

Regulation of levels of serum antibodies to ryegrass pollen allergen *Lol pIV* by an internal image anti-idiotypic monoclonal antibody

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SUMMARY

A murine monoclonal anti-idiotypic antibody (anti-Id), designated B1/1, was produced against an idiotope of a murine antibody (mAb91), which recognizes the epitope, site A, of allergen *Lol pIV*, one of the major groups of allergens in ryegrass (*Lolium perenne*) pollen. The ability of B1/1 to modulate the antibody responses to *Lol pIV* was investigated in murine model systems. In the first system, B1/1–keyhole limpet haemocyanin (KLH) conjugate was administered to treat three different strains of mice (C57BL/6, BALB/c and C3H). In the second and third model systems, a solution of B1/1 in phosphate-buffered saline (PBS) was used to treat syngeneic BALB/c mice at various doses and time intervals, respectively. The treatment with either form of B1/1, administered at doses ranging from 100 ng to 100 µg mouse, resulted in a reduction of the levels of the antibodies to *Lol pIV*. In particular, the level of IgE antibodies to *Lol pIV* was greatly reduced. The administration of a single intravenous (i.v.) injection of a solution of B1/1 8 weeks prior to the challenge with *Lol pIV* was still effective in reducing the level of antibodies to the allergen. Moreover, the level of antibodies to *Lol pIV* that expressed the idiotope mAb91 was also markedly decreased. By contrast, it was observed that the level of antibodies to *Lol pIV* in mice pretreated with B1/1 in PBS at a dose of 10 ng/mouse increased (albeit slightly) compared to that in mice treated with control mAb. These experimental models lend themselves for investigating the mechanism(s) by which an anti-Id modulates antibody responses to a grass pollen allergen.

INTRODUCTION

Idiotope (Id), which can be recognized as a unique epitope, is attributed to particular amino acid sequences expressed in the variable regions of an antibody molecule. Upon immunization with an antibody that contains a variety of idiotopes, the corresponding anti-idiotypic antibodies (anti-Id or Ab2) can be generated. This concept was first defined by Kunkel and Oudin in 1963.^{1,2} It paved the way for the Id network theory of immune regulation proposed by Jerne in 1974.³ The theory implies that an antibody response to an antigen is controlled by a series of Id–anti-Id interactions that may either up-regulate or down-regulate the immune response to the antigen. This complex set of interactions operates via feedback mechanisms to control the formation of antibodies to a given antigenic stimulation.

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Abbreviations: Ab2, anti-Id; Ab2β, internal image Ab2; ABTS, 2,2'-azino-bis (3-ethyl-benzthiazoline sulphonic acid; anti-Id, anti-idiotypic antibody; BBS, borate-buffered saline; HRP, horseradish peroxidase; Id91, Id of mAb91; KLH, keyhole limpet haemocyanin; *Lol pIV*, ryegrass (*Lolium perenne*) pollen allergen IV; PCA, passive cutaneous anaphylaxis; T-BBS, 0.2% Tween 20 in BBS.

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While the physiological significance of idiotypic interactions has not been firmly established, modulation of the immune responses via Id–anti-Id interactions has nevertheless been demonstrated. The observations^{4,5} that levels of anti-Id increased in allergic patients who had received hyposensitization treatment with allergenic extracts, stimulated the interest to investigate the use of anti-Id for modulating the levels of serum antibodies to allergens.

A monoclonal anti-Id, designated B1/1, was produced against an Id of mAb91 (designated Id91), which recognized a major epitope, referred to as site A of allergen *Lol pIV*, one of the major groups of allergens in ryegrass (*Lolium perenne*) pollen.⁶ B1/1 was characterized as an internal image of anti-Id that (1) inhibited the binding between the homologous antibody and the allergen; (2) was inhibited from binding to the Id91 in the presence of the allergen; and (3) recognized the Id91 that was common to human, mouse and rabbit antibodies to *Lol pIV*.⁷ Since administration of B1/1 to mice with an ongoing IgE antibody response to *Lol pIV* had no detectable effects on the levels of antibodies to the allergen, the initial attempts to develop a clinically relevant model were not successful. On the other hand, the protocol of administering B1/1 to mice prior to challenge with the allergen served as a model to evaluate the regulatory function of this internal image monoclonal anti-Id on the levels of serum antibodies to *Lol pIV* and their expression of the Id91. The experimental model employed in this study was used to investigate the role(s) and

mechanism(s) by which the anti-Id regulates the antibody responses to grass pollen allergens.

