

Human and murine antibodies to Rye grass pollen allergen *LolpIV* share a common idiotope

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SUMMARY

The possibility that a murine monoclonal antibody (mAb 12) to Rye grass pollen allergen *LolpIV* and *LolpIV*-specific antibodies in the sera of grass allergic individuals share a common idiotope (Id) was investigated. It was first established that mAb 12 and human IgE antibodies recognized the same (or similar) epitope(s) on *LolpIV*; i.e. mAb 12 could inhibit, to the extent of 35-60%, the binding of ^{125}I -*LolpIV* to the human IgE antibodies present in the sera of grass pollen-allergic individuals. Subsequently, a rabbit anti-Id antiserum was produced against mAb 12 and rendered Id-specific by appropriate immune absorptions, and its IgG antibody fraction was isolated (Rb-aId). The specificity of Rb-aId was demonstrated by the fact that the antibodies bound only to mAb 12 and not to any other murine monoclonal antibody tested. Observations that Rb-aId inhibited the binding of ^{125}I -*LolpIV* to mAb 12 indicated that the Id determinants recognized on mAb 12 were located at or near the antibody-combining sites. The Rb-aId also bound specifically to affinity-purified human anti-*LolpIV* antibodies isolated from human sera, but not to affinity-purified human anti-tetanus toxoid antibodies. This indicated that the human anti-*LolpIV* antibodies share a cross-reactive Id. The binding of Rb-aId to human anti-*LolpIV* antibody could also be inhibited by mAb 12. Therefore, it was concluded that the murine and human antibodies to *LolpIV* share a cross-reactive idiotope.

INTRODUCTION

It was first demonstrated by our Group (Bose *et al.*, 1984) that the serum of a Rye grass allergic-patient contained auto-anti-idiotypic (auto-aId) antibodies that cross-reacted with public idiotypic determinants (Id) expressed on human IgE and IgG antibodies to *LolpI*, a major allergen in Rye grass pollen. Further studies of *LolpI*-allergic patients, some of which received injections of grass pollen extract, have indicated that the levels of the auto-aId are influenced either by seasonal exposure to pollen or by immunotherapy, and in several cases an inverse relationship between serum levels of the auto-aId and those of IgE and IgG anti-*LolpI* antibodies was found (Bose, Marsh & Delespesse, 1986a).

Based on the interactions between idiotype (Id) and anti-idiotypic antibodies (aId) proposed originally by Neils Jerne (1974), studies in animal model systems strongly support the concept that Id-aId interactions play a significant role in

regulating humoral and cell-mediated immune responses (Blaser & de Weck, 1982). It is suggested that the IgE antibody responses could be modulated by Id-aId interactions as a consequence of immunotherapy.

Our studies on the isolation and characterization of grass pollen allergens have identified that *LolpIV* (this nomenclature was adopted according to the allergen nomenclature system recommended by WHO; Marsh *et al.*, 1986) (also known as HMBA) (Ekramoddoullah, Kisil & Sehon, 1983) is one of the major allergens in Rye grass pollen, i.e. sera of patients allergic to Rye grass pollen contain IgE and IgG antibodies to *LolpIV*. The availability of a murine monoclonal antibody (mAb 12) that recognized *LolpIV* provided an opportunity to examine the possibility that the murine and human anti-*LolpIV* antibodies share a common idiotypic specificity.

The findings confirmed our previous observations that mAb 12 is directed to an epitope which is recognized also by human IgE antibodies to *LolpIV* (Albuquerque *et al.*, 1985). Moreover, rabbit anti-idiotypic antibodies prepared against mAb 12 recognized idiotypic determinants present not only on this monoclonal antibody but also on human antibodies to *LolpIV* present in the sera of Rye grass allergic patients. These results indicated that an idiotope present on the murine monoclonal antibody and human antibodies to *LolpIV* possesses a cross-reactive specificity.

