Lol I] used to coat the microtitre wells, with saturation of the plates being attained at about 50 ng of Lol I per well (Fig. 1).

Ab epitope specificity determination

Since it is our intention to use the MAbs to block antigenic and/or allergenic epitopes on the Lol I molecule, it was necessary to investigate whether each MAB recognized a unique epitope. First, several MAbs were compared by an inhibition solid-phase RIA using ¹²⁵I-MAB No. 21.3 and rye-coated immunosorbent plates. The ability of six different MAbs, as well as MAB No. 21.3 itself, to inhibit the binding of ¹²⁵I-MAB No. 21.3 to the plates was investigated (Fig. 2). The inhibition curves indicate that the antigenic epitopes detected by MAB No. 21.3 vs MAbs (Nos 10 and 19) vs MAbs (Nos 11 and 12) are qualitatively different; other differences (e.g. between Nos 21.3, 3.2 and 13.2) may be quantitative.

Avidity determination of MAB No. 3.2

The binding of MAB No. 3.2 to ¹²⁵I-Lol I was studied by double-Ab RIA. The resultant Scatchard plot (Fig. 3) of "Ag bound/Ag free" vs "Ag bound" indicated values of 3.5×10^{-6} L/M. In other experi-

ments, we showed that the binding characteristics of a MAB obtained from ascites fluid bound ¹²⁵I-Lol I identically to the MAB found in the serum of the mouse in which the hybridoma line had been allowed to expand. Thus, for certain of the binding experiments, we were able to use purified MAB, ascites fluid and serum from the hybridoma-recipient mouse interchangeably.

MAB affinity column purification of Lol I

One and a half milligrams of pure Lol I were recovered following elution with citrate (pH 4.5) buffer of the MAB No. 3.2 immunoadsorbent column used to fractionate a crude rye preparation (see

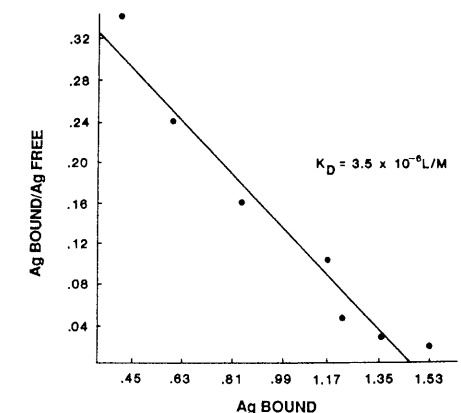


Fig. 3. Scatchard plot of "Ag bound/Ag free" vs "Ag bound", relating to the binding of ¹²⁵I-Lol I to MAB No. 3.2 in double-Ab RIA. The concn of antigen was held constant at 1.0×10^{-6} M and the concn of Ab was allowed to vary between 5.0×10^{-6} and 0.078×10^{-6} M.

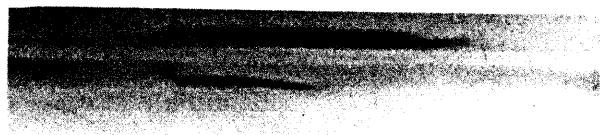


Fig. 4. IEP analysis of *Lol* I purified on an anti-*Lol* I (MAB No. 3.2) affinity column. Top well, DEAE cut of whole rye extract (approx. 25 μ g, containing 4 μ g *Lol* I); Bottom well, affinity-purified *Lol* I (3 μ g). The trough contains goat anti-whole rye extract (IgG cut).

Materials and Methods. The high degree of purity of the eluted *Lol* I is seen by IEP and CIE (Fig. 4) and by SDS-PAGE (Fig. 5). The eluted protein also retained its full immunoreactivity when assayed by a solid-phase RIA.

The cross-reactivity of MAB No. 3.2 with group I-containing allergenic extracts

The relative binding activity of MAB No. 3.2 to *Lol* I and to other Group I-containing grass pollen extracts was studied by direct binding of ^{125} I-MAB No. 3.2 to the immunosorbent plates of 10 different grass species (Fig. 6). Radiolabelled MAB No. 3.2 was found to bind effectively not only to *Lol* I but also to the Group I homologues of the nine other grass pollen extracts studied, including Bermuda Group I (*Cyn d* I), which is considered to be essentially non-cross-reactive with *Lol* I by conventional immunochemical analysis.

DISCUSSION

Thirteen mouse MABs toward the major rye grass pollen allergen, *Lol* I, were prepared and evidence presented for the high degree of purity of an isolate of one of these MABs (No. 3.2). Several others were isolated at a similar high level of purity. Most of the MABs were found to exist in at least two major isoelectrophoretic forms, differing by 0.05–0.30 pH units, possibly resulting from variable glycosylation of the protein moiety of the MAB (Awdeh *et al.*, 1970). All of the isoelectric forms were found to bind radiolabelled *Lol* I antigen.

The epitope specificities on *Lol* I that are recognized by seven of the MABs were investigated by cross-inhibition RIA's wherein the MABs themselves were used to inhibit the binding of one MAB (No. 21.3) to *Lol* I (Fig. 2). This assay provided evidence that certain MABs bound to different epitopes. In preliminary studies, we have examined the competitive binding between the murine MABs and human allergic sera. Each MAB appeared to have a unique binding pattern when it was allowed to compete for Ag with the IgE Abs in nine different patients' sera. It would, therefore, appear that murine MABs can also be used to investigate the molecular basis of interaction between allergens and human IgE Abs, which will be the subject of future studies.

