

A Murine Monoclonal Anti-Idiotypic Antibody Detects a Common Idiotope on Human, Mouse and Rabbit Antibodies to Allergen *Lol p IV*

E.-M. ZHOU, J. M. M. DZUBA-FISCHER, E. S. RECTOR, A. H. SEHON & F. T. KISIL

MRC Group for Allergy Research, Department of Immunology, University of Manitoba, Winnipeg, Manitoba, Canada

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A syngeneic mouse monoclonal anti-idiotypic antibody (anti-Id), designated as B1/1, was generated against a monoclonal antibody (MoAb 91) specific for Ryegrass pollen allergen *Lol p IV*. This anti-Id recognized an idiotope (Id) that was also present on other monoclonal antibodies with the same specificity as MoAb 91. Observations that (i) the anti-Id inhibited the binding of MoAb 91 to *Lol p IV* and (ii) the Id-anti-Id interaction could be inhibited by *Lol p IV* indicated that the Id was located within or near the antigen combining site. These properties served to characterize B1/1 as an internal image anti-Id. Evidence that an immune response in different species to *Lol p IV* elicits the formation of antibodies which express a common Id was provided by the observations that (i) the Id-anti-Id interactions could be inhibited by mouse, human and rabbit antisera to *Lol p IV* and (ii) the binding of these antisera to *Lol p IV* could be inhibited by the anti-Id. Interestingly, the internal image anti-Id B1/1 also recognized an Id on a monoclonal antibody which was directed to an epitope of *Lol p IV*, different from that recognized by MoAb 91.

Dr F. T. Kisil, Department of Immunology, University of Manitoba, 730 William Avenue, Winnipeg, Manitoba R3E 0W3, Canada

An idiotope (Id) is defined as an antigenic determinant within the variable regions of immunoglobulins and the antigen receptors of B and T lymphocytes. Idiotopes have also served as structural and functional markers for the variable regions. Upon immunization with an antibody which contains a variety of idiotopes, collectively referred to as idiotypes, the corresponding anti-idiotypic antibodies (anti-Id) can be generated. According to Jerne [1], anti-Id or Ab-2 can be classified into two major categories, Ab-2 α and Ab-2 β . The Ab-2 α recognizes the Id outside the antigen combining site. On the other hand, the Ab-2 β , also known as internal image anti-Id, has the capacity to mimic the three-dimensional structure of the antigenic determinants or epitopes and can substitute for the antigen in inducing or suppressing the immune response against those specific epitopes. It has been shown that antibodies which share antigen-binding

specificity often share the same idiotopes with one another [2-4].

In this report, we describe the generation and characterization of a mouse monoclonal anti-id, designated as B1/1, which bears the characteristics of Ab-2 β . This anti-Id was produced against a mouse monoclonal antibody MoAb 91 specific for Site A on *Lol p IV* [also known as high molecular weight basic allergen (HMBA) [5]. *Lol p IV* is one of the major allergens in Ryegrass pollen. Studies in our laboratory using monoclonal antibodies have so far identified four different epitopes on *Lol p IV* [6]. Three of these epitopes are also recognized by human IgE antibodies of grass pollen allergic patients. The monoclonal anti-Id B1/1 recognized an Id that was found to be common to mouse, human and rabbit anti-*Lol p IV*-specific antibodies. The Id was found to be located within or near the antigen combining sites of these antibodies. Furthermore,

the anti-Id also recognized the Id on monoclonal antibody MoAb 12 which recognized Site B of *Lol p IV*. Our studies demonstrated that an immune response to *Lol p IV* in three different species leads to the expression of a common Id on the resulting antibodies.

MATERIALS AND METHODS

Preparation of immunoabsorbent. Sepharose 4B gel (10 ml, Pharmacia, Uppsala, Sweden) was washed well in distilled water (dH₂O) and resuspended in 5 ml of dH₂O. Three grams of cyanogen bromide (Sigma, St Louis, Mo., USA) were dissolved in 5 ml of dH₂O and immediately mixed with the suspension of the Sepharose 4B gel. The reaction mixture was kept in an ice bath and the pH of the mixture was immediately raised and maintained at pH 11–12 with NaOH for a period of 12 min. The gel was then quickly washed with 200 ml of 0.2 M borate-buffered saline (BBS) pH 8.2 and transferred into a solution containing 30 mg of MoAb 91. This mixture was maintained at 25°C for 2 h and at 4°C overnight. Protein which had not bound was washed out. It was estimated that 97% of the protein was coupled to the gel. Finally, the immunoabsorbent was washed with a solution of 0.1 M acetic acid followed by BBS.

Isolation of allergen, *Lol p IV*. A solution of the retentate fraction of Ryegrass (*Lolium perenne*) pollen [5] prepared in BBS (20 mg/ml) was incubated with the MoAb 91-sepharose 4B immunoabsorbent at 4°C, overnight. After draining off the material which had not bound, the column was washed with BBS until the OD₂₈₀ was less than 0.1. A solution of 0.1 M glycine-HCl at pH 3.0 was employed to elute the protein from the immunosorbent. The fractions having an OD₂₈₀ 0.1 or higher were collected, dialysed against BBS and concentrated using the Amicon Ultrafiltration Systems (YM 5; Amicon Canada Limited, Oakville, Ontario, Canada). The purity and antigenicity of *Lol p IV* were tested utilizing sodium-dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE 12%) and enzyme-linked immunoassay (ELISA) respectively. The protein concentration of *Lol p IV* was determined by the method of Lowry et al. [7].