MATERIALS AND METHODS

Animals

Adult female mice of strains C57BL/6, BALB/c and C3H (H-2^b, H-2^d and H-2^k) were purchased from Charles River Breeding Laboratories (Wilmington, MA). Male Long Evans hooded rats were obtained from the local Central Animal Care Services (University of Manitoba, Winnipeg, Canada).

Preparation of allergen Lol pIV

Lol pIV from ryegrass pollen was purified by affinity chromatography with a mAb91 immunosorbent. For use in immunization, the allergen was precipitated in alum as described previously.^{7,8}

Preparation of the monoclonal internal image Anti-Id, B1/1

The syngeneic monoclonal anti-Id, B1/1 (IgG2b, κ), was generated, characterized as an internal image anti-Id according to the classification used by Bona & Kohler and Bona *et al.*,^{9,10} and purified from ascites as described previously.⁷ The monoclonal IgG2b specific for influenza virus (designated MCA) was obtained from Cedarlane Laboratories (Ontario, Canada). This monoclonal antibody (mAb) was used as a control Ab2.

Preparation of B1/1-KLH conjugate

The methods for coupling antibodies to keyhole limpet haemocyanin (KLH) has been described previously.⁷

Protocols for treatment with B1/1 and challenge with Lol pIV

Three model systems were employed to determine the regulatory function of B1/1 on the antibody responses to *Lol pIV*.

Treatment with B1/1-KLH conjugate. For the first model system, groups of five mice of each strain were treated with intraperitoneal (i.p.) administration of either B1/1 or MCA (25 μ g) conjugated to KLH and precipitated in alum. Fourteen days later, all mice were first challenged, and boosted 14 days later, by i.p. injections of *Lol pIV* (5 μ g) in alum. Blood was obtained 14 days after the boost.

Treatment with B1/1 alone at various times. For the second model system, a solution of B1/1 (25 μ g in phosphate-buffered saline; PBS) was administered intravenously (i.v.) to eight groups of adult female BALB/c mice (five mice/group). The intervals of time between the administration of B1/1 and the challenge with *Lol pIV* ranged from -70 to 0 days. For controls, eight groups of age-matched BALB/c mice similarly received MCA. In an alternate control, the mice received PBS at the same time intervals as indicated above. All mice were challenged and boosted with *Lol pIV* and bled as described for the first model system.

Treatment with B1/1 alone at various doses. In the third model system, eight groups of adult female BALB/c mice (five mice/group) received various doses (1 ng to 100 μ g/mouse) of B1/1 in PBS by an i.v. injection. MCA was used as a control. All mice were challenged and boosted with *Lol pIV* and bled as indicated above.

Serum from an individual mouse of each group was

collected and tested for the level of antibodies to *Lol pIV* and expression of the Id91. The range of difference in end-point titre between the five mice was less than twofold; therefore, sera from each group were pooled and used for the rest of assays. Sera obtained from the mice before treatment served as negative controls.

Biotinylation of mAb91 and B1/1

Antibody preparations were biotinylated according to methods described previously.^{8,11}

Preparation of immunoadsorbent

Freeze-dried cyanogen bromide-activated Sepharose 4B (2 g; Pharmacia, Uppsala, Sweden) was suspended in and washed with an aqueous solution of 1 mM HCl. The gel was mixed with the ligand, B1/1 (35 mg), in coupling buffer (0.1 M NaHCO₃, pH 8.3, containing 0.5 M NaCl). The mixture was maintained at 4° overnight. After washing out the free ligand with coupling buffer, any remaining reactive groups on the gel were blocked with 0.1 M Tris-HCl buffer, pH 8, for 16 hr at 4°. The immunoadsorbent was washed with 0.1 M acetic acid and finally borate-buffered saline (BBS).