Abbreviations: aId, anti-idiotypic; Id, idiotype; Ig, immunoglobulin; N BALB/c, normal BALB/c mice; N Rb-IgG, IgG fraction of normal rabbit serum; PBS, phosphate-buffered saline; Rb-aId, IgG fraction of rabbit anti-idiotypic antiserum; TT, tetanus toxoid.

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MATERIALS AND METHODS

Preparation of a rabbit anti-idiotypic antiserum to mAb 12

New Zealand white rabbits (Blue Farm, Lockport, Namibia) each received a total of three s.c. injections of mAb 12 (200 µg) in Freund's complete adjuvant (Difco Laboratories, Detroit, MI) administered at intervals of 1 month. The globulin fraction of the rabbit antiserum was obtained by precipitating twice with 50% (final saturation) $(\text{NH}_4)_2\text{SO}_4$ and dialysed against phosphate-buffered saline, pH 7.6 (PBS). This antibody preparation was extensively absorbed with (i) normal BALB/c (N/BALB/c) IgG-Sepharose 4B and (ii) P3 × 20 IgG-Sepharose 4B, in order to remove antibodies to the isotypic and allotypic specificities of mAb 12, respectively. This absorbed antibody preparation was passed through a column of protein A-Sepharose 4B and the IgG fraction was eluted with 4 M guanidine-HCl, pH 3.5, immediately neutralized with 1 M Tris-HCl, pH 8.5, and dialysed against PBS. For simplicity, this rabbit IgG anti-idiotypic antibody preparation is referred to as Rb-ald.

Human serum samples

Serum was harvested from blood obtained locally from grass pollen allergic individuals (nos 1–5); and from normal individuals (nos 6–9) who possessed no IgE antibodies to *LolpIV* detectable by RIA. Serum from local individuals hyperimmunized with tetanus toxoid (TT)s (nos 10 and 11) was kindly provided by Dr Guy Delespesse, formerly of this Department.

IgG preparations

The IgG fraction was isolated from the sera of N BALB/c mice, the ascitic fluids of mice inoculated i.p. with the MOPC-21A murine myeloma cell line, or with hybridoma monoclonal cell lines producing, respectively, monoclonal antibodies mAb 1 and mAb 12, which recognized Rye grass allergen to *LolpIV*, by a combination of precipitation with ammonium sulphate, followed by gel filtration on ACA-44 Utrogel (Bose *et al.*, 1986b). Ascitic fluids obtained from mice inoculated i.p. with a hybridoma monoclonal cell line producing mAb 27 (produced against the retentate fraction of Kentucky Bluegrass pollen; Kisil *et al.*, 1980) and the P3 × 20 myeloma cell line were fractionated on a Mono Q column using the FPLC system (Pharmacia Fine Chemicals, Montreal, Canada) (Ekramodoullah *et al.*, 1987) to obtain the IgG fractions. Normal rabbit IgG (N Rb-IgG) was isolated from pooled rabbit serum by adsorption onto protein A-Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden) as described above.

Rye grass allergen LolpIV

The allergen was isolated from the retentate fraction of Rye grass pollen by affinity purification on mAb 12-Sepharose 4B and its purity was assessed by SDS-PAGE (Albuquerque *et al.*, 1985). *LolpIV* was radiolabelled by the chloramine-T method (Klinman & Taylor, 1969).

Preparation of immunosorbents

LolpIV, mAb 12, TT (2440 unit) (Connaught Laboratories, Willowdale, ON), N BALB/c IgG, and P3 × 20 IgG were conjugated to cyanogen bromide-activated Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden) as described elsewhere (Axen, Porath & Ernback, 1967).