Preparation and characterization of monoclonal anti-*Lol p IV* antibodies. Monoclonal antibodies MoAb 90 to 94 (all IgG1 isotype) generated against *Lol p IV* have been characterized as recognizing an epitope designated as Site A [6]. MoAb 12 (IgG1) was produced against the retentate fraction of Kentucky Bluegrass (KBG) pollen allergen, and was identified to recognize an epitope referred to as Site B on *Lol p IV*. MoAb 95 to 100 recognized Site C and MoAb 102 recognized Site D [6]. In the present study, MoAb 91 was used to induce the production of the syngeneic anti-Id.

Purification and isolation of MoAb (IgG1 isotype) from ascitic fluid was performed as recommended by Dr M. R. Schick (Stanford University, personal communication). One volume of the ascitic fluid containing the MoAb was mixed with four volumes of a solution of 1.5

M glycine-HCl and 3 M NaCl, pH 8.9. The mixture was passed through a protein A-sepharose 4B (Pharmacia) affinity column which had been equilibrated with the glycine-HCl-NaCl buffer. This high salt-containing buffer was employed to enhance the binding of mouse IgG1 to protein A and resulted in the binding of 5 mg IgG1 per ml of the affinity gel. The column was washed with the buffer and then eluted with BBS and fractions were collected. The protein concentration was calculated using an extinction coefficient of 13.5 for a 1% preparation at OD₂₈₀ nm.

Generation of syngeneic monoclonal anti-Id. Female Balb/c mice (Jackson Laboratory, Bar Harbor, Maine, USA) were used in this study to generate the anti-Id against MoAb 91. Mice were given a total of three i.p. injections of 25 µg of MoAb 91 conjugated to keyhole limpet haemocyanin (KLH) (see below) and then precipitated in alum every two weeks. One month later, the mice were given the fourth injection. Two mice that had a high titre of serum anti-Id were boosted i.p. with 25 µg of MoAb 91 coupled to KLH, 5 days prior to using their spleen cells for the fusions. The hybridoma cells were prepared as described previously [8]. The hybridoma cell culture fluids were screened for anti-Id using a biotin-avidin ELISA (described below). Hybridomas secreting anti-Id were subcloned by the limiting dilution method to obtain monoclonal antibodies. A monoclonal anti-Id, designated as B1/1 was identified as an IgG2b(κ) using murine isotype-specific antibody reagents (BioRad Laboratories, Richmond, Calif., USA). The monoclonal anti-Id was isolated from ascitic fluid using a combination of salt fractionation and molecular sieve chromatography which have been described in detail elsewhere [9].

ELISA protocols. (i) **Antibody titres to *Lol p IV*.** The specific binding of antibodies in the mouse, human and rabbit antisera to *Lol p IV* was determined by a direct ELISA. The solid-phase was prepared by coating the wells of microtitre plates (Immulon II; Dynatech Laboratories Inc., Alexandria, Va., USA) with *Lol p IV* (200 ng in 50 µl BBS) at 4°C, overnight. For control, ovalbumin (200 ng in 50 µl BBS) was employed to coat wells. After blocking unbound sites with a solution of 2% gelatin at 37°C for 30 min, antisera obtained from mice, human and rabbits were diluted over a wide range and 50 µl samples were added to the microtitre wells and incubated at 37°C for 1 h. The wells were then washed three times with BBS containing 0.2% of Tween-20 (Tween-BBS, vol/vol). The antibodies which had bound to the solid-phase *Lol p IV* were detected by the addition of the corresponding horseradish peroxidase (HRP)-conjugated goat antibodies to mouse, human and rabbit immunoglobulins (BioRad Laboratories, Richmond, Calif., USA) at 37°C for 1 h. After washing the wells with Tween-BBS, the substrate, 2,2'-azino-di(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) in 0.1 M citrate buffer and 0.01% H₂O₂ were used to develop the colorimetric reaction. The reaction was stopped by adding 5% SDS and the optical density at 410 nm of each well was measured on an automatic ELISA plate reader (MR600; Dynatech Laboratory).

(ii) **Detection of Antibodies Specific to Site A of *Lol p IV*.** A biotin-avidin competitive inhibition ELISA procedure was employed to establish the relative levels of

antibodies in the different antisera, which recognized site A of *Lol p IV*. For this purpose, biotinylated MoAb 91 (40 ng in 100 μ l) (see below) was mixed with the various inhibitors e.g. MoAb (20 μ g in 100 μ l) and antisera (diluted ten-fold; 100 μ l), and volumes of 100 μ l of the mixtures were added to the wells which had been coated with *Lol p IV* (200 ng/well). After incubating at 37°C for 1 h, the wells were washed and a solution of Streptavidin-conjugated HRP was added and kept at 25°C for 25 min. The assay was continued as described above.

(iii) *Two-site ELISA for evaluating specificity of anti-Id B1/1*. The specificity of the anti-Id B1/1 for MoAb 91 was determined by the biotin-avidin two-site ELISA. Briefly, the wells of microtitre plates were coated with MoAb 91 (400 ng in 50 μ l BBS). For control, mouse monoclonal anti-ovalbumin (MoAb 2) and anti-*Poa p 1* (MoAb 60) antibodies were individually employed to coat the wells. After blocking unbound sites with gelatin, 100 μ l of the mouse antisera or hybridoma cell culture fluids or purified protein preparation containing the anti-Id was added to the wells. The anti-Id which had bound to the solid-phase MoAb 91 was detected by adding the biotinylated MoAb 91 (50 ng/well). The assay was continued as described above.