Monoclonal Abs offer a useful tool for allergy research in that allergens can be quickly and thoroughly isolated from contaminating proteins by a single adsorption on a MAB affinity column. *Lol* I was purified to apparent homogeneity by this method from a crude *Lol* I-containing fraction prepared by a single DEAE-cellulose chromatographic step. (It was decided that it was necessary to perform this simple,

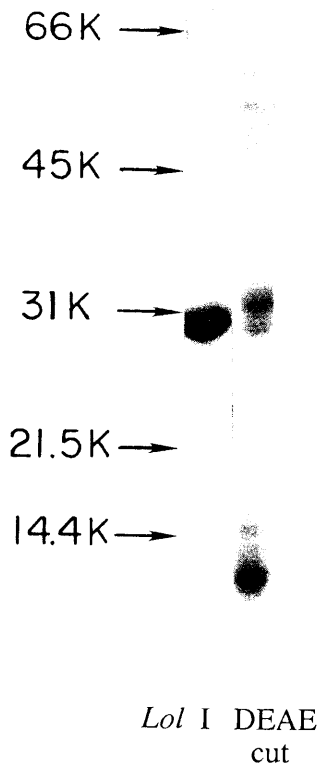


Fig. 5. SDS-PAGE showing mol. wt standards, affinity-purified *Lol* I (10 μ g) and the DEAE-cellulose fraction (10 μ g) from which the *Lol* I was derived.

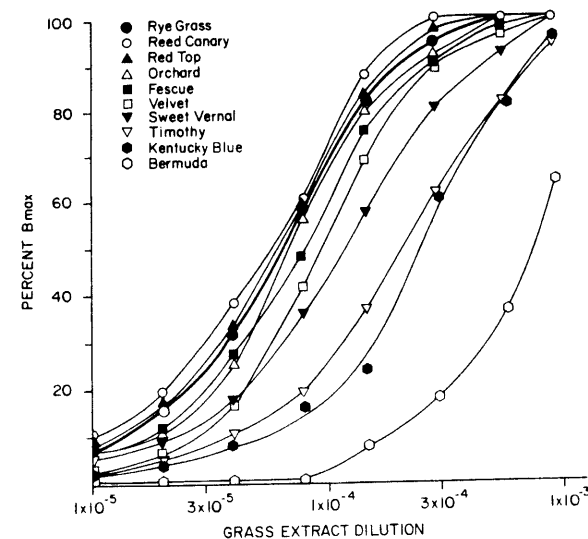


Fig. 6. Solid-phase RIA binding curves showing the direct binding of ^{125} I-MAB No. 3.2 to Group I components on immunosorbent plates coated with extracts of 10 different grass pollen species. Non-specific binding of MAB to ragweed coated plates was subtracted for each value. Non-specific binding was less than 5% of specifically bound MAB.

preliminary one-step DEAE-cellulose purification in order to remove pigments and large amounts of non-*Lol* I proteins and carbohydrates). The relatively low dissociation constant of MAB No. 3.2 (3.5×10^{-6} L/M) makes it an ideal ligand for this type of purification scheme since a gentle elution technique (pH 4.5 buffer) is sufficient to dissociate the Ab-Ag bond. Since MAB No. 3.2 was found to cross-react with all of nine other species of grass pollen extracts that we investigated, the affinity column could be used to isolate the various Group I molecules for subsequent immunochemical comparison studies. This has previously not been possible for certain grass species (Baer *et al.*, 1974) with conventional polyclonal Abs which cross-react poorly or not at all with the Group I homologues of the respective species.

As demonstrated in direct binding studies using radiolabelled anti-*Lol* I MAB No. 3.2, it should be possible to utilize MABs for the standardization of several different grass species according to their Group I contents. Of particular interest is our finding that MAB No. 3.2 binds well to the Group I homologue of Bermuda grass, a cross-reactivity which has not previously been detected using conventional polyclonal antisera (Marsh, 1975; Marsh *et al.*, 1970).

Krilis *et al.* (1981, 1983) were the first to suggest the use of MABs in allergen standardization, pointing out that the immortality of hybridoma cells offers the possibility of establishing reproducible primary standard reagents. Since our preliminary reports of anti-*Lol* I MABs (Kahn and Marsh, 1982, 1983). Esch and

Klapper (1984) have presented preliminary evidence of the utility of anti-*Lol* I MABs in molecular studies of Group I grass proteins. Also, Smart *et al.* (1983) have recently described the preparation of six MABs, raised against crude rye grass extract, which bind individual rye components, possibly including *Lol* I. It is already clear from the published studies that the use of purified allergens for the initial immunization of the mice greatly simplifies subsequent analyses of MAB specificity.

It is anticipated biochemically characterized anti-*Lol* I MABs will serve as useful probes for the dissection *in vitro* of Ab-combining sites. Their usefulness both in allergen isolation and standardization is already evident.

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