Radioimmunoassay (RIA)

RIA to determine if mAb 12 and human IgE antibodies recognize the same allergenic determinant on LolpIV. The ability of the mAb 12 to inhibit the binding of ^{125}I -*LolpIV* to human anti-*LolpIV* IgE was evaluated as described earlier (Bose *et al.*, 1986b). Briefly, polyvinyl microtitre plates (Dynatech Laboratories Inc., Alexandria, VA) were coated with a solution (100 µl/well) of murine monoclonal anti-human IgE (ascitic fluid from mice inoculated i.p. with hybridoma clone no. 89, prepared by the Hybridoma Unit of our Group, diluted 1:1000 in 0.01 M carbonate-bicarbonate buffer; the IgG concentration of this diluted preparation was 1 µg/ml) at room temperature overnight; the wells were washed and Rye grass allergic serum, neat or diluted in PBS containing 2% bovine serum albumin (BSA), was added (final volume 100 µl/well) and kept at room temperature overnight. After washing the plates with PBS, a mixture of either mAb 12, or for controls N BALB/c IgG or MOPC-21A IgG (at final concentrations of 50 µg/ml), and a solution of ^{125}I -*LolpIV* in BSA (10%) in PBS and Tween-20 (0.05%), containing $\sim 10^5$ c.p.m./100 µl/well, were incubated at room temperature for 3–4 hr and then added to the microtitre wells. After overnight incubation at room temperature, the plates were washed with PBS and the level of radioactivity in each well was measured in a Beckman 8000 Gamma Counter.

RIA to determine if the Id determinant recognized by Rb-ald was located at or adjacent to the antigen-combining site of mAb 12. The ability of Rb-ald to bind to mAb 12 and block subsequent binding of ^{125}I -*LolpIV* to mAb 12 was evaluated. Microtitre plates coated with mAb 12 (50 µg/ml in 0.01 M carbonate-bicarbonate buffer, 100 µl/well) were incubated with different concentrations of Rb-ald or N Rb-IgG (diluted in PBS containing 2% BSA and 1% N BALB/c serum; final volume 100 µl/well) at room temperature overnight. The plates were then washed with PBS and a solution of ^{125}I -*LolpIV* in PBS containing BSA (10%) and Tween-20 (0.05%) was added ($\sim 10^5$ c.p.m./100 µl/well). After an overnight incubation at room temperature, the plates were washed with PBS and the radioactivity in each well was measured.

Enzyme linked immunoassays (ELISA)

ELISA for determining the level of binding of Rb-ald to murine monoclonal antibody to LolpIV. Briefly, 96-well EIA plates (c. no. 3590, Costar, Cambridge, MA) were coated with a solution of mAb 12 in the carbonate-bicarbonate buffer (50 µg/ml; final volume 100 µl/well), blocked with gelatin 2% in PBS (200 µl/well), at room temperature for 2 hr, then incubated with different concentrations of Rb-ald [diluted in PBS containing BSA 2% and N BALB/c serum (1%) 100 µl/well] at room temperature for 18 hr. After washing with PBS, an alkaline phosphatase-conjugated preparation of affinity-purified F(ab')₂ fragment of sheep antibodies to rabbit IgG (Sigma Chemical Co., St Louis, MO), diluted 1000-fold with PBS containing BSA (1%), was added (100 µl/well). After incubation at room temperature for 1 hr, the enzyme substrate, *p*-nitrophenyl phosphate (Sigma Chemical Co.) was added (1 mg/ml in a diethanolamine buffer, pH 9.6), the colour reaction allowed to develop for 30 min and the optical density measured at 410 nm using the microplate reader (Model MR600, Dynatech Lab. Inc., Torrance, CA). N BALB/c IgG, MOPC-21A IgG, P3 × 20 IgG, mAb 1 IgG and mAb 27 IgG-coated microtitre plates were used for controls in place of mAb 12.

Table 1. Demonstration of the presence of IgE and/or IgG antibodies to *LolpIV* in human sera

Serum no.	IgE anti- <i>LolpIV</i> *	IgG anti- <i>LolpIV</i> †
1	3200	0.50
2	2145	0.25
3	3058	0.27
4	1115	0.31
5	976	0.40
6	0	0.82
7	0	0.71
8	0	0.86
9	0	0.60

* The IgE antibodies to *LolpIV* were detected by RIA using equal volumes of the human sera diluted two-fold (see the Materials and Methods). The figures are the values (c.p.m.) for ^{125}I -*LolpIV* which bound to the IgE antibodies.

