

Suppression of immune response to *Lol p I* by administration of idiotype

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Background: Allergic diseases are characterized by an increased production of specific IgE antibodies. Suppression of IgE antibody production may be accomplished through idiotypic manipulation.

Objective: Using an animal model, we explored the effects of anti-*Lol p I* monoclonal antibody administration on the subsequent IgE and IgG antibody response against *Lol p I*.

Methods: Mice were treated with an anti-*Lol p I* monoclonal antibody (290A-167), which resulted in the production of anti-idiotypic antibodies as evidenced by their ability to bind to the Fab fraction of 290A-167 and to inhibit the binding of rabbit polyclonal anti-idiotypic antibodies to 290A-167. The animals were then immunized with *Lol p I* adsorbed onto alum, and the immune response to the protein was analyzed.

Results: Antigen-specific IgG₁ and IgE responses were strongly suppressed as determined by immunoassay. Suppression of anti-*Lol p I* IgE antibodies was confirmed by a reduction of end-point titers measured by passive cutaneous anaphylaxis. The suppression of antigen-specific antibody was accompanied by a reduction of anti-*Lol p I* antibody-producing spleen cells.

Conclusion: These data indicate that pretreatment with 290A-167 can strongly downregulate the IgE response to the main allergen of ryegrass pollen, which is associated with an increase in anti-idiotypic antibodies. This approach could provide rapid, long-term hyposensitization in patients with grass pollen allergy. (*J ALLERGY CLIN IMMUNOL* 1995;95:751-8.)

Key words: Anti-idiotypic, downregulation, IgE, *Lol p I*, pretreatment

Ryegrass-induced allergic rhinitis is mediated by IgE antibodies provoked by exposure to allergenic proteins. The ryegrass allergen *Lol p I* is a major IgE binding protein, and responses to this allergen are of paramount importance.¹⁻⁴

Regulation of IgE synthesis to allergens, including *Lol p I*, by anti-idiotypic (aId) antibodies is suggested by studies comparing the levels of auto-aId antibodies in sensitive patients and nonatopic volunteers.⁵⁻⁸ Auto-aId antibody levels were reduced in untreated atopic patients compared with levels in normal subjects.^{7,8} Furthermore, in atopic pa-

Abbreviations used

aId:	Anti-idiotypic
BSA:	Bovine serum albumin
ELISPOT:	Enzyme-linked immunosorbent plaque assay
mAb:	Monoclonal antibody
PBS:	Phosphate buffered saline
PCA:	Passive cutaneous anaphylaxis

tients receiving specific immunotherapy, the levels of aId antibodies increased to the levels of normal volunteers.⁷ In addition, passive administration of intravenous immunoglobulins containing aId antibodies was shown to be effective in treating autoimmune diseases⁹⁻¹³ and, more recently, in treating a patient highly sensitive to bee venom.¹⁴ Furthermore, treatment of human subjects with immune complexes formed by reaction of autologous serum antibodies with dust mite allergen extracts produced significant clinical improvement, which was associated with the increased levels of auto-aId antibodies.^{15, 16}

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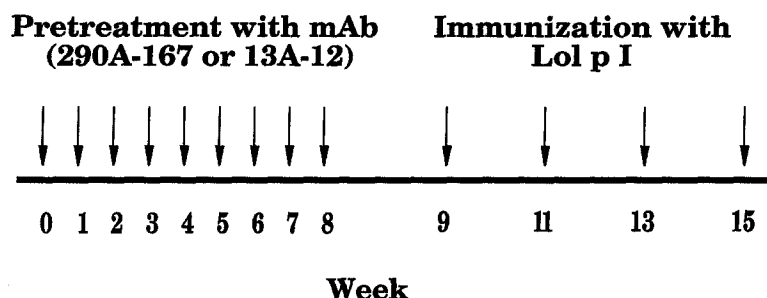


FIG. 1. Immunization schedule. Mice were first pretreated with anti-*Lol p I* mAb (290A-167) or anti-ferritin mAb (13A-12) and then received *Lol p I* adsorbed onto alum.

These findings, together with experimental studies showing that aId antibodies suppress IgE antibody response,^{17, 18} support the view that an idiotypic network can regulate immune response to allergens such as *Lol p I*.

