

## Characterization of anti-idiotypic antibodies and their use as probes for determination of shared idiotopes expressed on murine and human IgE anti-rye I antibodies

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### SUMMARY

This study describes the production and characterization of rabbit anti-idiotypic antibodies (anti-ID Abs) against three idiotypes of three mAbs with different specificities. The anti-ID Abs were rendered idiotypic specific by appropriate adsorption. Binding of labelled mAb to homologous anti-ID Ab bound to a polystyrene matrix was completely inhibited when the same mAb was added. In contrast, addition of other mAbs sharing the same isotype and the same light chain but with different specificity did not affect the binding reaction. Each anti-ID Ab inhibited completely and selectively the reaction between the allergen and the homologous mAb idiotypic. Labelled rye I binding to a given polystyrene-bound mAb idiotypic was completely blocked if the relevant anti-ID Ab was used as an inhibitor. Murine polyclonal anti-rye I antisera inhibited the reaction between all three mAbs and the antigens, as well as the reaction between all three mAb idiotypes and their homologous anti-ID Abs. On another hand, goat polyclonal anti-rye I antisera only inhibited the reaction between the mAbs and the antigens. These results suggest that the anti-ID Abs produced are directed against idiotopes located within the paratopes and such idiotopes are shared by murine monoclonal and polyclonal Abs. Human rye I-specific IgE and murine anti-rye I mAbs could share common idiotopes, since human IgE binding to the antigen was inhibited by the anti-ID Abs. These observations imply structural similarity in the V gene coding for the variable region of the antibody of two different species.

### INTRODUCTION

The specificity of an antibody (Ab) resides in the amino acid sequence of the variable region of the molecule, which expresses several determinants or idiotopes that are referred to collectively as the idiotypic. Regulation of a first Ab response can be monitored by the production of a second Ab population known as anti-idiotypic antibodies (anti-ID Abs) (Jerne, 1974). These anti-ID Abs can also be used to analyse the heterogeneity of the immune response and to study the idiotypic cross-reactivity. They can recognize idiotopes unique for one antibody (private) or shared by more than one antibody (public).

It has been postulated that Abs of different specificities do not share common idiotypes (Kunkel, Mannick & Williams, 1963; Kelus & Gell, 1969). It was also assumed that the same idiotopes would not normally be found on Abs of different

classes from the same subject, and even less so on Abs from unrelated individuals. But a growing body of evidence suggests that this is not the case. Indeed, in humans, using anti-ID Abs, idiotypic cross-reactivity has been found among Abs of different classes (IgE, IgM and IgG) of the same specificity on the same individual (Geha & Weinberg, 1978) and among different subjects (Bose *et al.*, 1984). Also, such cross-reactivity has been shown between rabbit polyclonal Abs of different specificities (Oudin & Cazenave, 1971) and between murine mAbs of different specificities (Zanetti, De Baets & Rogers, 1983).

Inter-species idiotypic cross-reactivity has also been demonstrated between goat and sheep Abs of the same specificity (Karol, Reichlin & Noble, 1977) and between mouse and rabbits (Francotte & Urbain, 1984). To our knowledge, there have only been a limited number of reports (Morgan *et al.*, 1985; Mourad & Hébert, 1986) showing idiotypic cross-reactivity between two phylogenetically different species, such as the mouse and man. We report here, firstly the production and characterization of rabbit anti-ID Abs against three anti-rye I mAbs of different specificity, and secondly, we used these anti-ID Abs as probes to study the idiotypic cross-reactivity between murine anti-rye I mAbs and human specific IgE Abs.

Abbreviations: Ab, antibody; anti-ID, anti-idiotypic; BSA, bovine serum albumin; mAb, monoclonal antibody; PBS, phosphate-buffered saline.