† The IgG antibodies to *LolpIV* were detected by ELISA using equal volumes of human sera diluted 10-fold (see the Materials and Methods). The values shown are the absorbancy at 410 nm.

ELISA to determine the level of binding of IgG antibodies in human sera to *LolpIV*. Briefly, 96-well EIA plates were coated with *LolpIV* (5 µg/ml; 100 µl/well). For control, wells were coated with gelatin (2% in PBS). After incubating the coated wells with human serum, diluted 10-fold in PBS containing BSA (2%), a preparation of affinity-purified F(ab')₂ fragment of goat anti-human IgG (γ-chain specific) conjugated to alkaline phosphatase (Sigma Chemical Co.), diluted 1000-fold with PBS containing BSA (1%), was added. The procedure was continued as described above.

ELISA to determine the level of binding of Rb-aId to human antibodies to *LolpIV*. The assay plates were coated with affinity-purified human antibodies to *LolpIV*, or for control, affinity-purified human antibodies to tetanus toxoid (TT) (these preparations were used at a protein concentration of 50 µg/ml in 0.01 M carbonate-bicarbonate buffer; 100 µl/well). The rest of the procedure was the same as described above.

Statistical analysis

Analysis of variance followed by Duncan's multiple comparison test were performed in order to identify if the binding of the Rb-aId to the human antibodies to *LolpIV* was significant in comparison with the binding which occurred between the Rb-aId and the human antibodies to the TT.

RESULTS

Detection of IgE and IgG antibodies to *LolpIV* in human sera

The human serum immunoglobulin preparations used in this study possessed either IgE and IgG antibodies to *LolpIV* (sera nos 1–5) or only IgG antibodies to *LolpIV* (i.e. sera nos 6–9 had no detectable IgE antibodies to *LolpIV*; Table 1).

Recognition of an allergenic epitope on *LolpIV* by mAb 12

The ability of mAb 12 to recognize an allergenic epitope on *LolpIV* was examined in terms of the capacity of mAb 12 to

Table 2. Demonstration * that mAb 12 recognizes an epitope on *LolpIV* which is recognized also by human IgE antibodies

Serum no.†	Anti- <i>LolpIV</i> IgE	% inhibition§		
	Maximum binding‡	N BALB/c IgG¶	MOPC 21A¶	mAb 12¶
1	2462	0	0	51.5 ± 11.0
2	1838	0	0	38.6 ± 6.5
3	2881	0	0	58.4 ± 2.8
4	1529	0	0	41.1 ± 8.7
5	789	0	5.8 ± 2.9	44.0 ± 2.9

* Demonstration in terms of the ability of mAb 12 to bind to *LolpIV* and inhibit its binding to human IgE antibodies to *LolpIV*.

† Sera from different grass-allergic individuals.

‡ C.p.m. of ^{125}I -*LolpIV* bound to human IgE anti-*LolpIV* in the presence of PBS containing 2% BSA.

§ Mean ± SD of quadruplicate determinations.

¶ Inhibitor used at a final concentration of 50 µg/ml.

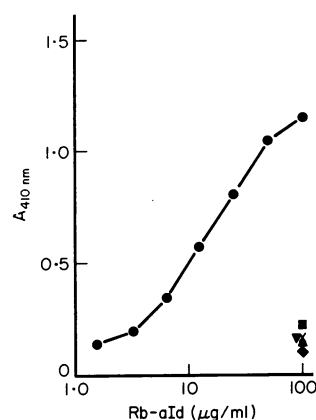


Figure 1. Demonstration by ELISA that Rb-aId binds specifically to mAb 12. Rb-aId, at different concentrations, was added to microtitre plates coated with: mAb 12 anti-*LolpIV* (●), and mAb 1 (anti-*LolpIV*) (X), normal BALB/c IgG (▲), MOPC-21A IgG (■), P3 × 20 IgG (♦), and mAb 27 (anti-KBG-R) (▼). The ELISA was performed as described in the Materials and Methods. Each point represents the mean of determinations made in quadruplicate of the absorbancy at 410 nm minus the control values obtained with normal rabbit IgG, which was 0.22 ± 0.03 .

inhibit the binding of human IgE anti-*LolpIV* to ^{125}I -*LolpIV*. The results, presented in Table 2, indicate that mAb 12 inhibited the binding of *LolpIV* to human IgE antibodies present in all five human sera. The extent of this inhibition ranged from 38% to 58%, whereas the same amount of N BALB/c IgG or MOPC-21A IgG had no inhibitory effect. These findings were interpreted to mean that mAb 12 recognizes an allergenic epitope on *LolpIV*.