In a previous study, we reported an idiotypic cross-reactivity between human IgE and IgG anti-*Lol p I* antibodies and murine anti-*Lol p I* monoclonal antibodies (mAbs).^{19, 20} We also showed that the idotype borne by one of these anti-*Lol p I* mAbs (290A-167) was expressed in 80% of grass-sensitive patients.²¹ These findings suggest that the idotype recognized by 290A-167 is an important public idotype and may represent a preferential target for selective immunoregulation.

With the use of an animal model, this study was undertaken in an attempt to analyze the effects of anti-*Lol p I* mAb administration on the subsequent antibody response against *Lol p I*.

METHODS

Animals

Female 8- to 10-week-old BALB/c mice and female Sprague-Darley rats used in this study were purchased from Charles River (St. Constant, Quebec, Canada).

Antibodies and antigens

The anti-*Lol p I* mAb 290A-167 ($\gamma 2b, \kappa$) was produced in our laboratory as previously reported.²² Anti-ferritin mAb (13A-12) and anti-gonococcus mAb (10H11) with the same isotype as 290A-167, produced in our laboratory, were used as controls. All these mAbs were purified from ascitic fluids or cell culture supernatants by affinity chromatography on protein A. Rabbit polyclonal aId antiserum to 290A-167 was prepared and highly purified as previously described.²¹ All mAbs originated from BALB/c mice, and the same mouse strain was used throughout this study to avoid the possibility of antiallotype response.

Lol p I was purified from commercial extracts (Omega Laboratories, Montréal, Quebec, Canada) by an immu-

nosorbent technique with anti-*Lol p I* mAb (290A-167) fixed on solid phase, as previously described.³

Immunization schedule

Groups of five mice were injected intraperitoneally (at weekly intervals with anti-*Lol p I* mAb (290A-167; 0.01, 0.1, 1, or 10 μ g) or unrelated mAb (13A-12). The first two injections were given in Freund's complete and incomplete adjuvant, respectively; and for subsequent injections, mAbs were injected with saline solution. After nine mAb injections, the mice were immunized at 2-week intervals by intraperitoneal injections of 2 μ g of *Lol p I* adsorbed onto 2 mg of alum. Mice were regularly bled for specific antibody determination. The entire immunization protocol is summarized in Fig. 1.

Measurement of aId antibodies to 290A-167

Direct binding on Fab fraction of 290A-167. The Fab fraction of mAb 290A-167 was adsorbed to microtiter plates at 100 ng/well. After saturation with bovine serum albumin (BSA), different serum dilutions were added and incubated for 2 hours at room temperature. The plates were then washed, and an anti-mouse Fc-specific IgG labeled with alkaline phosphatase (Zymed, San Francisco, Calif.) was added to each well. Incubation was carried out for 2 hours at room temperature, and p-nitrophenyl phosphate (Boehringer Mannheim GmbH, Mannheim, Germany) in diethanolamine buffer was then added. The absorbance at 410 nm was read on a multiscanner ELISA plate reader (Dynateck, Chantilly, Va.). Anti-idiotypic antibodies to anti-ferritin mAb (13A-12) or anti-gonococcus mAb (10H11) were also measured with Fab fractions of these mAbs, as described above.

Binding-inhibition of the 290A-167 to purified rabbit polyclonal aId antibodies. Microtiter plates were coated with rabbit polyclonal aId antibodies (100 ng/well) and saturated with Tris-buffered saline solution containing 3% BSA. After the washes, iodine 125-labeled 290A-167, at a concentration corresponding to 50% of maximum binding, was incubated with various dilutions of test sera for 2 hours at room temperature. The mixture was then incubated for 2 hours at 37°C on polyclonal aId antibody-coated plates, the radioactivity was

TABLE I. Production of ald antibodies by injection of anti-*Lol p I* mAb (290A-167) or control mAb

Pretreatment*	Direct binding to†		
	290A-167	13A-12	10H11
290A-167	1:12800	≤ 1:50	≤ 1:50
13A-12	≤ 1:50	1:6400	≤ 1:50

*BALB/c mice (five per group) received nine weekly injections of mAb.

†Anti-idiotypic antibodies were measured by direct binding of Fab fraction of mAb.

counted, and the percentage of inhibition was determined.

