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Biological Activity of Monoclonal Anti-Idiotypic Antibody Representing the Internal Image of the Major Allergenic Component of *Lolium perenne* Pollen

Key Words

Anti-idiotypic
Internal image
Biological activity

Abstract

Upon immunization with an anti-Lol p I (major allergenic component of *Lolium perenne* pollen) monoclonal antibody, we have previously produced anti-idiotypic monoclonal antibody (A7H2) displaying some internal image properties. The present study was designated to evaluate the capacity of this anti-idiotypic monoclonal antibody to mimic functionally the antigen by triggering histamine release from basophils of patients allergic to Lol p I. Anti-idiotypic monoclonal antibody, as the antigen, could induce histamine release in a dose-response fashion in all of the atopic patients (6/6). The inhibition of this histamine release by the addition of the idiotype (290A-167) confirmed the specificity of the reaction. Binding inhibition of human IgE to Lol p I demonstrated that the anti-idiotypic antibody recognized an idiotope expressed in the antigen-combining site of IgE molecules. Altogether, these data confirmed the internal properties of our anti-idiotypic antibody and it can mimic the original antigen in its capacity to trigger histamine release.

Introduction

The immunoglobulin molecules produced by any one B cell contain hypervariable regions that are different from those in immunoglobulin molecules produced by most or all other B cells. The unique determinants of immunoglobulin hypervariable regions are called idiotopes. Collectively, the idiotopes define the idiotype (Id) of the molecule [1]. Id can be shared by antibodies (Ab) of different isotypes in the same individual or can be also expressed by Ab from different species [2, 3]. During any immune response, the Id becomes immunogenic, leading to the pro-

duction of Ab directed against it [4, 5] which are termed anti-idiotypes (aId). There are markers that distinguished the variable regions of specific Ab molecules [6, 7] and play an essential regulatory role in the immune network system [1]. Using polyclonal aId Ab against IgG anti-tetanus toxoid, it was demonstrated that human IgG, IgM and IgE Ab to tetanus toxoid shared the same Id [8]. Using polyclonal aId, we [9, 10] and other [11] demonstrated the idiotype cross-reactivity between human IgG, IgE and murine monoclonal Ab (mAb) directed against Lol p I (major allergenic determinant of *Lolium perenne* pollen). Histamine release experiments extended these data [12].

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We have demonstrated that polyclonal aId directed against murine anti-Lol p I mAb could interact with IgE on the surface of basophils resulting in the release of histamine in a dose-response fashion.

We also reported [13] the production and the characterization of aId mAb (Ab2) upon immunization of mice with an anti-Lol p I mAb (290A-167). This Ab2 displayed internal image properties of Lol p I. It could be affinity purified on an immobilized Id column. Its binding to the anti-Lol p I mAb (290A-167) was inhibited by Lol p I and it inhibited in a dose-response fashion the binding of the specific Id to antigen (Ag). It was recognized by anti-Lol p I antisera from different species (mouse, human and goat). The concept of internal image implies that aId could elicit anti-aId Ab (Ab3) that recognize the original Ag. In fact, we found in the fusion leading to the production of aId, an anti-Lol p I mAb (Ab3) that bound both aId and Ag and share common Id with Ab1. Indirectly, these data suggested that our aId mAb really bear the internal image of Lol p I.

In this report, using binding-inhibition studies, we demonstrated that this aId mAb recognized idiotopes found in human IgE specific to Lol p I. Also, since aId should mimic stereospecifically and functionally the original Ag, we compared the ability of aId to induce histamine release from basophils to that of allergen. To insure the specificity of the assay, we assessed the ability of Ab1 to inhibit the aId induced-histamine released.

Materials and Methods

Study Populations

Six grass-sensitive subjects aged 19–56 years were included in this study after written informed consent was obtained. They were selected from the Allergy and Immunology Clinic of the Centre Hospitalier de l'Université Laval according to the following characteristics: a clear-cut history of grass-allergic rhinitis without asthma for at least 5 years, a positive skin prick test and positive RAST to *L. perenne*, normal routine physical exam and normal laboratory workup and no previous immunotherapy to grass pollens. Four nonatopic volunteers with no history of allergic rhinitis and negative skin tests and RAST to common inhalants were also included in this study.