(iv) *Detection of a common Id on antibodies to Lol p IV*. Antibodies to *Lol p IV* that possessed an Id in common with MoAb 91 were detected in terms of their capacity to compete with MoAb 91 and inhibit its binding to the anti-Id. For this purpose, solid-phase was prepared by coating individual wells of the ELISA plate with the purified preparation of MoAb 91 (200 ng in 50 μ l BBS) at 4°C, overnight. The wells were blocked with gelatin as described above. A constant amount of the anti-Id B1/1 preparation (200 ng/100 μ l) was mixed with the various inhibitors i.e. MoAbs or antisera from mouse and rabbit to *Lol p IV* and sera from grass pollen allergic and non-allergic individuals and then volumes of 100 μ l of the mixtures were added to the wells and kept at 37°C for 1 h followed by washing three times with Tween-BBS. HRP-labelled goat anti-mouse IgG2b (Cedarlane Laboratories Ltd., Ontario, Canada) was used to determine the extent to which the anti-Id had bound to the solid-phase. These values were compared with the maximum binding of the anti-Id which occurred in the absence of a competing antibody or antiserum. The assays were performed in triplicate.

(v) *Inhibition of the binding of antibodies to Lol p IV by the anti-Id*. To ascertain whether the anti-Id could bind to antibodies against *Lol p IV* and inhibit their binding to *Lol p IV*, a competitive inhibition assay was performed in triplicate. For this purpose, solid-phase was prepared by coating individual wells of ELISA plates with *Lol p IV* (200 ng in 50 μ l BBS) at 4°C overnight. Mixtures of the anti-Id and the MoAb or antisera to *Lol p IV* were prepared prior to addition to *Lol p IV* coated assay plates. The anti-Id was used at various amounts (μ g/well); the final amounts of the MoAb per well was 100 ng for MoAbs 90 and 91; 200 ng for MoAbs 12 and 98; the antisera were used at the final dilutions of: 1/10,000 for mouse antisera no. 1, no. 3; 1/5,000 for mouse antisera no. 2, no. 4, no. 5; 1/500 for human serum Hu1; 1/100 for sera Hu2, Hu3, and the serum Hu5; 1/50 for Hu4; 1/5,000 for rabbit antiserum no. 1; 1/2,500 for rabbit antisera no. 2, no. 3. HRP-

labelled goat antibodies to mouse IgG1, human and rabbit immunoglobulins were used to detect the levels of the antibodies which bound to the solid-phase. The addition of the substrate and quantitation of the colorimetric reaction was performed as described above.

(vi) *Inhibition of the binding of anti-Id to the Id MoAb 91 by Lol p IV*. To determine whether the allergen *Lol p IV* could interfere with the binding between the anti-Id B1/1 and the Id MoAb 91, a competitive inhibition assay was employed. The assays were performed in triplicate as described in section (iv) except that the inhibitor employed in this assay was *Lol p IV*.

The degree of inhibition in the respective assays was calculated according to the formula: per cent inhibition = $1 - [\text{OD}_{410} \text{ with inhibitor minus background OD} / \text{OD}_{410} \text{ without inhibitor minus background OD}] \times 100$.

Preparation of antibody-KLH conjugate. The method for coupling antibodies to KLH has been described in detail elsewhere [10]. Briefly, the purified IgG preparation of MoAb 91 (5 mg/ml) in BBS cooled to 4°C was mixed with 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDAC) (Sigma Chemical Co., St. Louis, MO., USA) at a molar ratio of 1:10,000. After incubation at 4°C for 30 s, KLH (Calbiochem Corporation, La Jolla, Calif., USA) was added to the IgG preparation at a molar ratio of 1:50 and incubated for 2 h at 25°C and at 4°C, overnight. After dialysing against BBS at 4°C, overnight, the IgG-KLH conjugate was then precipitated in alum.

Biotinylation of antibodies. The method used for biotinylation of antibodies has been described in detail elsewhere [11]. Briefly, 2 mg of purified monoclonal antibody preparation was incubated with the mixture of 0.5 M NaHCO₃, pH 9.0 and 0.5 mg of biotin-N-hydroxysuccinimide ester (Pierce Chemical Co., Rockford, Ill., USA) at 25°C for 1 h. A volume of 25 μ l of a solution of NH₄Cl (1 M) was added to stop the reaction and the mixture was dialysed against BBS. Biotinylated monoclonal antibody was mixed with glycerol at a ratio of 1:1 (vol/vol) and stored at -20°C for further use.

RESULTS

Characterization of antibodies against Lol p IV

The relative levels of serum antibodies to *Lol p IV* from allergic and non-allergic human sera, and mouse and rabbit antisera are reported in Table I. Non-allergic human serum, Hu6, contained antibodies of the IgG class to Ryegrass pollen and KBG allergens. However, this serum was found to be devoid of detectable IgE antibodies, in the P-K and RAST assays [6]. Normal sera from human, mouse and rabbit lacked antibodies of the IgG and IgE classes to Ryegrass and KBG pollen allergens.