Detection of anti-*Lol p I* antibody levels

Test sera were first adsorbed on 290A-167 cross-linked to Sepharose gel (Pharmacia, Piscataway, N.J.) to deplete them of aId antibody activity. For specific IgG₁ determinations, each well was coated with 50 ng of *Lol p I*; 200 ng/well were needed for specific IgE determinations. After saturation with BSA, various dilutions of test sera were added to the wells. After a 2-hour incubation at 37° C, biotinylated goat anti-mouse IgE (Binding Site, Birmingham, U.K.) or IgG₁ (Jackson ImmunoResearch, West Grove, Pa.) was added. After a 90-minute incubation at 37° C, streptavidin-conjugated alkaline phosphatase (BRL, Gaithersburg, Md.) was added and incubated for 30 minutes at 37° C. After the washes, the plates were incubated with p-nitrophenyl phosphate. The absorbance at 410 nm was read on a multiscanner ELISA plate reader.

Passive cutaneous anaphylaxis (PCA)

Murine IgE antibodies to *Lol p I* were also measured by the PCA technique. Rats were sensitized by intradermal injections in several sites with various serum dilutions. After 24 hours, they were challenged with 1 ml of a solution containing 1 mg of *Lol p I* and 1% Evans blue dye. The titers were expressed as the reciprocal of serum dilutions yielding reactions of 5 mm in diameter.

Measurement of anti-*Lol p I*-secreting spleen cells in mice pretreated with 290A-167 or control mAb and immunized with *Lol p I*

After nine injections of 290A-167 or control mAb (10 µg) and four immunizations with *Lol p I* adsorbed to alum, the mice were killed. Single cell suspensions of spleen cells were prepared and finally resuspended in RPMI-1640 (Gibco, Grand Island, N.Y.) with 10% fetal calf serum. The enumeration of cells secreting anti-*Lol p I* antibodies was done by enzyme-linked immunosorbent plaque assay (ELISPOT).²³ Twenty-four-well, tissue culture plates (Nunc, Roskilde, Denmark) were coated with purified *Lol p I* in carbonate buffer, pH 9.6,

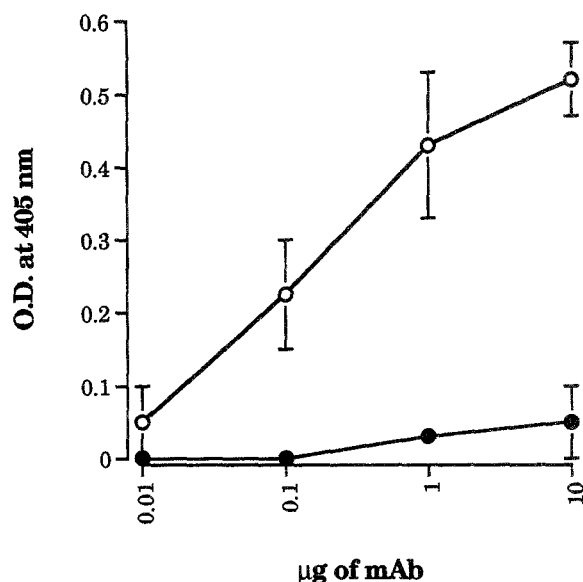


FIG. 2. Dose response to immunization of various concentrations of idiotype (290A-167). Levels of ald antibodies to 290A-167 mAb in mice treated with various doses of idiotype mAb (○) or control mAb (●) were measured by direct binding. Groups of five mice, each received nine intraperitoneal injections at weekly intervals. After the last immunization, serum samples were obtained by retro-orbital bleeding and were tested individually (dilution, 1:100) for ald antibody response. Experimental data represent geometric means with standard error.

at a concentration of 10 µg/well, and the plates were incubated overnight at 4° C. The wells were then washed in phosphate-buffered saline (PBS) with Tween-20 and blocked by adding 1 ml of 3% BSA in PBS. Various concentrations of mouse spleen cells, ranging from 20 to 150 cells/well in a volume of 1 ml/well, were added, and the plates were incubated overnight at 37° C under 5% CO₂. After this incubation, the wells were flicked empty and washed with cold PBS-Tween-20. Then 0.4 ml/well of biotinylated anti-Ig (Zymed, San Francisco, Calif.) antibody diluted in PBS-Tween 0.1% with 1% BSA was added to the wells. After incubation for 2 hours at room temperature, 0.4 ml of alkaline phosphatase conjugated to streptavidin (BRL) was added to each well. Plates were incubated for 30 minutes at room temperature and washed, and warm 5-bromo-4-chloro-3-indolyl phosphate substrate solution was added. After incubation for 15 to 30 minutes, the blue spots were counted and expressed as spots per 10⁶ spleen cells.