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## MATERIALS AND METHODS

### *Antigens and antisera*

Rye I allergen was purified by immunosorbent techniques from crude rye grass extract (Omega Laboratories, Montréal) as described previously (Mourad *et al.*, 1986). Ragweed allergen (AgE) was obtained from the National Institutes of Health (Bethesda, MD). Murine polyclonal anti-rye I antisera were produced by immunization of mice with purified rye I (100 µg/mouse, emulsified in complete Freund's adjuvant; Difco Laboratories, Detroit, MI). After three weekly injections, mice were bled and sera collected and frozen at  $-80^{\circ}$  until further use. Goat anti-rye I antisera were kindly provided by Dr D. G. Marsh (Johns Hopkins Hospital, Baltimore).

Human sera were obtained from two groups of allergic donors from Le Centre Hospitalier Université Laval, Chul, Quebec, after informed consent was obtained. The first group had clinical symptoms of allergic rhinitis during the grass pollen season (June, July) and positive skin tests (prick technique) to purified rye I. The second group included subjects with no history of allergy to grasses and negative skin tests, but they were clinically sensitive to ragweed (August, September), with positive skin tests to ragweed.

The preparation of murine anti-rye mAbs [290A-167 (γ2b, K), 348A-6 (γ1, K) and 539A-6 (γ2a, K)] has been reported previously (Mourad *et al.*, 1986). These mAbs are directed against three distinct non-overlapping and non-repetitive epitopes on rye I (W. Mourad, G. Pelletier and J. Hébert, submitted for publication). Murine anti-ferritin mAbs 3E10 (γ1, K), 3E3 (γ2a, K) and 13A-12 (γ2b, K) were kindly provided by Dr J. P. Valet (Chul, Québec). 7H8 anti-human IgE mAb was produced previously in our laboratory (J. Hébert, D. Bernier and W. Mourad, submitted for publication). All of these mAbs were purified from mouse ascitic fluid by passing the fluid through a protein A-Sepharose CL4B column.

### *Radioiodination*

Protein A-purified mAbs (290A-167, 348A-6, 539A-6, 3E3, 3E10, 13A-12 and 7H8) and purified rye I were iodinated with  $^{125}\text{I}$  by using Iodo-Gen or Iodo Beads (Pierce Chemical Co., Rockford, IL). Free  $^{125}\text{I}$  was separated from  $^{125}\text{I}$ -labelled protein by passage through a Sephadex G-50 column. Specific activities were estimated following the instructions of the company and were in the range of  $5\text{--}12 \times 10^6$  c.p.m./µg.

### *Immunosorbent*

Purified human IgG (depleted of rye I antibody activity), 3E10, 3E3 and 13A-12 mAbs were dialysed against 0.1 M  $\text{NaHCO}_3$  and cross-linked separately to cyanogen bromide-activated Sepharose CL4B (Pharmacia, Piscataway, NJ) using the standard procedure (Cuatrecasas, 1970).

### *Purification of $\text{F(ab')}_2$ fragments of anti-rye I mAbs*

$\text{F(ab')}_2$  fragments of each anti-rye I mAb (290A-167, 348A-6 and 539A-6) were purified as described previously (Mourad & Hébert, 1986). Briefly, protein A-purified mAbs were rendered highly specific by adsorption over rye I coupled to Sepharose CL4B. The adsorbed mAbs were eluted and hydrolysed with immobilized pepsin (Pierce Chemical Co.).  $\text{F(ab')}_2$  was separated from Fc and undigested mAb by passing through a protein A-Sepharose CL4B column. The unbound material was con-

centrated and dialysed; this fraction contained exclusively  $\text{F(ab')}_2$  fragments as assessed by Ouchterlony immunodiffusion.

### *Immunization of rabbits*

After 10 ml of blood were obtained, rabbits were first immunized with 1 mg of a given  $\text{F(ab')}_2$  anti-rye I mAb in 1 ml of phosphate-buffered saline (PBS) (pH 7.4) emulsified in an equal volume of complete Freund's adjuvant (Difco Laboratories). Three booster immunizations of 150 µg of  $\text{F(ab')}_2$  in incomplete Freund's adjuvant were then given at weekly intervals, and the rabbits were bled 2 weeks after the last booster dose. All immunizations were performed in multiple 0.1 ml intradermal and subcutaneous injections.