The relative specificities of antibodies of different sources to *Lol p IV* were compared with that of MoAb 91. The specificities were evaluated in

TABLE I. Antibody titres to *Lol p IV**

Antisera	Specificity	End point titre
Mouse no. 1	Anti- <i>Lol p IV</i>	10,000
Mouse no. 2	Anti- <i>Lol p IV</i>	5,000
Mouse no. 3	Anti- <i>Lol p IV</i>	10,000
Mouse no. 4	Anti- <i>Lol p IV</i>	5,000
Mouse no. 5	Anti- <i>Lol p IV</i>	5,000
Mouse no. 6	Normal serum	0
Allergic Human Serum:		
Hu1	Grass pollens	500
Hu2	Grass pollens	100
Hu3	Grass pollens	100
Hu4	Grass pollens	50
Hu5	Grass pollens	100
Nonallergic Human Serum:		
Hu6	Grass pollens	100
Hu7	Normal serum	0
Rabbit no. 1	Anti- <i>Lol p IV</i>	5,000
Rabbit no. 2	Anti- <i>Lol p IV</i>	2,500
Rabbit no. 3	Anti- <i>Lol p IV</i>	2,500
Rabbit no. 4	Normal sera	0

* A direct ELISA was utilized to determine the relative total levels of the serum antibodies to *Lol p IV*. The values under the heading 'End point titre' refer to the final dilution of the antiserum which gives an OD₄₁₀ value three times higher than that obtained with normal sera.

terms of the capacities of the antibodies to inhibit the binding of MoAb 91 to *Lol p IV*. As shown in Fig. 1, neither MoAb 98 nor MoAb 12 was able to inhibit the binding of MoAb 91 to *Lol p IV*. These observations confirmed the results of previous studies in this laboratory which have shown that (i) MoAb 90 and 91; (ii) MoAb 98; and (iii) MoAb 12, respectively recognized three distinctly different epitopes on *Lol p IV* [6]. Sera from five individuals allergic to Ryegrass and KBG pollen all inhibited, to different degrees, the binding of MoAb 91 to *Lol p IV*. One non-allergic human serum, Hu6, also partially inhibited (to the extent of 35%) the binding of MoAb 91 to *Lol p IV*. Similarly, five mouse and three rabbit antisera produced against *Lol p IV* also inhibited the binding of MoAb 91 to *Lol p IV* to an appreciable extent. On the other hand, this binding was not affected by MoAb 60 (anti-*Poa p I*) and the sera obtained either from normal human or mouse and rabbit obtained prior to their immunization

with *Lol p IV*. The results of these inhibition experiments indicated that at least part of the repertoire of the mouse, human and rabbit antibodies to *Lol p IV* was directed to Site A of *Lol p IV*, which was also recognized by MoAb 91.

Specificity of the monoclonal anti-Id B1/1

The specificity of the mouse monoclonal anti-Id B1/1 was tested by a biotin-avidin two-site ELISA. The results are illustrated in Fig. 2. The lowest concentration of anti-Id B1/1 which bound to solid-phase MoAb 91 was of the order of 500 ng/ml. Monoclonal anti-Id B1/1 did not bind to other IgG1 monoclonal antibodies directed to ovalbumin (MoAb 2) or to *Poa p I* (MoAb 60). These results indicated that B1/1 recognized an Id on an antibody specific for *Lol p IV*.

*Monoclonal anti-Id recognizes an Id common to antibodies to *Lol p IV**

To examine the possibility that antibodies to *Lol p IV* present in mouse, human and rabbit antisera share an Id in common with MoAb 91, a competitive inhibition assay was employed. In this assay, the abilities of antibodies from the different species to inhibit the binding between the Id (MoAb 91) and anti-Id (B1/1) were evaluated over a range of doses. The results are shown in Fig. 3a-d. The homologous monoclonal antibody, MoAb 91 and another monoclonal antibody, MoAb 90, which also recognized Site A of *Lol p IV*, inhibited the Id-anti-Id interaction in a dose-dependent fashion and total inhibition was achieved at an amount of 20 µg/well (Fig 3a). By comparison, a ten-fold increase in the amount of MoAb 12 which recognized Site B of *Lol p IV* [6], was required to inhibit the binding to the extent of 80%. On the other hand, an anti-*Lol p IV* monoclonal antibody, MoAb 98 specific for Site C, and an anti-*Poa p I*, MoAb 60, did not inhibit the binding between the Id and anti-Id even at high amounts.

All five murine antisera to *Lol p IV* inhibited the binding between the Id and anti-Id (Fig. 3b). The degrees of inhibition ranged from 25-65% at the serum dilution of 1 to 10 and increased to 50-85% when the amount of the antisera was increased five-fold.

All five Ryegrass pollen allergic human sera and one non-allergic serum, diluted 10-fold were