RESULTS

Production of ald antibodies to 290A-167

The levels of aId antibodies were measured by direct binding of test sera to Fab fractions of 290A-167. The animals were first injected with 290A-167 or control mAb mixed with complete Freund's adjuvant. One week later, mice received

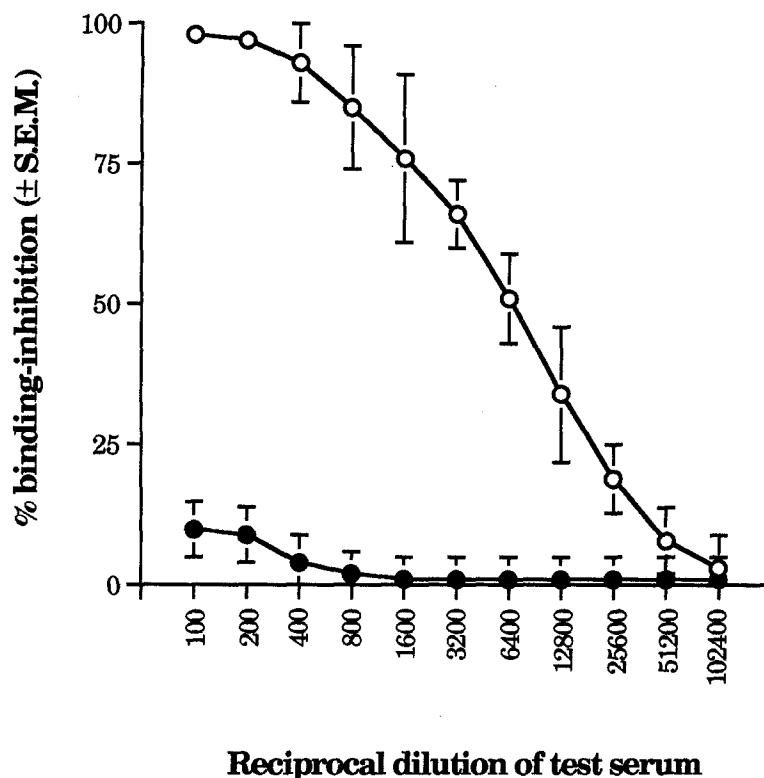


FIG. 3. Binding inhibition of idiotype (290A-167) to rabbit polyclonal ald antibodies by test sera. Groups of five mice, each received nine intraperitoneal injections of 10 μ g of idiotype mAb (○) or control mAb (●) at weekly intervals. Test sera were serially diluted. Experimental points represent geometric means with standard error.

TABLE II. Depletion of ald activity in sera from mice pretreated with mAb

Pretreatment*	Titer of ald antibodies to 290A-167†	
	Before ald depletion	After ald depletion
290A-167	1:12800	1:100
13A-12	$\leq$ 1:50	$\leq$ 1:50

*BALB/c mice (five per group) received nine weekly injections of mAb.

†Anti-idiotypic antibodies were measured by direct binding of Fab fraction of 290A-167 mAb.

mAb with incomplete Freund's adjuvant. Thereafter, the animals received seven other injections with mAb in saline solution at weekly intervals. The serum samples were drawn 1 week after the last injection of mAb. Table I shows the titers of aId antibodies in mice injected with 10 μ g of 290A-167 or control mAb. Only mice immunized with anti-*Lol p* I had aId antibodies to 290A-167. Mice that received 13A-12 had aId antibodies to

this idiotype, but aId antibodies to 290A-167 were not detected in the sera of these mice. The absence of binding of sera from 290A-167- or 13A-12-immunized mice on Fab fractions of an unrelated mAb (anti-*Neisseria gonorrhoeae*) with the same isotype as 290A-167 supported the specificity of the assay.

Production of aId antibodies was dose-dependent, as shown in Fig. 2. Mice were immunized with different concentrations of mAb (0.01 to 10 μ g per mouse) as described earlier. During week 8, doses of 0.1, 1, and 10 μ g of anti-*Lol p* I induced significant levels of aId antibodies to 290A-167.