Demonstration that Rb-aId specifically detects idiotopes on mAb 12

In ELISA, the Rb-aId bound to mAb 12 in a dose-dependent manner (Fig. 1). By contrast, Rb-aId did not bind to normal

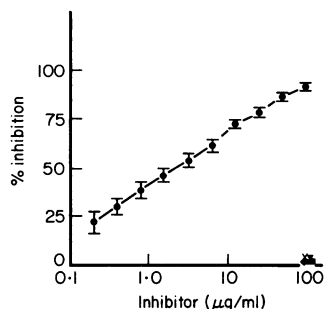


Figure 2. Demonstration by inhibition of ELISA that Rb-aId binds specifically to mAb 12. Microtitre plates coated with mAb 12 were incubated with a mixture of Rb-aId and mAb 12 were incubated with a mixture of Rb-aId and mAb 12 (●), normal BALB/c IgG (▲), MOPC-21A IgG (■), P3 × 20 IgG (♦), mAb 27 (▼) or mAb 1 (X). The ELISA inhibitions were performed as described in the Materials and Methods. Each point represents the mean ± SD of determinations made in quadruplicate.

Table 3. Demonstration * that Rb-aId recognizes a paratopic idiotope on mAb 12

Inhibitor†	Amount (µg)	% inhibition‡
N Rb-IgG	100	0.0
N Rb-IgG	50	0.0
Rb-aId IgG	100	47.4 ± 3.5
Rb-aId IgG	50	42.2 ± 3.8
Rb-aId IgG	25	13.5 ± 4.7
Rb-aId IgG	12.5	0.0

* Demonstrated in terms of the ability of Rb-aId to inhibit the binding of ^{125}I -LolpIV to mAb 12. Microtitre plates coated with mAb 12 (50 µg/ml; 100 µl/well) were incubated with different amounts of N Rb-IgG or Rb-aId overnight. After washing, ^{125}I -LolpIV (~10⁵ c.p.m./well) was added.

† In the absence of an inhibitor (i.e. N Rb-IgG or Rb-aId), 2577 ± 287 c.p.m. of ^{125}I -LolpIV bound to mAb 12.

‡ Mean ± SD determined from two to three experiments performed in duplicate.

BALB/c IgG, MOPC-21A IgG (IgG1, k), P3 × 20 IgG (IgG1, k), mAb 27 (IgG1, k) and mAb 1 (IgG1, k). Furthermore, normal rabbit IgG did not bind to mAb 12 (data not shown). The specificity of the binding of Rb-aId to mAb 12 was demonstrated by the inhibition of ELISA (Fig. 2). The degree of inhibition of the binding of Rb-aId to mAb 12 was dose dependant; the maximum inhibition of 91% was achieved in the presence of mAb 12 at a concentration of 100 µg/ml. On the other hand, at the same concentration, N BALB/c IgG MOPC-21A IgG, P3 × 20 IgG, mAb 1 or mAb 27, had no inhibitory effect.