### *Purification of rabbit anti-ID Abs*

Rabbit blood was allowed to clot at  $37^{\circ}$  for 1 hr, then at  $4^{\circ}$  overnight. The serum was separated, dialysed against PBS (pH 8.0) and passed through a protein A-Sepharose column. The IgG was eluted with 100 mM glycine-HCl (pH 2.8), dialysed, concentrated to 10 mg/ml and rendered idiotype specific by several adsorptions with 3E3, 3E10, 13A-12 mAbs, rye I, and non-specific human IgG coupled to Sepharose CL4B. The IgG fraction that did not attach to the protein column was referred to as anti-ID Abs.

### *Assay for anti-ID Abs specificity*

The specificity of the anti-ID Abs was assessed by the ability of the corresponding mAb to inhibit completely the reaction between the anti-ID Ab and the homologous mAb idiotype. This was performed as follows: polystyrene removable strips (Dynatech Laboratories Inc., Chantilly, VI) were first coated with a given anti-ID Ab (200 ng/well), followed by saturation of protein binding sites by adding 200 µl of 3% bovine serum albumin (BSA)/well. Wells were washed and  $^{125}\text{I}$ -labelled mAb ( $10^5$  c.p.m./well) added to the homologous polystyrene-bound anti-ID Ab. In inhibition assays, labelled mAb was added in the presence of various amounts (6.25–500 ng/well) of cold mAb of the same origin or of different specificity but sharing the same isotype and light chain. After overnight incubation, wells were washed, the radioactivity counted in a Gamma Counter (LKB, Sweden) and the percentage inhibition calculated as follows:

$$1 - \left( \frac{\text{c.p.m. bound in presence of inhibitor}}{\text{c.p.m. bound in absence of inhibitor}} \right) \times 100.$$

### *Assay for anti-ID Ab activity*

Anti-ID Ab activity was determined by the ability of anti-ID Abs to inhibit the binding between the idiotype and the rye I allergen. This was performed by two different methods. First, the ability of anti-ID Abs to inhibit the binding of labelled mAbs to polystyrene-bound rye I was assessed. Polystyrene removable strips were first coated with rye I (12.5 ng/well), followed by 200 µl of 3% BSA/well to saturate additional protein-binding sites. Wells were washed and labelled mAb (290A-167, 348A-6 or 539A-6) ( $10^5$  c.p.m./well) was added, alone or in the presence of various amounts (0.25–250 ng/well) of anti-ID Abs or control rabbit IgG previously adsorbed on rye I, 3E3, 3E10 and 13A-12 mAbs coupled to Sepharose CL4B in order to remove any naturally occurring anti-mouse IgG and anti-rye I antibodies. After overnight incubation, wells were washed, the radioactivity counted and the percentage inhibition calculated as just de-

scribed. Second, the ability of anti-ID Abs to inhibit the binding of labelled rye I to polystyrene-bound mAbs was also assessed, polystyrene removawell strips were coated with a given anti-rye I mAb (290A-167, 348A-6 or 539A-6) (100 ng/well) followed by saturation with 3% BSA. After washing, labelled rye I ( $10^5$  c.p.m./well) was added alone or in the presence of various amounts (0.5–500 ng/well) of anti-ID Ab or control rabbit IgG and the assay was carried out as just described.

#### Specificity of polyclonal murine and goat anti-rye I antisera

Epitope specificity of polyclonal murine (pool of three mice) or goat anti-rye I antisera was analysed by inhibition of the binding of labelled mAb to polystyrene-bound rye I. Polystyrene removawell strips were first coated with rye I (12.5 ng/well) followed by incubation with 3% BSA. After washing, labelled mAb (290A-167, 348A-6 or 539A-6) ( $10^5$  c.p.m./well) was added alone or in the presence of various dilutions of goat or murine anti-rye I antisera. The latter polyclonal antibodies were adsorbed previously with 3E3, 3E10 and 13A-12 mAbs coupled to Sepharose CL4B in order to remove any naturally occurring anti-mouse IgG subclasses. Goat and mouse non-immune sera were used as negative controls. After overnight incubation wells were washed, radioactivity counted and the percentage inhibition calculated.