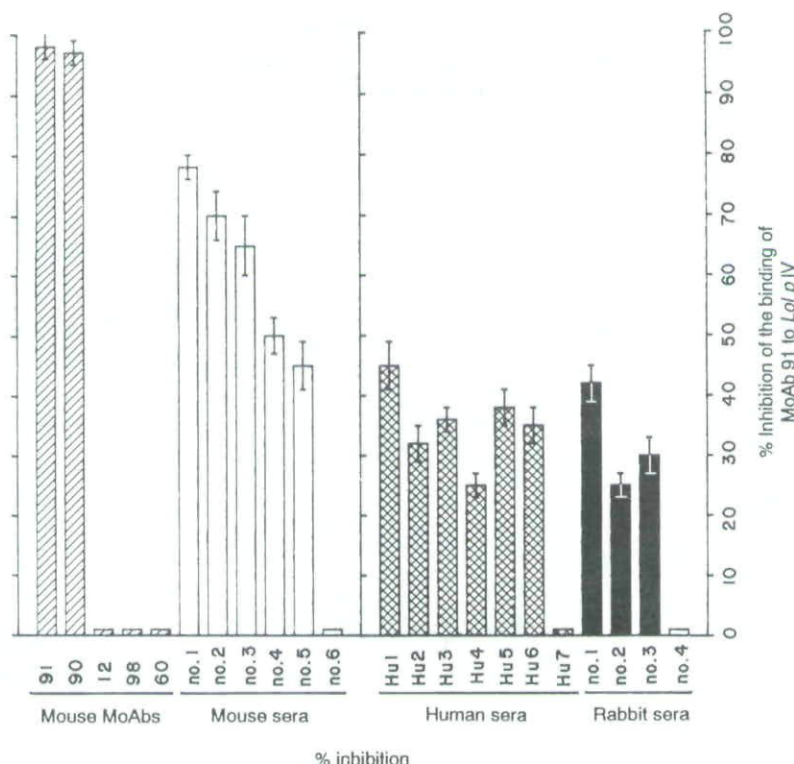


FIG. 1. Determination of relative antibody specificities by competitive inhibition assays. Biotinylated MoAb 91 (40 ng in 100 μ l) was mixed with various inhibitors. MoAbs were used at 20 μ g in 100 μ l and the anti-*Lol p IV* sera were diluted ten-fold. The maximum binding of the antibodies to solid-phase *Lol p IV* was determined in the absence of inhibitor. The degree of inhibition (%) of the binding of the biotinylated MoAb 91 to a solid-phase *Lol p IV* was calculated from the mean values ($\pm$ SEM) of ELISA assays performed in triplicate.

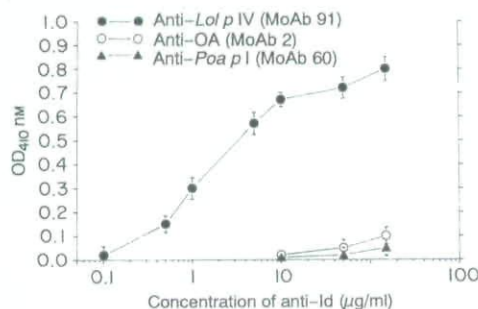


FIG. 2. Specificity of the monoclonal anti-Id B1/1. The biotin-avidin two-site ELISA was used to determine the extent to which the anti-Id B1/1 bound to: MoAb 91 anti-*Lol p IV* (the homologous Id) and control MoAb 2 anti-ovalbumin, and MoAb 60 anti-*Poa p I*. Each point represents the mean value ($\pm$ SEM) of ELISA assays performed in triplicate.

also partially able to inhibit (25–42%) the murine Id-anti-Id interaction. The degrees of inhibition increased to 55–68% when these sera were used at a two-fold dilution (Fig. 3c).

Of three rabbit antisera to *Lol p IV*, diluted 10-fold, two of them partially inhibited (25% and 55%) the binding between the Id and anti-Id. The degrees of inhibition increased to 55% and 85%, respectively, when the sera were used at a dilution of 1:2 (Fig. 3d).

The specificity of the inhibitions was demonstrated by the observations that the sera obtained from mice and rabbits prior to immunization and from a normal human individual had no effect on the Id-anti-Id interaction. Moreover, after removing the anti-*Lol p IV* antibodies from the mouse, human and rabbit antisera by affinity

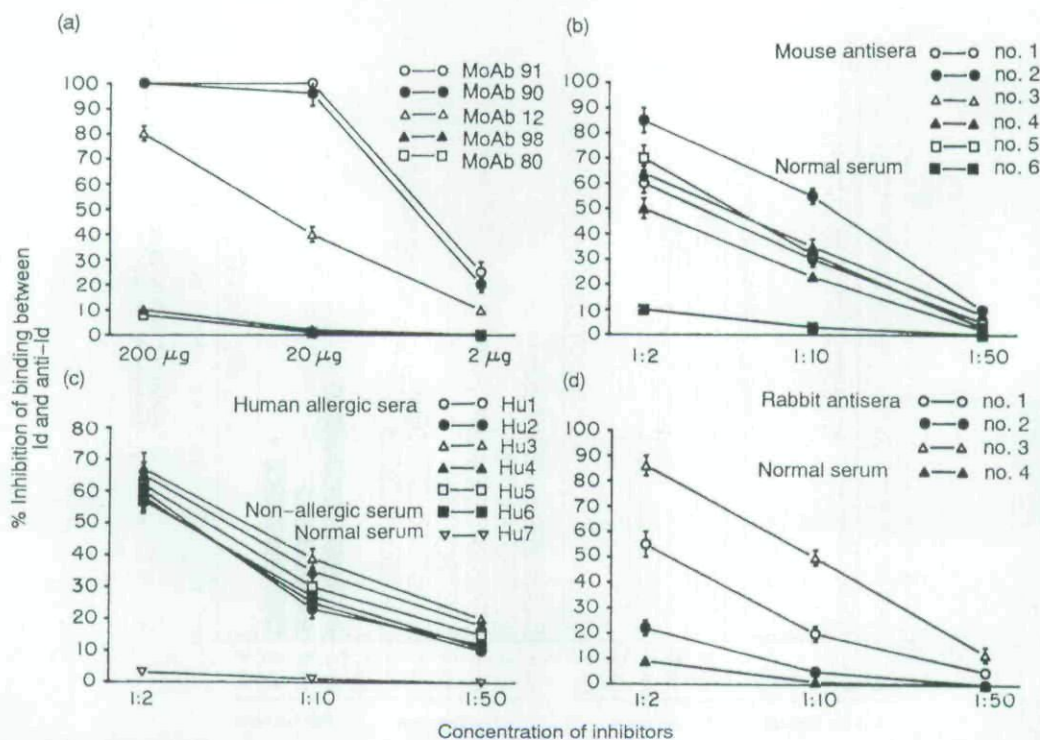


FIG. 3. Demonstration that antibodies to *Lol p IV* and MoAb 91 share a common Id. The extent to which antibodies from different sources (i.e. the inhibitors) were capable of inhibiting the binding between the Id (MoAb 91 solid-phase) and anti-Id B1/1 (200 ng/100 μ l) was elevated. (a) Monoclonal antibodies were used at various amounts (μ g/100 μ l); (b-d) antisera were used at various dilutions. The maximum binding of the anti-Id to the solid-phase MoAb 91 was determined in the absence of inhibitor. The per cent inhibition was determined from the mean values ($\pm$ SEM) of the ELISA assays performed in triplicate.

absorption with *Lol p IV* conjugated to Sepharose 4B, the capacity of the absorbed antisera to inhibit the Id-anti-Id interaction was markedly reduced (Fig. 4). Thus, on the basis of these collective results, the monoclonal anti-Id B1/1 was considered to recognize an Id that was common to antibodies in the mouse, human and rabbit antisera to *Lol p IV*.