Determination of levels of antibodies to Lol pIV by ELISA

The levels of antibodies to *Lol pIV* in murine antisera were determined by an indirect enzyme-linked immunosorbent assay (ELISA). Briefly, the solid-phase was prepared by coating the wells of microtitre plates (Immunolon II; Dynatech Laboratories Inc., Alexandria, VA) with the *Lol pIV* 200 ng in 50 μ l of BBS at 4° overnight. For control, ovalbumin (OVA) was similarly employed to coat the wells. After blocking unbound sites with a solution of gelatin (2%) at 37° for 30 min, 50 μ l of the respective pool of antisera diluted in bovine serum albumin (BSA; 1% in BBS) was added to the microtitre wells and incubated at 37° for 1 hr, followed by washing three times with 0.2% Tween in BBS (T-BBS). The antibodies to *Lol pIV* that had bound to the solid-phase *Lol pIV* were detected by incubating the wells with horseradish peroxidase (HRP)-goat anti-mouse IgG heavy and light chains (Cedarlane Laboratories) at 37° for 1 hr. For detection of the levels of the individual classes of antibodies to *Lol pIV*, individual specific HRP-goat anti-mouse IgG1, IgG2b and IgM (Cedarlane Laboratories) were used. After washing the wells with T-BBS three times, the substrate 2,2'-azino-bis (3-ethyl-benzthiazoline sulphonic acid) (ABTS), in 0.1 M citrate buffer and 0.01% H₂O₂ (v/v), was used to develop the colorimetric reaction. The reaction was stopped by adding 5% sodium dodecyl sulphate (SDS) and the optical density (OD) of each well was measured in 410 nm on an automatic ELISA plate reader (Dynatech Instruments Inc., Torrance, CA). The assay was performed in triplicate. Serum levels of antibodies to *Lol pIV* were quantified as an end-point titre, and represented the final dilution of the antisera that yielded a differential OD₄₁₀ of 3 compared with the OD₄₁₀ obtained with preimmune sera.

Passive cutaneous anaphylaxis (PCA)

The PCA procedure was used to determine the levels of serum IgE antibodies to *Lol pIV*. Twofold serial dilutions of the mouse antisera were made in 0.9% saline and volumes of 50 μ l were injected intradermally into rats. Twenty-four hours later, 1 ml of *Lol pIV* (0.5 mg in saline) mixed with Evans blue dye (at

a final concentration of 0.25%) was injected i.v. and 20 min later the animals were killed. The size of the reactions (extravasation of the dye) which appeared on the undersurface of the skin was measured. The highest dilution of antisera that gave a reaction size of 3 mm in diameter was considered as the end-point titre. Each sample was tested in duplicate.

Inhibition ELISA

Detection of antibodies to site A of *Lol pIV*. The levels of antibodies specifically directed to site A of *Lol pIV* were evaluated in terms of the capacity of the murine antisera to inhibit the binding of the biotinylated mAb91 to *Lol pIV*. Microtitre plates were coated with *Lol pIV* in BBS at 200 ng/well, at 4° overnight. After blocking unbound sites with a solution of gelatin (2%), biotinylated mAb91 at a concentration of 5 µg/ml was mixed with the individual pool of murine antisera diluted in BSA (1% in BBS), placed into the wells and incubated at 37° for 1 hr. The levels of biotinylated mAb91 that bound to the solid-phase *Lol pIV* were determined by adding streptavidin-conjugated HRP in BSA. After incubating at 25° for 25 min, the substrate was used as described above. The assay was performed in triplicate.

Detection of antibodies that possess the Id91. To determine the levels of Id91⁺ antibodies to *Lol pIV* in murine antisera, a competitive ELISA was performed. The capacity of these antibodies to interfere with the Id-anti-Id interaction was examined in a manner similar to the inhibition procedure described above, with the following modifications; the solid-phase was prepared by coating each well of the microtitre plate with a solution of mAb91 (200 ng) in BBS at 4° overnight. After blocking any unbound sites with gelatin, biotinylated B1/1 (8 µg/ml), premixed with an individual pool of antisera to *Lol pIV* diluted in BSA, was added to the wells and incubated at 37° for 1 hr. After washing the plates, the extent to which the biotinylated B1/1 had bound to the solid-phase mAb91 was determined by adding streptavidin-conjugated HRP in BSA. The addition of substrate and quantification of the colorimetric reaction were performed as described above. The extent of the inhibition was calculated according to the formula: inhibition (%) = $1 - [\text{OD}_{410} \text{ with inhibitor} - \text{background} / \text{OD}_{410} \text{ without inhibitor} - \text{background}] \times 100$.

RESULTS

Effects of anti-Id B1/1 on the levels of serum antibodies to *Lol pIV*

Levels of antibodies to *Lol pIV*. The effects of treatment with B1/1-KLH in alum on three strains of mice (C57BL/6, BALB/c and C3H) on the levels of serum antibodies to *Lol pIV* were studied in one model system. Similarly, the effects of treatment of syngeneic BALB/c mice with B1/1 in PBS at (1) different intervals of time and (2) different doses, prior to their challenge with *Lol pIV*, were also investigated. The serum antibodies that bound to the solid-phase *Lol pIV* were detected using HRP-goat anti-mouse IgG heavy and light chains. Treatment of the three strains of mice with B1/1 (25 µg)-KLH conjugate precipitated in alum (Fig. 1) 14 days prior to the challenge with *Lol pIV*, or treatment of BALB/c mice with B1/1 (25 µg) in PBS at various times (from day -70 to day 0) (Table 1) or at various doses (100 ng to 100 µg) (Table 2), prior to their