#### Specificity of human rye I-specific IgE

The capacity of anti-rye I mAbs to inhibit the reaction between human serum IgE and the allergen was assessed. Polystyrene removawell strips were coated with rye I (200 ng/well) and saturated with 3% BSA. The plates were incubated overnight with human serum diluted 1/4 alone or in the presence of anti-rye I mAb (290A-167, 348A-6 or 539A-6;  $1 \mu\text{g/ml}$ ). 13A-12 mAb was used as a negative control. After washing, labelled 7H8 anti-human IgE mAb was added ( $10^5$  c.p.m./well) and incubated for 18 hr. Wells were extensively washed, the radioactivity counted and the percentage inhibition calculated.

#### Idiotypic cross-reactivity

Firstly, idiotypic cross-reactivity between anti-rye I mAbs and murine or goat polyclonal anti-rye I antisera was examined by competitive inhibition of the binding of labelled mAb to its homologous polystyrene-bound anti-ID Ab bound to polystyrene. Briefly, polystyrene strips were coated with a given anti-ID Ab (200 ng/well) followed by saturation with 3% BSA. Labelled mAb ( $10^5$  c.p.m./well) was added in competition with various dilutions of polyclonal anti-rye I antisera obtained from mice or goats. Non-immune mice and goat sera were used as negative controls. After overnight incubation, wells were washed, radioactivity counted and the percentage inhibition calculated.

Secondly, idiotypic cross-reactivity between anti-rye I mAb and human rye I serum-specific IgE was assessed by the ability of anti-ID Abs directed against each mAb to inhibit the reaction between serum IgE and rye I- or ragweed-coated plates. Human sera (at a dilution found in preliminary experiments to bind approximately 50% of the added labelled 7H8 anti-human IgE mAb) were first mixed with various amounts of a given anti-ID Ab, or normal rabbit IgG as a negative control. After 2 hr of incubation with constant agitation, the mixtures were transferred to polystyrene wells previously coated with rye I or

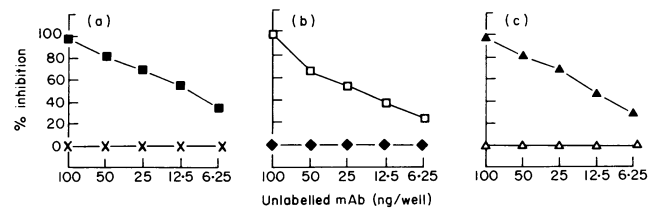


**Figure 1.** Inhibition by unlabelled mAbs (■—■, 290A-167; □—□, 348A-6; and ▲—▲, 539A-6) of the binding of labelled 290A-167 (a), 348A-6 (b) and 539A-6 (c) mAbs to their homologous polystyrene-bound anti-ID Abs. 13A-12 (×—×), 3E12 (◆—◆), and 3E-3 (△—△) mAbs, were used as negative controls. Each point represents the mean value of the three experimental determinations.

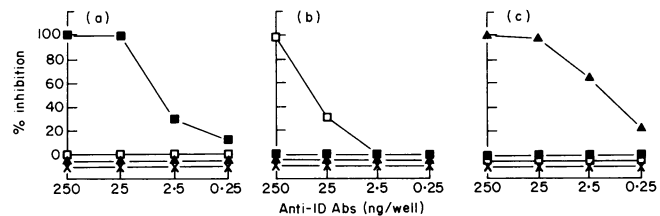


**Figure 2.** Inhibition by anti-ID Abs (■—■, anti-ID of 290A-167; □—□, anti-ID of 348A-6; and ▲—▲, anti-ID of 539A-6 mAbs) of the binding of labelled 290A-167 (a), 348A-6 (b), and 539A-6 (c) mAbs to polystyrene-bound rye I. Normal rabbit IgG was used as negative control (×—×). Each point represents the mean value of three experimental determinations.

ragweed (Ag-E) (200 ng/well) and saturated with 3% BSA. After overnight incubation, wells were washed, labelled 7H8 anti-human IgE ( $10^5$  c.p.m./well) added, and incubated overnight. Wells were washed, radioactivity counted and the percentage inhibition calculated.