The aId activity was also assessed by binding-inhibition studies in which sera from mice immunized with 290A-167 or control mAb were also analyzed as to their capacity to inhibit the binding of rabbit polyclonal anti-290A-167 to the idiotype (Fig. 3). Sera from mice immunized with anti-*Lol p* I mAb (290A-167) were able to inhibit the binding of polyclonal aId antibodies to 290A-167 up to 98%. The dilution of serum that led to 50% of binding inhibition was 1:6400. The percentage of inhibition with sera from control mice reached no

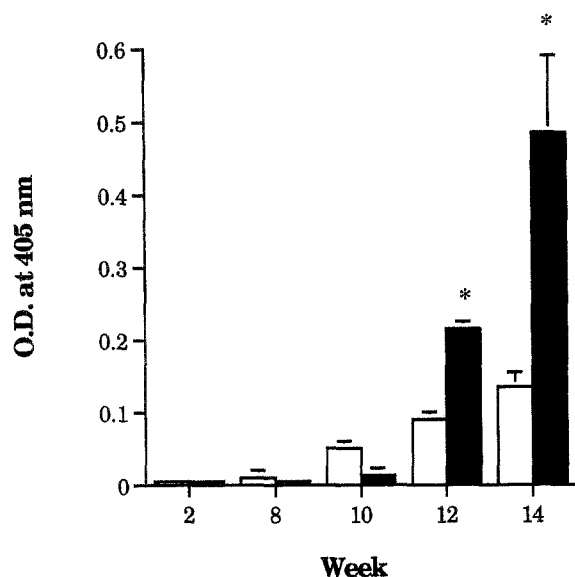


FIG. 4. Levels of specific IgE anti-*Lol p I* at weeks 2, 8, 10, 12, and 14 in mice pretreated with 10 µg of idiotype mAb (□) or control mAb (■). Mice (five mice/group) received 2 µg of *Lol p I* adsorbed onto alum at weeks 9, 11, and 13. Experimental points represent geometric means with standard error. * $p \leq 0.05$.

TABLE III. PCA titer on pretreatment of mice with anti-*Lol p I* mAb (290A-167) or control mAb

Week No.	PCA titer*	
	Idiotype-pretreated	Control mAb-pretreated
8	≤ 40	≤ 40
10	≤ 40	≤ 40
12	80	320
14	320	5120

*Sera were depleted of aId activity.

more than 10% of inhibition with a dilution of 1:100. These results indicate that significant levels of aId antibodies were produced after nine injections with 290A-167. These aId antibodies bound to Fab fractions of idiotype and also inhibited the binding of idiotype to rabbit polyclonal aId antibodies, supporting the specificity of aId antibodies for the idiotype borne by 290A-167 mAb.

Anti-*Lol p I* response after pretreatment with 290A-167

To determine the effects of idiotype immunization on subsequent anti-*Lol p I* antibody response, mice that received idiotype or control mAb were then immunized 1 week after the final dose of mAb 290A-167 or control mAb with *Lol p I* (2 µg)

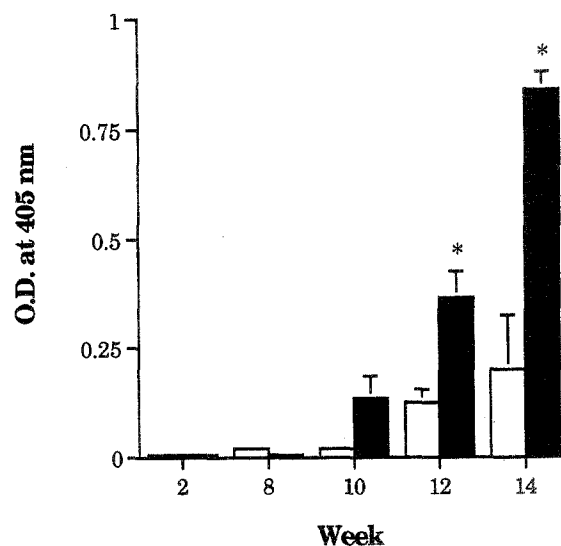


FIG. 5. Levels of specific IgG anti-*Lol p I* at weeks 2, 8, 10, 12, and 14 in mice pretreated with 10 µg of idiotype mAb (□) or control mAb (■). Mice (five mice/group) received 2 µg of *Lol p I* adsorbed onto alum at weeks 9, 11, and 13. Experimental points represent geometric means with standard error. * $p \leq 0.05$.