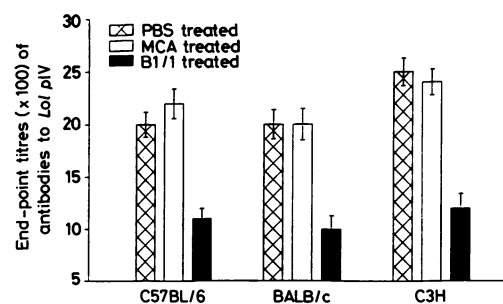


Figure 1. Effect of treatment of three strains of mice with B1/1-KLH on the levels of antibodies to *Lol pIV*. The end-point titre refers to the final dilution of the antisera which gave an OD₄₁₀ value three times higher than that obtained with pre-sera. The values represent mean (± SEM) of an ELISA performed in triplicate.

challenge with *Lol pIV*, resulted in a 50% reduction in the levels of antibodies to *Lol pIV* compared to control groups. By contrast, about a 30% elevation of the levels of antibodies to *Lol pIV* in the antisera of mice that had received B1/1 at a dose of 10 ng was observed (Table 2) compared to the groups that received a control mAb preparation. Treatment with 1 ng of B1/1 had no effect on the levels of antibodies to *Lol pIV*.

On the basis of these results, it was concluded that (1) anti-Id B1/1 could induce the reduction of antibodies to *Lol pIV* and the expression of Id91 in three different strains of mice; (2) a single treatment with soluble B1/1 at 25 µg 8 weeks prior to challenge with *Lol pIV* was effective in reducing the level of antibodies to *Lol pIV*; and (3) high doses (100 ng to 100 µg) of B1/1 induced a reduction in the level of antibodies, whereas a slight elevation of the level of the antibodies to *Lol pIV* was observed from animals that received a low dose (10 ng) of anti-Id.

Table 1. Effect of *in vivo* treatment with anti-Id, B1/1, at different intervals of time on the levels of serum antibodies to *Lol pIV*

Interval between treatment with B1/1 and challenge with <i>Lol pIV</i> * (days)	Level of antibodies to <i>Lol pIV</i> following the challenge and boost with <i>Lol pIV</i> †		
	PBS	MCA	B1/1
-70	0.50	0.48	0.50
-63	0.52	0.50	0.53
-56	0.50	0.53	0.20
-42	0.50	0.48	0.25
-28	0.49	0.48	0.20
-14	0.50	0.51	0.25
-7	0.45	0.52	0.24
0	0.50	0.52	0.25

* Each group of mice (five mice/group) received either PBS or 25 µg MCA or B1/1 at different time intervals prior to challenge with 5 µg of *Lol pIV* in alum. All mice were boosted with *Lol pIV* 14 days later.

† The individual pool of antisera obtained 14 days after the challenge with *Lol pIV* was diluted 1000-fold and the levels of antibodies to *Lol pIV* were evaluated by an ELISA. The values represent the mean of OD₄₁₀ nm of an ELISA performed in triplicate.

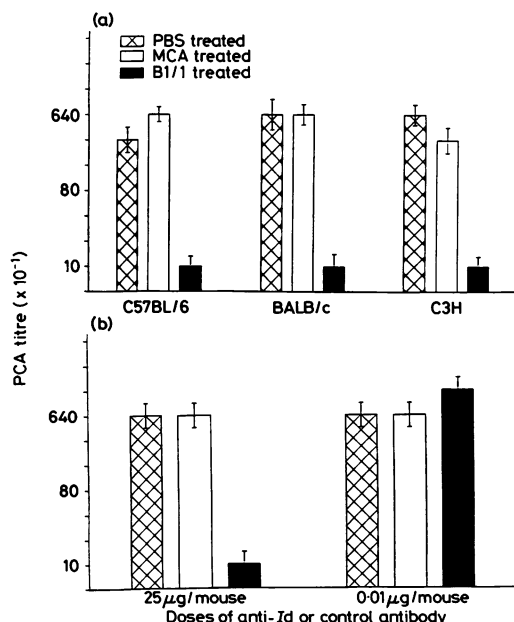
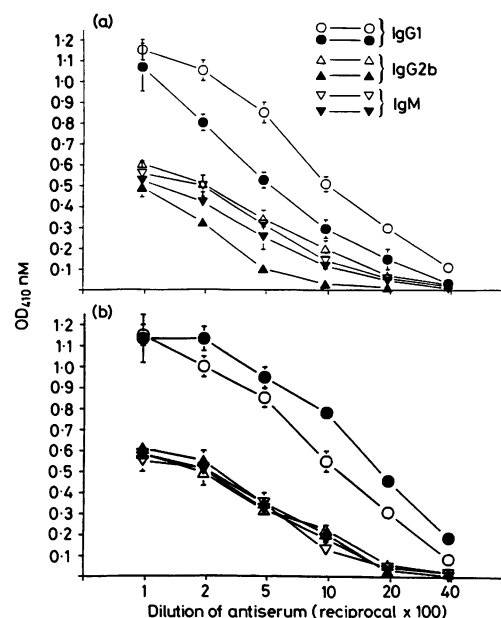
Table 2. Effect of *in vivo* treatment with B1/1 at various doses on the levels of serum antibodies to *Lol pIV**