Monoclonal anti-Id possesses the characteristics of an internal image anti-Id

The anti-Id B1/1 was examined to determine if it possessed the properties of an internal image anti-Id, i.e. whether or not: (i) anti-Id B1/1 could inhibit the binding of antibodies to *Lol p IV* and, (ii) the binding of anti-Id B1/1 to the Id could be inhibited by *Lol p IV*. The degrees of inhibition were found to be dose dependent. In the presence

of anti-Id B1/1 (25 μ g/well), the binding of MoAb 90 and 91 to solid-phase *Lol p IV* was inhibited to the extent of 100% (Fig. 5a). Similarly, it was found that the antibodies in murine antisera to *Lol p IV* were inhibited from binding to *Lol p IV* by anti-Id B1/1 to the extent of 60-85% (Fig. 5b); the binding of antibodies in human allergic and non-allergic sera to *Lol p IV* was inhibited by 60-80% (Fig. 5c) and by 75-90% for the rabbit antisera (Fig. 5d). In the presence of *Lol p IV* (final concentration of 100 μ g/ml), the binding of anti-Id B1/1 to solid-phase MoAb 91 was inhibited by 50%, whereas no inhibition was achieved with ovalbumin even at a concentration of 1 mg/ml (Fig. 6). Together, these results indicate that the anti-Id B1/1 possesses the characteristics of an internal image anti-Id which recognizes the Id located within or near the antigen combining sites of the antibodies to *Lol p IV*.

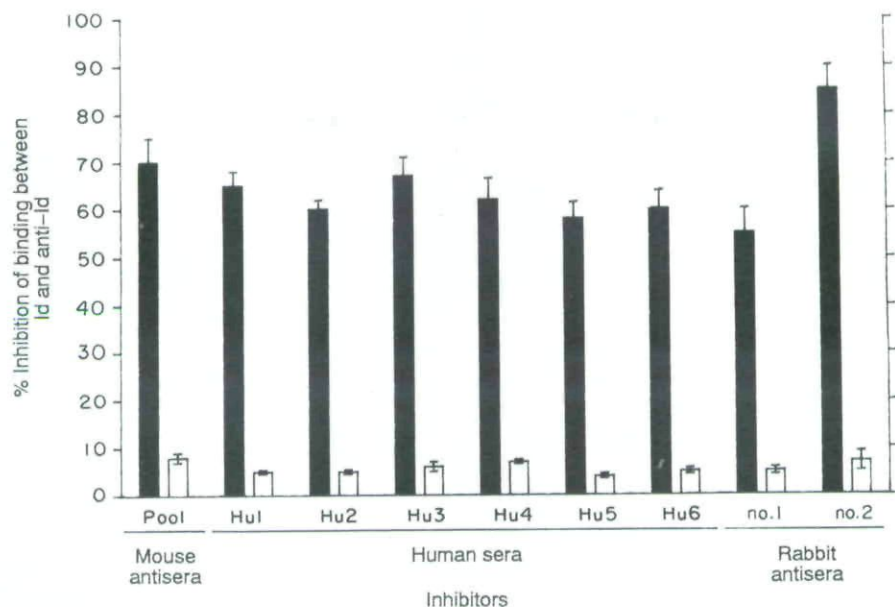


FIG. 4. Effect of absorption of anti-Lol p IV antibodies on the ability of antisera to inhibit the Id-anti-Id interaction. The extent to which the Id-anti-Id interaction was inhibited by antisera, before (solid columns) and after (open columns) removing anti-Lol p IV antibodies by affinity absorption on Lol p IV sepharose 4B. Antisera were diluted 10-fold. The per cent inhibition was determined from the mean values ($\pm$ SEM) of the ELISA assays performed in triplicate.