TABLE IV. Number of anti-*Lol p I*-secreting cells after pretreatment with anti-*Lol p I* mAb (290A-167) or control mAb

Spot-forming cells/10 ⁶ spleen cells	
Idiotype-pretreated	Control mAb-pretreated
3.75 ± 1.5	23 ± 2.6

adsorbed onto alum. *Lol p I* immunization was given four times at 2-week intervals. To avoid binding inhibition of specific antibodies to *Lol p I* by actively produced aId antibodies, test sera were first absorbed with 290A-167. After depletion, the presence of aId antibodies was measured by direct ELISA on Fab fractions of 290A-167, as described earlier. No detectable levels of aId antibodies were noted, as shown in Table II, suggesting that this treatment removed any residual aId antibody activity from the serum.

Anti-*Lol p I*-specific IgE and IgG₁ antibodies were then determined. As illustrated in Fig. 4, the anti-*Lol p I* IgE response was measured by ELISA. This response was greatly reduced in mice pretreated with anti-*Lol p I* mAb (290A-167) compared with mice immunized with control mAb. *Lol p I*-induced IgE response was further assessed with the PCA technique. As shown in Table III, the PCA titers of sera from mice pretreated with idiotype mAb during week 14 were 16 times lower

than those with sera from mice initially treated with control mAb. As measured by ELISA, anti-*Lol p I* IgG₁ antibody responses were also markedly reduced in anti-*Lol p I*-pretreated mice subsequently immunized with *Lol p I* (Fig. 5). These data indicated that immunization with 290A-167 resulted in a significant suppression of both IgE and IgG₁ antibody response to *Lol p I*.

Effect of 290A-167 pretreatment on specific antibody-secreting cells

It was of interest to establish whether this suppression of anti-*Lol p I* antibody response in 290A-167-pretreated mice was the result of a diminished capacity of cells to secrete anti-*Lol p I* antibodies or of a reduction in the number of cells that produced specific antibodies. To address this issue, spleen cells from 290A-167- or control mAb-pretreated mice were removed 1 week after the fourth immunization with *Lol p I*. The number of anti-*Lol p I*-secreting cells was then evaluated with ELISPOT. The results (Table IV) indicated that the number of spot-forming cells in mice pretreated with 290A-167 was significantly lower than that in control mAb-treated mice. These data clearly indicated that the suppression of anti-*Lol p I* was associated with a reduction of the number of cells secreting anti-*Lol p I*-specific antibodies.

DISCUSSION

Anti-idiotypic antibodies could exert an efficient suppressive effect on the synthesis of IgE antibodies.^{17, 18, 24, 25} In these studies, simple chemical components acting like haptenic determinants were used rather than complex proteins such as allergens. Although few studies with such complex antigens have been conducted, evidence of idio-type regulation in these responses has been gathered.^{26, 27} One of the major problems with idiotypic manipulation of the immune network is selecting the proper idio-type with the capacity to modify the immune response.

The possibility of inducing specific unresponsiveness through autologous idiotypic antibody immunization or passive administration of aId antibody was also demonstrated in various systems, especially in spontaneous or experimentally induced autoimmune diseases.²⁸⁻³⁴ Evidence has been provided that idio-type regulation involves the interaction of idio-type and aId antibodies with T cells. Idio-type-specific T-cell lines have been prepared in various models,³⁵⁻³⁸ and different effects on B-cell responses have been demonstrated.^{26, 34, 39} Some of these idio-type-specific T cells

were T-suppressor cells that could downregulate the antibody response. One might speculate that T-suppressor cells can directly bind to B cells bearing regulatory idio-type and influence the production of specific antibodies.

In this study, administration of idiotypic antibody (290A167) suppressed subsequent anti-*Lol p I*-specific response. However, further investigation is needed to determine the exact role of aId antibodies in the suppression of anti-*Lol p I* response. It will also be interesting to identify and characterize idio-type-binding cells in the suppression of specific antibody response after passive transfer of 290A-167.

However, our data indicate that the administration of a major idio-type has a potential application in therapy because this treatment has the capacity to reduce considerably the immune response to *Lol p I*. In human beings, immunotherapy with autologous immune complexes reduced skin and bronchial reactivity to house dust mites.¹⁶ The mechanisms of action for this treatment remain to be clarified, but a role for aId antibodies cannot be excluded, because it is known that immune complexes potentiate aId responses, which are usually suppressive.^{40, 41} On the other hand, we and others have demonstrated that administration of intravenous immunoglobulins containing both idio-type and aId antibodies can reduce symptoms associated with allergic diseases.^{14, 42, 43} Therefore use of an idio-type alone or in complex form may offer potential therapeutic advantages. This approach is effective and bears no risk of systemic reactions if idiotypes of human origin or humanized antibodies are used.

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