Dose of antibody ($\mu\text{g/ml}$)	Level of antibodies to <i>Lol pIV</i> following challenge and boost with <i>Lol pIV</i> †	
	MCA	B1/1
100	0.53	0.25
50	0.48	0.26
25	0.50	0.22
10	0.52	0.21
1	0.59	0.24
0.1	0.50	0.21
0.01	0.52	0.68
0.001	0.50	0.48

* Each group of mice received various doses of either MCA or B1/1. All animals were challenged 14 days later with *Lol pIV* (5 $\mu\text{g}/\text{mouse}$) in alum and were boosted 2 weeks later.

† See Table 1.

Level of IgE antibodies to *Lol pIV*. The level of serum IgE antibodies in the control group of mice treated with MCA was the same as the group that had been treated with PBS. However, the IgE antibody titre to *Lol pIV* was 160–320-fold lower in mice treated with B1/1–KLH (Fig. 2a) and with B1/1 in PBS at a dose of 25 $\mu\text{g}/\text{mouse}$ (Fig. 2b). On the other hand, the IgE antibody titre was twofold higher for mice treated with B1/1 at a dose of 10 ng in PBS.

**Figure 2.** Effect of *in vivo* treatment with B1/1 on the IgE antibody response to *Lol pIV*. A PCA was used to test the IgE antibodies from mice that received (a) 25 μg of antibody–KLH in alum in three strains of mice, and (b) high and low doses of antibodies in PBS. The values represent the mean ($\pm$ SEM) of PCA titre performed in duplicate.**Figure 3.** Effect of *in vivo* treatment with B1/1 on the levels of IgG1, IgG2b and IgM antibodies to *Lol pIV*. An indirect ELISA was used to test IgG1, IgG2b and IgM anti-*Lol pIV* antibodies obtained from mice that received MCA (open symbols) or B1/1 (solid symbols) at a dose of (a) 25 $\mu\text{g}/\text{mouse}$ or (b) 10 ng/mouse. OD values represent the mean ($\pm$ SEM) of an ELISA performed in triplicate.

The subsequent studies of the effects of administering anti-Id B1/1 on the levels of antibodies to *Lol pIV* employed B1/1 at a dose of 25 μg (referred to as a high dose) and 10 ng (referred to as a low dose), respectively, per mouse.

Level of IgG1, IgG2b and IgM antibodies to *Lol pIV*. Prior treatment with a high dose of B1/1 in PBS (Fig. 3a) resulted in a reduction of the level of antibodies of the IgG1 and IgG2b isotypes. On the other hand, after administration of B1/1 at a dose of 10 ng, a slight elevation of the level of antibodies of the IgG1 class to *Lol pIV* was observed, whereas the levels of antibodies of the IgG2b and IgM classes did not change (Fig. 3b).

Levels of antibodies directed to site A of *Lol pIV*. An inhibition ELISA was employed to determine whether the administration of B1/1 had any effect on the level of antibodies with a specificity either identical or similar to that of mAb91, which is directed to site A of *Lol pIV*.⁶ In this assay, the capacity of antisera to inhibit the binding of mAb91 to solid-phase *Lol pIV* was evaluated. The results presented in Fig. 4 indicate that the antisera (diluted 20-fold) obtained from the mice treated with a high dose of B1/1 gave an inhibition value of less than 30%, whereas the antisera from the control groups had inhibition values in the range of 70–80%. Therefore, as a corollary of these observations, it was concluded that the level of a major portion of the antibodies to site A of *Lol pIV* had been reduced in the anti-Id-treated animals. On the other hand, the antisera from mice treated with a low dose of B1/1 completely inhibited the binding between mAb91 and *Lol pIV*. Collectively, these results indicate that the levels of antibodies with specificity related to mAb91 were modulated by treatment with anti-Id B1/1.