## RESULTS

### Characterization of anti-ID Abs

Rabbit anti-ID Abs produced against each anti-rye I mAb were rendered idiotypic specific by subsequent adsorption with 3E3, 3E10 and 13A-12 mAbs coupled to Sepharose CL4B. These three mAbs have the same isotype and light chains as the three anti-rye I mAbs, but they are of different specificity.

Figure 1 shows the specificity of the resulting anti-ID Abs. The binding of labelled mAb to homologous polystyrene-bound anti-ID Ab was completely inhibited when the same cold mAb was added. In contrast, this reaction was not affected when other mAbs of the same isotype and light chain but of different specificity were used. The anti-ID Ab can only completely inhibit the binding of the homologous mAb idiotypic to polystyrene-bound rye I (Fig. 2).

In order to verify whether or not the idiotypic determinants recognized by the anti-ID Ab were associated with the antigenic combining site, the ability of the anti-ID Ab to inhibit the binding of labelled rye I to a polystyrene-bound mAb idiotypic was assessed. The results are shown in Fig. 3. Binding of labelled rye I to mAb idiotypic was completely inhibited only by the corresponding anti-ID Ab.

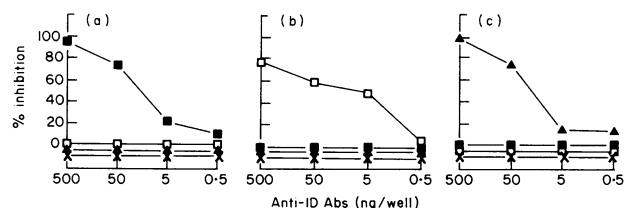


**Figure 3.** Inhibition by anti-ID Abs (■—■, anti-ID of 290A-167; □—□, anti-ID of 348A-6; and ▲—▲, anti-ID of 539A-6) of the binding of labelled rye I to polystyrene-bound 290A-167 (a), 348A-6 (b), and 539A-6 (c) mAbs. Normal rabbit IgG was used as negative control (x—x). Each point represents the mean value of three experimental determinations.

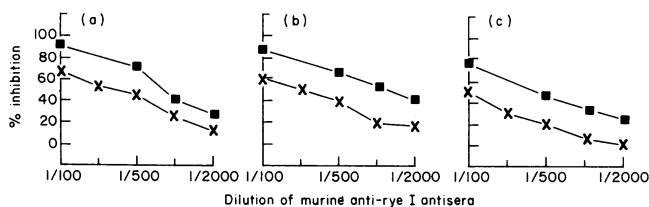


**Figure 4.** Inhibition by murine anti-rye I antisera of the binding of labelled 290A-167 (a), 348A-6 (b), and 539A-6 (c) mAbs to polystyrene-bound rye I (■—■) or to their homologous anti-ID Ab-coated polystyrene (x—x). Normal murine sera were used as negative controls. Each point represents the mean value of three experimental determinations.

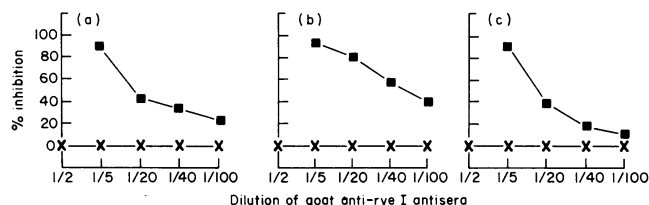


**Figure 5.** Inhibition by goat anti-rye I antisera of the binding of labelled 290A-167 (a), 348A-6 (b), and 539A-6 (c) mAbs to polystyrene-bound rye I (■—■) or to their homologous anti-ID Ab-coated polystyrene (x—x). Normal goat sera were used as negative control. Each point represents the mean value of three experimental determinations.