Antigens and Antisera

Lol p I was purified by immunosorbent techniques from crude extracts of *L. perenne* (Omega Laboratories, Montréal) as described previously [14]. Anti-Lol p I mAb 290A-167 (γ 2b, κ), 348A-6 (γ 1, κ) and 539A-6 (γ 2a, κ) were produced in our laboratory and previously characterized [9, 14]. aId mAb, A7H2, (γ 1, κ) directed against the Id 290A-167 and anti-aId mAb, 1H4, (γ 1, κ) directed against the latter Ab2 were recently produced and characterized [13]. An antihuman IgE mAb also produced in our laboratory was used in binding-inhibition assay and histamine-release experiments. All mAb used in this

study were purified from murine ascitic fluids by passing the fluids through a protein A column. Antiferritin mAb, 13A-12 (γ 2b, κ) and 3E12 (γ 1, κ) were used as control.

Radiolabeling of Proteins

Purified mAb (30 μ g) was iodinated by using IODOGEN (Pierce Chemical Co., Rockford, Ill.), and free ^{125} I was separated from ^{125} I-labeled mAb by passage through an Econo-Pac 10DG column (Bio-Rad, Richmond, Calif.).

Functional Activity of aId mAb: Ab2-Induced Histamine Release by Human Leukocytes

The aim of the present experiment was to verify the biological activity of aId mAb (A7H2) by its ability to trigger histamine release by leukocytes of *L. perenne*-sensitive patients or nonatopic subjects. Leukocytes were isolated by dextran sedimentation as described by Lichtenstein and Osler [15]. These preparations generally contained about 0.5% basophils. The cells were washed, divided into aliquots (4×10^6 cells/1.0 ml/tube), and incubated alone or with various concentrations (0.08, 0.4 and 2 μ g/ml) of aId mAb (A7H2), Lol p I, anti-IgE mAb or control mAb for 45 min at 37 °C with constant agitation. At the end of the incubation, tubes were centrifuged at 130 g for 5 min at 4 °C and the supernatants were collected for determination of histamine content by using a radioenzymatic assay as previously reported [16]. All values were corrected for spontaneous release and the percentage of histamine release was calculated based on the total cellular histamine content determined after cell disruption by boiling. The specificity of the reaction will be ensured by verifying the capacity of anti-Lol p I mAb to inhibit the histamine release.

Inhibition by Id of Histamine Release Induced by aId mAb

We assessed the capacity of Id (290A-167) to block the release of histamine to verify the specificity of histamine release induced by aId mAb (A7H2). An equal volume of fixed concentration of A7H2 (0.4 μ g/ml) was incubated alone, with various concentrations (0.08 and 0.4 μ g/ml) of 290A-167 or control mAb for 1 h at room temperature before triggering histamine release by leukocytes as described above. Percentage of inhibition of histamine release was calculated as following:

$$\% \text{ of inhibition of histamine release} = \frac{\text{HRO} - \text{HRI}}{\text{HRO}} \times 100,$$

where HRO and HRI represent respectively the percentage of histamine release without and in presence of inhibitors.

Binding Inhibition of Human IgE Ab to Lol p I by Ab2

The capacity of aId mAb to inhibit the reaction between human serum IgE and the allergen was assessed. Lol p I-coated cellulose disks were incubated overnight with human serum (pooled or individual) diluted 1/4 alone or in the presence of various concentrations of aId (A7H2) as well as Lol p I, anti-Lol p I mAb (290A-167), anti-aId mAb (1H4) or antiferritin mAb (13A-12 and 3E12). After washing, ^{125} I-labeled antihuman IgE mAb was added (10⁵ cpm/well) and incubated for 18 h. Wells were extensively washed, the radioactivity counted and the percentage inhibition calculated as follows:

$$\left[1 - \frac{\text{cpm bound in presence of inhibitor}}{\text{cpm bound in absence of inhibitor}} \right] \times 100.$$