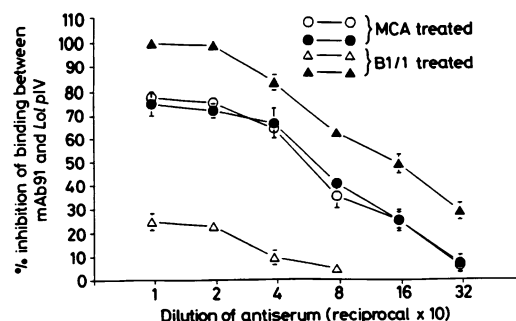


Figure 4. Effect of *in vivo* treatment with B1/1 on the levels of antibodies to site A of *Lol pIV*. An inhibition ELISA was used to test the ability of antisera to compete with biotin-mAb91 for binding to *Lol pIV*. Antisera were obtained from mice that received B1/1 at a dose of 25 µg (open symbols) or 10 ng/mouse (solid symbols). The percentage inhibition was determined from the mean ($\pm$ SEM) of an ELISA performed in triplicate.

Effects of treatment with B1/1 on the level of Id91⁺ antibodies

An inhibition ELISA procedure was used to determine whether the treatment with B1/1 affected the levels of Id91⁺ antibodies to *Lol pIV*. In this assay, the capacities of antisera from different groups of mice to inhibit the interaction between mAb91 and B1/1 were evaluated. The antisera from mice treated with MCA followed by challenge with *Lol pIV* inhibited, in a dose-dependent manner, the interaction between the mAb91 and B1/1 (Fig. 5). Treatment of the animals with a high dose of B1/1, followed by challenge with *Lol pIV*, produced antisera that were, at best, only poorly capable of inhibiting (< 15%) the interaction between mAb91 and B1/1. By comparison, the antibodies from the mice treated with a low dose of B1/1 expressed higher levels of antibodies carrying the Id91 (inhibition values increased approximately 30% relative to that of the control group). These results indicate that administration of B1/1 regulated the levels of antibodies to *Lol pIV* that possessed the Id91.

The proportion of Id⁺Ag⁺ and Id⁻Ag⁺ antibodies to *Lol pIV*

To establish what proportion of antibodies to site A of *Lol pIV* (i.e. Ag⁺) also possessed Id91 (i.e. Id⁺), the antisera from mice treated with a low dose of B1/1 or MCA and challenged with *Lol pIV* were passed through a reversed-immunoabsorbent prepared with B1/1. The adsorption was repeated using recycled adsorbent until the effluent was devoid of detectable Id⁺ antibodies. The adsorbed antisera were concentrated to their original volume and evaluated by the relevant assays in order to establish (1) the levels of Id⁺ antibodies (in terms of their capacity to inhibit the interaction between mAb91 and B1/1); (2) the levels of total antibodies to *Lol pIV* (by direct binding to solid-phase *Lol pIV*) and IgE antibodies to *Lol pIV* (by PCA); and (3) the levels of Ag⁺ antibodies (in terms of their capacity to inhibit the binding of mAb91 to *Lol pIV*). The results shown in Fig. 6 reveal: that (1) before the adsorption, the antisera could inhibit completely the binding between mAb91 and B1/1, whereas after removing the Id⁺ antibodies from the antisera the remaining antibodies were not able to inhibit this interaction (Fig. 6a); and (2) the levels of antibodies

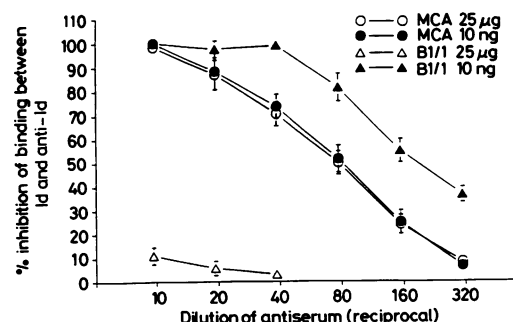


Figure 5. Effect of *in vivo* treatment with B1/1 on the level of Id91⁺ antibodies. The extent to which antibodies from mice that received B1/1 or MCA were capable of inhibiting the binding between mAb91 and B1/1 was evaluated in an inhibition ELISA. The percentage inhibition was determined from the mean ($\pm$ SEM) of an ELISA performed in triplicate.