### Idiotypic cross-reactivity

In order to verify whether or not polyclonal murine and goat anti-rye I shared common idiotopes with murine anti-rye I mAbs, it was first important to compare the epitope specificity of polyclonal Abs to that of mAbs. Such specificity was confirmed by the ability of polyclonal Abs to inhibit the binding of labelled mAbs to polystyrene-bound rye I. Then idiotypic cross-reactivity was analysed by the ability of polyclonal Abs to inhibit the binding of homologous idiotypic and anti-ID Abs. Figure 4 shows that murine polyclonal anti-rye I can inhibit the reaction between labelled mAbs and the allergen as well as the ID-anti-ID reaction. In contrast, goat anti-rye I antisera can inhibit only the reaction between labelled mAbs and the allergen (Fig. 5). Idiotypic cross-reactivity between human specific IgE Abs and mAbs was assessed. The ability of each anti-rye I mAb to inhibit the reaction between rye I-specific IgE and the allergen

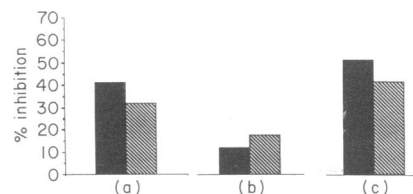


**Figure 6.** Inhibition by anti-rye I mAbs (■) [290A-167 (a), 348A-6 (b) and 539A-6 (c)] or their homologous anti-ID Abs (▨) of the binding of human IgE to polystyrene-bound rye I. The concentration of each mAb was 1 µg/ml and for anti-ID Ab was 250 µg/ml, to give such inhibition.

were analysed first. Then the idiotypic cross-reactivity was studied by the ability of each anti-ID Ab to inhibit the same reaction. Figure 6 shows the inhibition obtained with each anti-rye I mAb and with their respective anti-ID Abs. Human IgE binding to rye I is inhibited by all three anti-rye I mAbs, although binding is much more strongly inhibited with two of them (290A-167 and 348A-6). Approximately the same degree of inhibition was observed when the homologous anti-ID Abs were used. No additive inhibition was observed when two or three mAbs of different specificities, or their respective anti-ID Abs, were mixed (not shown). The three anti-rye I mAbs and their homologous anti-ID Abs did not affect the binding of IgE contained in the sera of patients sensitive to ragweed allergen (not shown).

### DISCUSSION

This paper describes the idiotypic specificity of rabbit anti-ID Abs produced against idiotypes of three different anti-rye I mAbs. After appropriate adsorptions of the rabbit antisera, the resulting anti-ID Abs recognized idiotypic determinants either very close to, or identical with, the paratopic (antigen-binding) region of the binding site. This conclusion is based on the observation that the binding of labelled antigen to a given polystyrene-bound anti-rye I mAb idiotype was completely blocked by the homologous anti-ID Ab. Such inhibition is a necessary, but not sufficient condition, to conclude that the anti-ID Abs contain an antibody fraction representing the internal image of the antigen. Recent reports have shown that anti-ID Abs representing the internal image of the antigen must react with an idiotype of the same specificity from different species (Urbain *et al.*, 1980; Thanavala *et al.*, 1985). Our data showed that goat anti-rye I antisera could strongly inhibit the binding of all three mAbs to the antigen, which suggests that goat antisera may contain Abs directed against the same or closely related epitopes as those recognized by anti-rye I mAbs. In contrast, these antisera did not affect the ID-anti-ID reaction. Therefore, it is not possible to conclude from these data if the anti-ID Abs used do represent or not the internal image of the antigen, even though they are directed against idiotopes located within the paratope, and study is in progress in order to clarify this point. However, it is reasonable to believe that the goat anti-rye I Abs used in this study did not express idiotopes shared with anti-rye I mAbs. In contrast, polyclonal antibodies obtained from rye I-immunized BALB/c mice (W. Mourad, G. Pelletier and J. Hébert, manuscript submitted for publication) share idiotypic determinants with anti-rye I mAbs originating from the same strain of mice, due to the fact that the former Abs could inhibit the ID-anti-ID reaction.