It was also demonstrated by RIA that Rb-aId inhibited the binding of ^{125}I -LolpIV to mAb 12 by 47% when 100 µg/well of Rb-aId was used as the inhibitor (Table 3). At the same

Table 4. Demonstration* that Rb-aId recognizes an idiotope on human anti-LolpIV

Antibody on solid phase†	Absorbance at 410 nm‡
Anti-LolpIV Ig no. 1	1.69 ± 0.23 ($P < 0.01$)§
Anti-LolpIV Ig no. 2	1.32 ± 0.14 ($P < 0.01$)
Anti-LolpIV Ig no. 6	0.93 ± 0.19 ($P < 0.01$)
Anti-LolpIV Ig no. 7	1.03 ± 0.15 ($P < 0.01$)
Anti-LolpIV Ig no. 8	1.4 ± 0.14 ($P < 0.01$)
Anti-LolpIV Ig no. 9	1.19 ± 0.35 ($P < 0.01$)
Anti-TT Ig no. 10	0.46 ± 0.05
Anti-TT Ig no. 11	0.20 ± 0.04

* Demonstrated in terms of the ability of Rb-aId to bind to the affinity-purified human antibodies.

† The microtitre plates were coated with the affinity-purified antibodies (50 µg/ml; 100 µl/well) isolated from different human sera (see Table 1).

‡ The figures are the mean ± SD of the values from two experiments performed in duplicate. The absorbance using N Rb-IgG, in place of Rb-aId, was 0.25 ± 0.04.

§ Refers to the statistical significance of the comparison made between the value for the binding of Rb-aId to the LolpIV-purified antibodies on one hand to the TT affinity-purified antibodies (anti-TT) on the other.

concentration normal rabbit IgG had no inhibitory effect. These data suggested that some of the idiotypic determinant(s) recognized by Rb-aId is(are) located in or near the antigen combining site of mAb 12.

Demonstration that Rb-aId recognizes idiotypic determinant(s) on human anti-LolpIV antibodies

By ELISA (Table 4), the Rb-aId was demonstrated to bind to affinity-purified human anti-LolpIV antibodies isolated from grass allergic individuals (sera nos 1 and 2) or isolated from the sera of non-allergic individuals (sera nos 6–9). On the other hand, Rb-aId only bound weakly to affinity-purified human anti-TT antibodies (sera nos 10 and 11). Statistical analysis showed that the binding of Rb-aId to human anti-LolpIV was indeed significant ($P < 0.01$) when compared with the binding to human anti-tetanus antibodies. The capacity of Rb-aId to bind to human anti-LolpIV antibodies isolated from three different sera was inhibited not only by homologous human anti-LolpIV antibodies but also by antibodies isolated from the sera of different individuals, i.e. cross-inhibition (Table 5). However, affinity-purified anti-TT antibodies were not effective for these inhibitions. It is interesting to note that the degree of inhibition of the binding of Rb-aId to anti-LolpIV no. 6 obtained with anti-LolpIV no. 9 was greater than the inhibition obtained with anti-LolpIV no. 6 itself. Consistent with this observation, the anti-LolpIV no. 6 was unable to inhibit the binding of Rb-aId to the anti-LolpIV no. 9.

Table 5. Demonstration * that human anti-*LolpIV* antibodies possess cross-reactive idiotope(s)

Plates coated with affinity-purified human <i>LolpIV</i> Ig, no.	Affinity-purified human antibodies used for the inhibition†							
	Anti- <i>LolpIV</i> Ig no.						Anti-TT Ig no.	
	1	2	6	7	8	9	10	11
1 (1.05)†	49 ± 1	30 ± 10	21 ± 6	33 ± 3	38 ± 4	28 ± 3	5 ± 2	6 ± 5
6 (0.49)	48 ± 13	53 ± 7	41 ± 5	43 ± 1	60 ± 2	57 ± 2	12 ± 7	0
9 (0.60)	40 ± 1	45 ± 9	6 ± 3	29 ± 4	43 ± 7	29 ± 6	0	2 ± 2

* Demonstration in terms of the ability of a given preparation of affinity-purified human anti-*LolpIV* antibodies to inhibit the binding of Rb-aId to (i) the same preparation of human anti-*LolpIV* Ig (i.e. homologous inhibition) and (ii) preparations of human anti-*LolpIV* Ig from different individuals (i.e. cross-inhibition).

† The values indicated in parenthesis are the absorbances at 410 nm for the binding of Rb-aId to human anti-*LolpIV*-coated plates, in the absence of inhibitor.