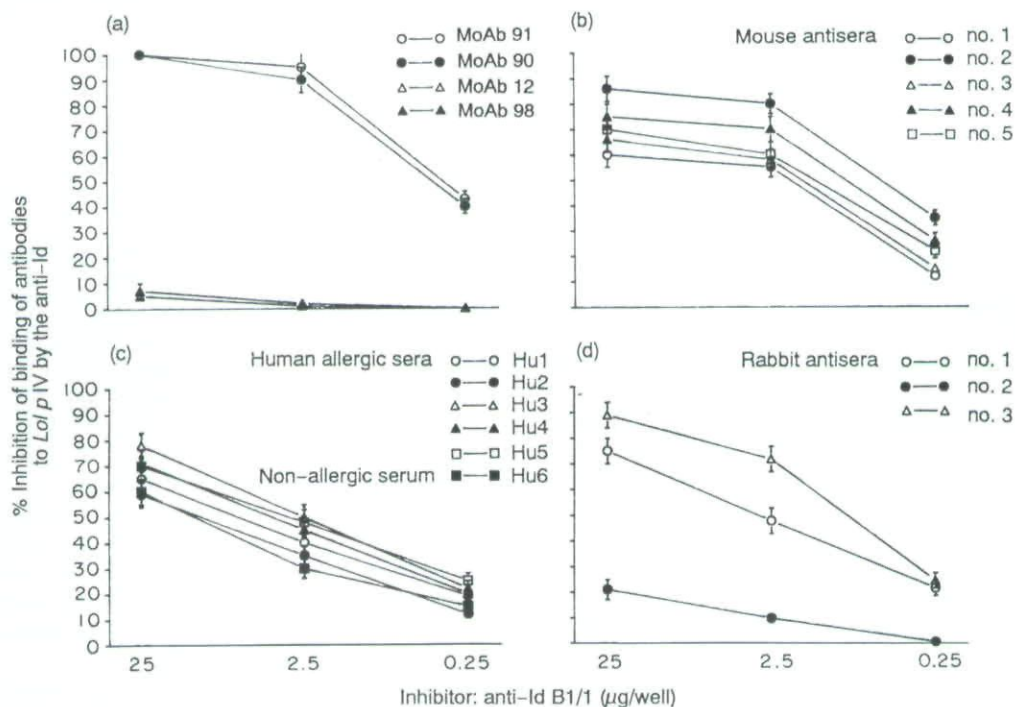


FIG. 5. Demonstration that the binding of antibodies to Lol p IV is inhibitable by the anti-Id B1/1. The extent to which the anti-Id B1/1 recognized the Id on mouse MoAbs and antibodies present in mouse, human and rabbit antisera to Lol p IV and interfered with their binding to Lol p IV was evaluated. The maximum binding of the antibodies to the solid-phase Lol p IV was determined in the absence of the anti-Id B1/1. The per cent inhibition was determined from the mean value ($\pm$ SEM) of the ELISA assays performed in triplicate.

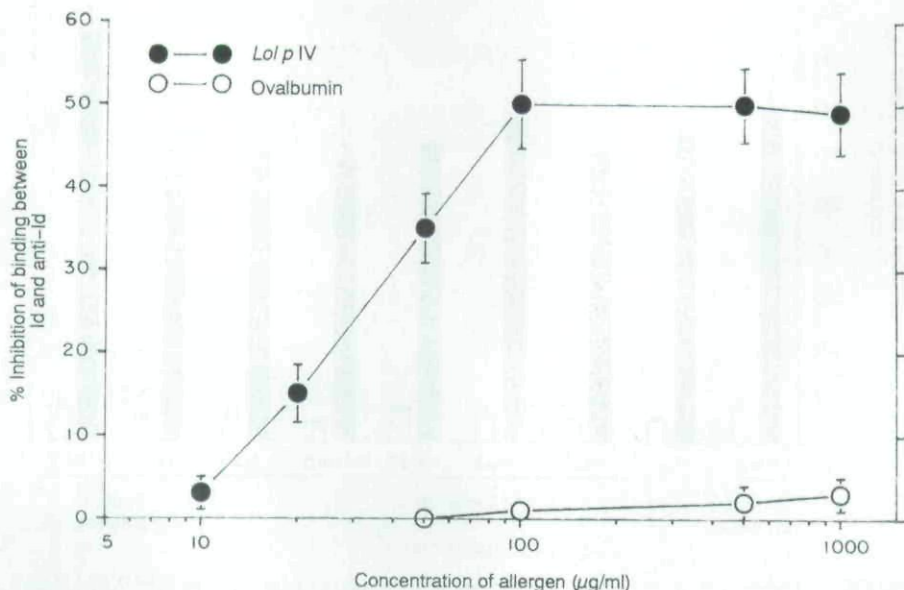


Fig. 6. Demonstration that the Id-anti-Id interaction is inhibitable by the homologous antigen. The extent to which the presence of *Lol p IV* or ovalbumin inhibited the binding of the anti-Id B1/1 (100 ng/well) to the solid-phase MoAb 91 (200 ng/well) is illustrated. The maximum binding of the anti-Id to the solid-phase MoAb 91 was determined from the mean values ($\pm$ SEM) of the ELISA assays performed in triplicate.

DISCUSSION

This report describes the generation and characterization of a mouse monoclonal anti-Id, designated as B1/1, directed to an Id on monoclonal antibody, MoAb 91. MoAb 91 recognized an epitope referred to as Site A on the Ryegrass pollen allergen *Lol p IV* [6]. The finding that the Id (MoAb 91) and anti-Id (B1/1) interaction could be inhibited, in a dose-dependent manner, by mouse, human and rabbit antisera to *Lol p IV* (Fig. 3) and that the anti-Id could inhibit these antibodies from binding to *Lol p IV* (Fig. 5) indicated that the Id is common to antibodies that are formed to *Lol p IV* in unrelated species. Moreover, evidence was also obtained which revealed that the interaction between the Id and anti-Id could be inhibited by *Lol p IV* (Figure 6). From these observations, it was inferred that anti-Id B1/1 recognized an Id either within or near the antigen combining sites of MoAb 91. According to the classification of anti-idiotypic antibodies [12, 13], the anti-Id B1/1 has the characteristics of an internal image anti-Id. Although the expression of the Id recognized by B1/1 on the antibodies of various species to *Lol p IV* varied, it was evident that these antibodies all

possessed an Id which is same or similar to that present on MoAb 91.