A number of studies have demonstrated that Abs of different specificities do not share common idiotopes (Kunkel *et al.*, 1963; Oudin & Michel, 1963, 1969; Kelus & Gell, 1969). However, Oudin & Cazenave (1971) have reported idiotypic cross-reactivity between polyclonal Abs of different specificities. Recently, Zanetti *et al.* (1983) further analysed such idiotypic cross-reactivity in producing anti-ID Abs against mAb idiotypes, and they reported that mAbs of different specificities may share common idiotopes. In our system, such cross-reactive idiotopes are not shared by anti-rye I mAbs of different specificity. This discrepancy could be explained by the extensive adsorption of our anti-ID Abs over immobilized anti-ferritin mAbs sharing the same isotype and light chain with anti-rye I mAbs, which could render them highly specific to the idiotype used for immunization of rabbits.

Inhibition of human IgE binding to the antigen by mAbs directed against the same antigen is a classical assay used in order to establish whether or not the epitopes recognized by mAbs also react with human IgE Abs. In spite of differences in the genetic make-up of mice and human, previous investigators using this assay have reported that Abs of both sources could be directed against the same epitopes (Bose *et al.*, 1986; Eseh & Klapper, 1985; Kahn & Marsh, 1982; Olson & Klapper, 1986). Our data show that mAbs can inhibit the binding of human IgE to the antigen, and a high level of inhibition was observed when 539A-6 mAb was used. However, we cannot conclude that both Abs (human IgE and mAb) are directed against the same epitopes, since no additive inhibition was observed when two mAbs of different specificities were mixed and used as inhibitors. Therefore, these data suggest that the site where human IgE bind to the antigen is partially shared by each epitope recognized by the three mAbs.

Limited reports have shown idiotypic cross-reactivity between Abs of different species. Morgan *et al.* (1985) and Eilat, Fischel & Zlotnik (1985) have shown such cross-reactivity between human and murine anti-DNA autoantibodies. Recently, using rabbit anti-ID Abs directed against human rye I-specific IgG, we have reported that murine anti-rye I mAbs may express idiotopes shared by human rye I-specific IgG (Mourad & Hébert, 1986). We report here that rye I-specific IgE may also share common idiotopes with mAbs, since anti-ID Abs directed against mAb idiotypes can inhibit the reaction between IgE and the antigen. This observation may suggest a structural similarity in the V gene coding for the variable regions of the Abs from these two different species which has been conserved during phylogenetic evolution. Finally, this study will make possible the use of such anti-ID Abs recognizing public idiotopes on human rye I-specific IgE to analyse their modulating effects on the *in vitro* synthesis of IgE Ab by peripheral blood lymphocytes from grass-sensitive patients.