which bound to the solid-phase *Lol pIV* decreased after the adsorption of Id⁺ antibodies (Fig. 6b). It was of interest to establish to what extent the Id⁺ antibodies were also Ag⁺. From the results shown in Fig. 6c, it is clear that adsorption of the Id⁺ antibodies markedly reduced the inhibitory capacity of the antisera in the assay. On the basis of this observation, it was deduced that a major portion of the Id⁺ antibodies were directed to site A of *Lol pIV*. Since the PCA titres of the antisera from the control group and the group treated with the low dose of B1/1 dropped from levels of 640 and 1280, respectively, down to 10 (Fig. 6d) after passage through the B1/1 immunoabsorbent, it was considered that practically the entire repertoire of antibodies of the IgE class to *Lol pIV* was Id⁺.

DISCUSSION

Anti-Id, which could potentially serve as vaccines for infectious diseases and as therapeutic anti-tumour agents, have been studied over the past few years.¹²⁻¹⁶ However, potential therapeutic approaches using anti-Id to regulate the antibody responses to allergens, such as ryegrass pollen allergen *Lol pIV*, have not yet been explored to the same extent.

This study demonstrates that it is possible to modulate antibody responses to the allergen *Lol pIV* by a single injection of mice with the internal image monoclonal anti-Id, B1/1. The results indicate that B1/1-KLH could be used to reduce the antibody responses to *Lol pIV*, i.e. the level of antibodies to *Lol pIV* and the expression of Id91. The reason for the use of the B1/1-KLH conjugate was to compare with B1/1 alone for the effect of reducing the level of antibodies to *Lol pIV*. The possibility that KLH had some influence on the effect was ruled out by the observation that B1/1 in PBS had a similar effect as the B1/1-KLH conjugate. The results obtained from three different strains of mice, C57BL/6, BALB/c and C3H (their haplocytes are H-2^b, H-2^d and H-2^k, respectively), indicate that there may be no restriction of the modulating properties of B1/1 at the level of the major histocompatibility complex (MHC).

A major criterion for evaluating the effectiveness of any therapy for pollen allergies is that a reduction of the IgE antibodies to the offending allergens could occur. Concomitantly,

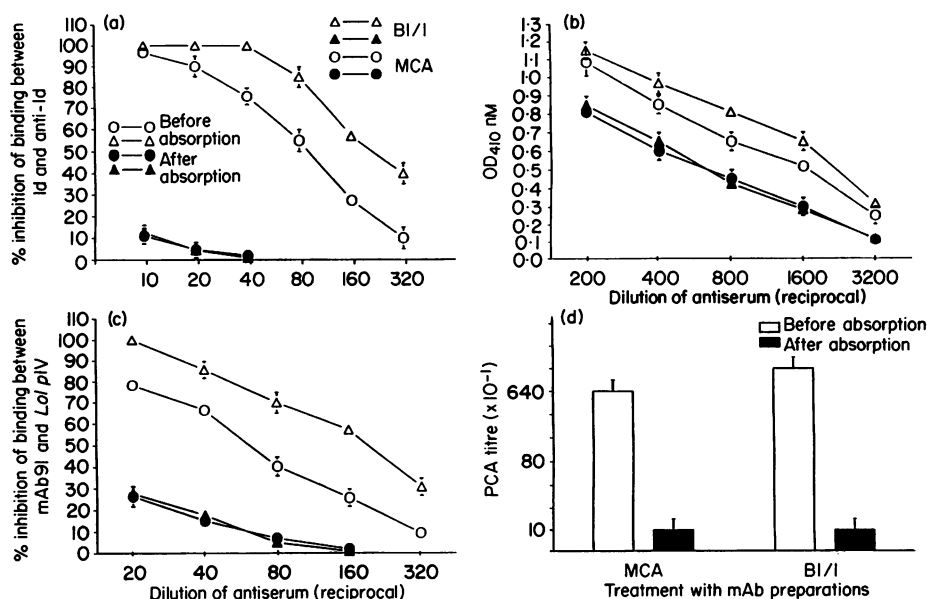


Figure 6. The proportion of Id⁺Ag⁺ and Id⁻Ag⁺ antibodies to *Lol pIV*. Before and after the removal of Id⁺ antibodies from antisera obtained from mice that received 10 ng of either B1/1 or MCA, the antisera were evaluated by various assays to determine: (a) levels of Id⁺ antibodies by an inhibition ELISA; (b) levels of total antibodies to *Lol pIV* by an indirect ELISA; (c) levels of anti-site A antibodies by an inhibition ELISA; and (d) levels of IgE antibodies to *Lol pIV* by a PCA. The values represent the mean ($\pm$ SEM) of assays performed in triplicate.