‡ Rb-aId (100 µg/ml) was mixed with various inhibitors (affinity-purified human antibodies; 50 µg/ml) at room temperature for 4 hr. The mixtures were added to the wells coated with human anti-*LolpIV* antibodies. Bound Rb-aId was detected by adding goat anti-rabbit IgG-alkaline phosphatase conjugate. The values given in the table are the mean ± SD of percentage inhibition and were calculated according to the formula: % inhibition = (A - B)/A × 100; A = absorbancy at 410 nm in the absence of inhibitor and B = absorbancy in the presence of inhibitor.

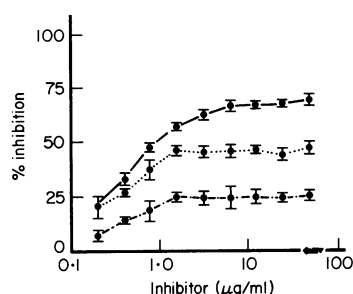


Figure 3. Inhibition of ELISA. Demonstration that murine monoclonal antibody, mAb 12, inhibits specifically the binding of Rb-aId to human antibodies to *LolpIV*. The microtitre plates were coated with affinity-purified human antibodies to *LolpIV* obtained from serum no. 1 (—), 6 (---) 7 (----). Fifty micrograms per millilitre of Rb-aId were mixed with varying concentrations of an inhibitor, i.e. mAb 12 (●), normal BALB/c IgG (▲), MOPC-21A (■), mAb 27 (♦) or mAb 1 (▼), for 4–5 hr at room temperature. A volume of 100 µl of the mixture was added to the coated plates. The mean values, of determinations made in quadruplicate, of the absorbancy at 410 nm, in the absence of any inhibitor were 0.89, 0.85 and 1.18, respectively.

Demonstration that human and murine anti-*LolpIV* share a common Id determinant(s)

The possibility that human antibodies and a murine monoclonal antibody to *LolpIV* possessed idiotypic determinants which could be recognized by Rb-aId was evaluated by ELISA in terms of the ability of mAb 12 to inhibit the binding of Rb-aId to human anti-*LolpIV* antibodies. Thus, the binding of Rb-aId to three different affinity-purified human anti-*LolpIV* antibody preparations was inhibited strongly by mAb 12 but not by murine antibodies mAb 1, and mAb 27, nor by normal BALB/c IgG or MOPC-21A IgG (Fig. 3). The maximum degree of inhibition differed for each of the three human anti-*LolpIV*

antibodies tested and ranged from 25% to 67%. This finding suggested that the concentrations of the specific idiotypic determinants recognized by the Rb-aId varied from one individual to another.

DISCUSSION

In order to investigate the possibility that a murine monoclonal antibody and human antibodies to *LolpIV* share common idiotypic determinants, it was first established that the murine monoclonal antibody, mAb 12, recognized an allergenic epitope on *LolpIV*. The ability of mAb 12 to inhibit the binding of ¹²⁵I-*LolpIV* to human IgE anti-*LolpIV* revealed that mAb 12 recognizes an epitope on *LolpIV* that is identical or close to the one recognized by at least some of the human IgE antibodies to the *LolpIV*. The rabbit antiserum produced against mAb 12 was rendered idiotype specific by extensive adsorptions with normal BALB/c immunoglobulins and with P3 × 20 myeloma IgG. The Rb-aId bound specifically to mAb 12, but not to another anti-*LolpIV* monoclonal antibody (mAb 1) or to normal BALB/c IgG or to the P3 × 20 myeloma IgG. Moreover, the specificity of Rb-aId was confirmed by observations that the binding of Rb-aId to mAb 12 could be inhibited exclusively by mAb 12 and not by other murine monoclonal antibodies or IgG preparations.