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#### REFERENCES

- BOSE R., MARSH D.G., DUCHATEAU J., SEHON A.S. & DELESPESE G. (1984) Demonstration of auto-anti-idiotypic antibody cross-reactivity with public idiotypic determinants in the serum of rye-grass allergic patients. *J. Immunol.* **135**, 2474.
- BOSE R., RECTOR E.S., FISHER J., TARONNO R. & DELESPESE G. (1986) Production and characterization of mouse monoclonal antibodies to allergenic epitopes on Lolp I (Rye I). *Immunology*, **59**, 309.
- CUATRECASAS P. (1970) Protein purification by affinity chromatography. Derivations of agarose and polyacrylamide beads. *J. biol. Chem.* **245**, 3095.
- EILAT D., FISCHEL R. & ZLOTNIK A. (1985) A central anti-DNA idiotype in human and murine systemic lupus erythematosus. *Eur. J. Immunol.* **15**, 368.
- ESCH R.E. & KLAPPER D.G. (1985) An allergenically important, cross-reactive epitope on grass pollen Group I antigens. *Fed. Proc.* **44**, 1879.
- FRANCOTTE M. & URBAIN J. (1984) Induction of anti-tobacco mosaic virus antibodies in mice by rabbit anti-idiotypic antibodies. *J. exp. Med.* **160**, 1484.
- GEHA R.S. & WEINBERG R.P. (1978) Anti-idiotypic antisera in man. I. Production and immunochemical characterization of anti-idiotypic antisera to human antitetanus antibodies. *J. Immunol.* **121**, 1518.
- JERNE N.K. (1974) Toward a network theory of the immune system. *Ann. Immunol. (Inst. Pasteur)* **125C**, 373.
- KAHN C.R. & MARSH D.G. (1982) Monoclonal antibodies as probes for allergenic and antigenic determinants on the rye I allergen. *Fed. Proc.* **41**, 826.
- KAROL R.A., REICHLIN M. & NOBLE R.W. (1977) Evolution of an idiotypic determinant: anti-Val. *J. exp. Med.* **146**, 435.
- KELUS A.S. & GELL P.G.H. (1969) Immunological analysis of rabbit anti-antibody systems. *J. exp. Med.* **127**, 215.
- KUNKEL H.G., MANNIK M. & WILLIAMS R.C. (1963) Individual antigenic specificity of isolated antibodies. *Science*, **140**, 1218.
- MORGAN A., ISANBERG D.A., NAPARSTEK Y., RANCH J., DUGAN D., KHIROYA R., STAINES N.A. & SCHATTNER A. (1985) Shared idiotypes are expressed on mouse and human anti-DNA autoantibodies. *Immunology*, **56**, 393.
- MOURAD W. & HÉBERT J. (1986) Human IgG and murine monoclonal antibodies share common idiotopes as determined by competitive binding to polystyrene and nitrocellulose-bound antigens. *Immunol. Invest.* **15**, 801.
- MOURAD W., PELLETIER G., BOULET A., ISLAM N., VALET J.P. & HÉBERT J. (1986) Allergenicity and cross-reactivity of rye grass pollen extracts revealed by monoclonal antibodies. *J. immunol. Meth.* **89**, 53.
- OLSEN J.R. & KLAPPER D.G. (1986) Two major human allergenic sites on ragweed pollen allergen antigen E identified by using monoclonal antibodies. *J. Immunol.* **136**, 2109.
- UDIN J. & CAZENAVE P.A. (1971) Similar idiotypic specificities in immunoglobulin fractions with different antibody functions or even without detectable antibody function. *Proc. natl. Acad. Sci. U.S.A.* **68**, 2616.
- UDIN J. & MICHEL M. (1963) Une nouvelle forme d'allotypie des globulines et du sérum de lapin apparemment liée à la fonction et à la spécificité anticorps. *C.R. Acad. Sci. (Paris)*, **257**, 805.
- UDIN J. & MICHEL M. (1969) Idiotype of rabbit antibodies. I. Comparison of idiotype of antibodies against *Salmonella typhi* with that of antibodies against other bacteria in the same rabbits, or antibodies against salmonella typhi in various rabbits. *J. exp. Med.* **130**, 595.
- THANAVALA Y.M., TEDDER A.B.R., HAY F.C. & ROITT I.M. (1985) Monoclonal 'internal image' anti-idiotypic antibodies of hepatitis B surface antigen. *Immunology*, **55**, 197.
- URBAIN J., CAZENAVE P.A., WILKER M., FRANSSSEN J.D., MARIANNE B. & LEO O. (1980) Idiotype induction and immune network. In: *Progress in Immunology IV* (eds M. Fougereau and J. Dausset), p. 81, Academic Press, New York.
- ZANETTI M., DE BAETS M. & ROGERS J. (1983) High degree of idiotypic cross-reactivity among murine monoclonal antibodies to thyroglobulin. *J. Immunol.* **131**, 2452.