the level of IgG antibodies should rise following the therapy. The reason for determining each isotype of antibody to *Lol pIV* was used to delineate the effectiveness of anti-Id treatment on the repertoire of antibody responses to this allergen. In the present study, it was found that the level of IgE antibodies to *Lol pIV* was dramatically reduced (Fig. 2) in the mice that had received treatment with B1/1 at a high dose. By comparison, the levels of other classes of antibodies (IgG1 and IgG2b) to *Lol pIV* were only reduced to the maximum extent of 50% of the control values (Fig. 3). This suggests that the IgE antibody response may be more susceptible to modulation by B1/1 compared to other classes of antibodies. Other studies have also demonstrated the suppressive effect of anti-Id. For example, the IgE and IgG antibody responses to benzylpenicilloyl (BPO) can be specifically suppressed in BALB/c mice for a period of 2–3 weeks by a single i.v. injection of anti-BPO anti-Id.¹⁷ In this study, the effects of B1/1 on the antibody responses persisted for a period of 8 weeks (data not shown). In another murine system, the anti-Id to antigen-specific T-helper factor and IgE antibody has been used to specifically suppress the IgE antibody response to an allergen of timothy grass pollen.^{18,19} These studies collectively indicate that anti-Id could be used to suppress the IgE antibody responses to allergens.

The suppression of antibody responses by anti-Id has been studied in other antigen–antibody systems. An anti-Id has been used to suppress the murine antibody responses to a defined peptide of tobacco mosaic virus protein.²⁰ In this case, administration of the anti-Id suppressed the level of Id⁺ antibodies to the peptide; however, the level of the antibodies to the virus protein was not affected.²¹ By contrast, in the present study, not only was the level of Id91⁺ antibodies to the site A of *Lol pIV* affected (Fig. 4), but the levels of the IgG1 and IgG2b antibodies to *Lol pIV* were also altered (Fig. 3).

The observation that the level of antibodies to site A of *Lol pIV* was not completely reduced (Fig. 4) cannot be attributed simply to an inadequate amount of anti-Id administered, since doses of B1/1 which differed a thousand-fold (100 ng to 100 μ g) had similar effects on the formation of antibodies to *Lol pIV* (Table 2). To account for this observation, it may be speculated that at least two families of B-cell clones which recognize the same epitope (site A) are generated by challenge with *Lol pIV*. One of these clones produced antibodies which possess Id91 and is referred to as Id⁺Ag⁺, whereas the other clones produce antibodies which lack Id91 and are referred to as Id⁻Ag⁺. It is suggested that administration of a high dose of B1/1 suppressed the Id⁺Ag⁺ clone and resulted in a reduction in the level of antibodies to *Lol pIV*. On the other hand, the Id⁻Ag⁺ clones were not affected and produced antibodies by challenge with *Lol pIV*. The observation that the level of Id⁺Ag⁺ antibodies to *Lol pIV* was elevated by treatment of the mice with a low dose of B1/1 may be accounted for by the preferential activation of the Id⁺Ag⁺ clones. Clearly, the direction of modulating properties of B1/1 (i.e. up- or down-regulation) was dose-dependent.

In an early study, Kelsoe *et al.*,²² using anti-Id to modulate the immune response to the hapten NP, have shown that administration of 10 μ g of anti-Id to antibodies against NP suppressed the Id⁺ antibodies, whereas the total antibody level to NP was not affected. They proposed that the Id⁺ clones were suppressed but that the Id⁻ clones were still secreting the Id⁻ antibodies to NP. Another study²³ has shown that cloned anti-Id-producing B cells possessed the capacity of modulating the *in vitro* Id⁺ anti-dextran antibody response. This effect was dose dependent; thus, at a high dose (1000 cells) suppression was induced, whereas at a low dose (10–100 cells), the Id⁺ antibody response was enhanced.

Observations that passive transfer of non-atopic beekeeper's plasma containing the anti-Id to antibodies against bee venom protected sensitive patients from a subsequent systemic reaction to bee venom,²⁴ and that the auto-anti-Id were detected in non-reactive multi-sting beekeepers and in patients accepting the immunotherapy,²⁵ suggest that anti-Id may have a therapeutic potential for the treatment of allergy.

In summary, this study has demonstrated that in murine model systems the antibody responses to allergen *Lol* pIV could be effectively modulated by the use of the internal image monoclonal anti-Id B1/1; high doses suppressed and low doses enhanced the levels of serum antibodies.

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