The levels of the Id on the antibodies to *Lol p IV* were evaluated in terms of the ability of the antisera to inhibit the binding between the Id (MoAb 91) and the anti-Id (MoAb B1/1). It was clear that in the presence of homologous Id MoAb 91 (or MoAb 90 which had identical specificity to MoAb 91) the Id-anti-Id interaction was completely inhibited. On the other hand, none of the mouse and rabbit antisera and the human allergic sera at the maximum dose employed (diluted 2-fold) was able to completely inhibit the Id-anti-Id interaction (Fig. 3). Since the antibodies to *Lol p IV* in the different sera are of polyclonal origin and therefore are capable of recognizing any of the determinants of *Lol p IV* e.g. Sites A, B, C, D, [6] or other as yet unidentified epitopes, it is evident that only that fraction of the entire repertoire of antibodies which is (i) directed to Site A of *Lol p IV* and (ii) possesses the Id which is the same or similar to that of MoAb 91, has the capacity to inhibit the Id-anti-Id interaction. Therefore, it was not surprising to find that the sera were not able to completely inhibit the Id-anti-Id interaction.

Similar observations were made in evaluating

the capacity of anti-Id B1/1 to inhibit the binding of antibodies of various sources to *Lol p IV*. It was demonstrated that in the homologous system the binding of MoAb 91 and MoAb 90 to *Lol p IV* were completely inhibitable by the anti-Id, whereas antibodies to *Lol p IV* in the various antisera, were capable of being inhibited from binding to *Lol p IV* to a maximum of approximately 70% (Fig. 5). In these assays, the polyclonal antibodies which lacked the Id of MoAb 91, would not be expected to be inhibited from binding to *Lol p IV* by the anti-Id, as was indeed the case.

A previous study in this laboratory [14] has shown that a rabbit polyclonal anti-Id antiserum that was rendered specific for the Id of a murine monoclonal anti-*Poa p IV* antibody (which recognized epitope Site B of Ryegrass pollen allergen *Lol p IV*) also recognized an Id on human antibodies to *Lol p IV*. Thus, the human antibodies and murine MoAbs to *Lol p IV* possessed Ids of the same or similar specificity. Other studies have shown that mouse monoclonal and rabbit polyclonal anti-Id produced to antibodies against the Ryegrass pollen group I allergen [3, 4] inhibited the binding of the mouse and human antibodies to the allergen. In our investigations, the anti-Id recognized not only the Id on mouse antibodies to *Lol p IV* but also the Id on human and rabbit antibodies to *Lol p IV*. This finding was confirmed by the observations that removal of the anti-*Lol p IV* antibodies from the mouse, human and rabbit antisera by affinity absorption, removed also the capacity of these absorbed antisera to inhibit the binding between the Id and anti-Id (Fig. 4). The antibodies to *Lol p IV* in one of three rabbit anti-*Lol p IV* antisera appeared to lack the Id recognized by anti-Id B1/1 since the anti-Id had no effect on the binding of the antibodies to *Lol p IV* and the antibodies were not able to inhibit the Id-anti-Id interaction. It was concluded that the expression of an idiotype specificity can vary from one individual to another.

The observations that in the presence of the anti-Id B1/1, both monoclonal and polyclonal antibodies to *Lol p IV* were inhibited from binding to *Lol p IV* (Fig. 5) and that *Lol p IV* inhibited the Id-anti-Id interaction (Fig. 6), indicated that B1/1 has the characteristics of an internal image anti-Id. The reasons for the finding that *Lol p IV* could inhibit the Id-anti-Id reaction only to the extent of 50%, are not presently known. It may be speculated that the

affinity between the Id and anti-Id in the assay system employed was significantly higher than the affinity between the anti-*Lol p IV* antibodies and *Lol p IV*.

Interestingly, even though MoAb 91 and MoAb 12 recognized different epitopes on *Lol p IV* (i.e. Site A and B, respectively) [6], MoAb 12 was also capable of inhibiting the binding between the Id and anti-Id. This fact indicated that MoAb 12 possessed an Id with a specificity which is the same or similar to that found on MoAb 91. Observations of a comparable nature have been reported by other investigators who found that monoclonal antibodies which recognized different epitopes of an antigen (e.g. GAT, [15], hen egg lysozyme [16], whale myoglobin [17], lipopolysaccharide [18], haemagglutinin of influenza virus [19], and herpes simplex virus [9]) possessed the same or a similar idiotype specificity. In the present study, MoAb 91 (anti-Site A) which bears the homologous Id, was clearly superior to MoAb 12 (anti-Site B) in its ability to inhibit the Id-anti-Id interaction. These observations may be interpreted to indicate that the Id on MoAb 12 is either not identical or as accessible as the homologous Id for binding the anti-Id. Moreover, MoAb 12 could not be inhibited by the anti-Id from binding to *Lol p IV*. On the basis of these observations, it is suggested that the Id on MoAb 12 which is recognized by anti-Id B1/1 is not located within the antigen binding sites. By comparison, the rabbit anti-Id produced against MoAb 12 partially inhibited the binding of this Mab to *Lol p IV* [14]. This result is not surprising since the rabbit anti-Id antibodies are of polyclonal origin and are, therefore, capable of recognizing collectively the idiotypes on MoAb 12 and of inhibiting, at least partially, the binding between this MoAb and *Lol p IV*.

One prevailing concept in immunology developed from the idiotype network theory [1] is that the immune response to a given antigen can be regulated by a series of Id-anti-Id interactions. The expression of a given Id is under the control of an anti-Id and the anti-Id can also be regulated by an anti-anti-Id. This complex set of interactions operates via a feedback mechanism to either enhance or suppress the immune response (i.e. formation of antibodies with a particular idiotype). The investigations on the immune response to grass pollen allergens [4, 14, 20] indicate the existence of the Id networks. However, the role(s) of the Id-anti-Id networks in controlling the

immune responses to Ryegrass pollen allergens is (are) not known at this time. Investigations using a murine model system are in progress to determine whether the anti-Id B1/1 can modulate the immune response to Ryegrass pollen allergen *Lol p IV*.

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