The ability of Rb-aId to bind polyclonal human anti-*LolpIV* antibodies from different grass allergic individuals indicated that human anti-*LolpIV* antibodies possess cross-reactive idiotopes. This reactivity appeared to be specific, since the binding could be inhibited only by anti-*LolpIV* antibodies and not by human antibodies to tetanus toxoid. Moreover, it was concluded that only some of the antibodies present in Rb-aId recognize the paratopic idiotope (i.e. an idiotypic determinant at or near the antibody combining site) of mAb 12. This conclusion was made on the basis that Rb-aId, at a dose of 50 µg, inhibited

the binding of mAb 12 to ^{125}I -*LolpIV* to the extent of 42% and that doubling the dose to 100 μg only minimally increased the inhibition to 47% (Table 3). On the other hand, Rb-aId was unable to inhibit the binding of human IgE and IgG antibodies to *LolpIV* (data not shown). However, this observation, which was not unexpected, does not rule out the possibility that the Rb-aId contained antibodies specific for a paratopic idiotope on some of the human anti-*LolpIV* antibodies. Since we are dealing with polyclonal antibody responses, that fraction of the human IgE and IgG antibodies to *LolpIV* possessing the paratopic idiotope recognized by Rb-aId, may simply have been present in amounts far too low to be detected. Another explanation could be that the Rb-aId recognized the idiotope located outside the antibody-combining site of human IgE and IgG antibodies to *LolpIV*.

The ability of mAb 12 to inhibit the binding of Rb-aId to human antibodies to *LolpIV* (Fig. 3) revealed that the murine monoclonal antibody and the human antibodies to *LolpIV* share a cross-reactive idiotypic determinant(s). The variation in the maximum degree of inhibition of the binding of Rb-aId to the antibodies to *LolpIV* isolated from different individuals suggested that the levels of the antibodies which possess the cross-reactive idiotypic determinant varies from one individual to another. The results of the cross-inhibition experiments (Table 5) indicated (i) that the affinity-purified antibodies to *LolpIV* of any one allergic individual differed in their ability to inhibit the binding of Rb-aId to antibodies to *LolpIV* isolated from other individuals; (ii) the data add further support to the concept that the level of the idiotypic determinants can vary from one individual to another; and (iii) the idiotype present in individual sera may also have different affinity for the Rb-aId, which may explain the higher inhibition obtained with the heterologous antibody (no. 9) when the antibody no. 6 was used on the solid phase.

Previous studies from this Group have demonstrated the existence of public idiotypic determinants on human IgE and IgG antibodies to *LolpI*, a major allergen of Rye grass pollen (Bose *et al.*, 1984, 1986a). In the present study, it was found that Rb-aId, produced and rendered specific to a murine monoclonal antibody to *LolpIV*, also recognized an idiotypic determinant(s) on human antibodies to *LolpIV*. The results also demonstrated an *interspecies* cross-reactivity between idiotypic determinants. Indeed, *interspecies* idiotypic cross-reactions have been demonstrated in other antigen-antibody systems as well. For example, a rabbit anti-idiotypic antiserum generated against human antibodies to hepatitis B surface antigen could detect a common idiotype in sera from humans who had been infected previously with hepatitis B virus. This common idiotype was also identified in sera from rabbits, mice, guinea-pigs, swine, goats and chimpanzees that had been immunized with hepatitis B surface antigen (Kennedy *et al.*, 1983). In another study (Schwartz *et al.*, 1978) an anti-idiotypic antiserum (anti-anti-acetylcholine receptor), prepared in C57BL/65 mice, bound specifically to anti-acetylcholine receptor antibodies produced in different strains of mice, as well as in other species such as the rat, rabbit and monkey. In the present study, in addition to the demonstration of an *interspecies* cross-reactivity between idiotypic determinants, an *intraspecies* cross-reactivity was observed between an idiotype present on human antibodies to *LolpIV*.

Another interesting observation made in this study was the detection of *LolpIV*-specific IgG antibodies in human sera in the

absence of detectable IgE antibodies to *LolpIV*. This finding suggests that the formation of specific IgE anti-*LolpIV* antibodies is suppressed in the non-allergic individuals. The role, if indeed any, of anti-idiotypic antibodies in this suppression is not known at the present time. A murine reagenic model system for investigating the effects of anti-idiotypic antibodies on antibody responses to a well-defined allergen such as *LolpIV* is currently being developed.

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