Table 1. Histamine release from human basophils triggered by aId mAb (A7H2) or by Lol p I

	aId mAb			Lol p I			Anti-IgE mAb			Control mAb		
	2 ^a	0.4	0.08	2	0.4	0.08	2	0.4	0.08	2	0.4	0.08
<i>Allergic</i>												
A.V.	71 ^b	54	38	68	74	79	27	14	13	10	0	10
C.M.	34	9	8	77	74	71	71	72	41	13	16	11
C.P.	100	89	50	100	100	100	57	33	22	0	0	0
C.L.	100	84	50	100	100	100	62	60	53	7	5	0
M.B.	58	91	61	76	76	51	63	46	23	32	20	10
D.S.	28	11	1	54	53	49	30	12	4	16	18	3
<i>Nonatopic</i>												
Y.B.	8	2	3	9	n.d.	3	18	6	2	7	6	5
L.H.	0	0	0	0	0	0	21	18	0	0	0	0
S.A.	0	0	0	0	0	0	5	6	0	0	0	0
N.B.	7	11	3	9	8	3	23	22	9	5	21	10

^a Concentration of protein (µg/ml).
^b Percentage of histamine release.
n.d. = Not determined.

Table 2. Inhibition by anti-Lol p I mAb (290A-167) of histamine release (HR) induce by 0.4 µg of aId mAb (A7H2)

Patients	% inhibition of HR by Id (290A-167)	
	0.4 µg	0.08 µg
A.V.	59 ^a	43
C.L.	53	29
C.M.	32	34
C.P.	37	25

^a Numbers represent the percentage of inhibition.

Results

Functional Activity of aId mAb: Ab2-Induced Histamine Release by Human Leukocytes

The expression of the idiotope recognized by our aId mAb on human IgE was studied in a functional assay where the ability of A7H2 to induce histamine release from basophils of 6 different atopic patients and 4 normal subjects was compared to that of the Ag itself or anti-IgE mAb. We used as controls mAb of different specificity but carrying the same isotype. It could be seen that aId mAb induced release of histamine in a dose-dependent fashion (table 1) as seen for Lol p I itself. No significant release was seen with a mAb of unrelated specificity. Furthermore, no significant histamine release was observed using either Lol p I or aId mAb with basophils from nonatopic

subjects. These results confirm the presence of shared idiotopes between basophil-bound IgE and murine anti-Lol p I mAb.

We assessed the ability of the Id (290A-167) to inhibit the histamine release induced by the aId to verify the specificity of aId mAb-induced histamine release. As shown in table 2, the anti-Lol p I (290A-167) could inhibit significantly (up to 59%) the histamine release by leukocytes from atopic patients whereas 13A-12, a control mAb with the same isotype of 290A-167, had no effect on the histamine release induced by aId mAb [not shown]. The percentage of inhibition varied from patient to patient, reflecting the cellular sensitivity of the different patients.

Inhibition of IgE Binding to Lol p I by mAb aId

Histamine release experiments did not indicate if the idiotopes recognized by aId mAb were located in the Ag-binding site of the IgE molecule. To answer to that question, we analyzed the ability of aId mAb to inhibit the binding of pooled human IgE Ab to allergen. Figure 1 shows the inhibition obtained with the Ag, anti-Lol p I (290A-167), aId mAb (A7H2) and anti-aId mAb (1H4). The specificity of these reactions was shown by the absence of inhibition when other mAb of the same isotype and light chain but of different specificity were used.

Maximal inhibition obtained with the aId was approximately 80% while the percentage of binding-inhibition observed with the Lol p I was 100%. Anti-Lol p I and anti-aId mAb inhibited no more than 40% of human IgE-binding

to solid-phase fixed Lol p I at the concentrations used herein. Furthermore, the profiles of inhibition of anti-Lol p I (290A-167) and anti-aId mAb (1H4) were comparable. Although A7H2 was an anti-290A-167, the maximal inhibition obtained with this aId mAb was higher than that obtained with the Id. Binding-inhibition studies of individual serum IgE Ab were also assessed. As shown in table 3, the quantity of aId required to inhibit 50% of the binding of IgE to Lol p I ranged from 63 to 117 ng/ml except for 1 patient (> 250 ng/ml). This variability reflects probably the affinity and specificity of the human polyclonal IgE Ab.

Discussion

We have previously produced mAb against Lol p I, the major allergenic determinant of *L. perenne*, and shown that these idiotopes are shared also by human IgG and IgE by using rabbit aId Ab [10]. However, to study the regulation of anti-Lol p I Ab responses in animal and human, we produced aId mAb directed against an anti-Lol p I mAb produced and characterized in our laboratory. This aId mAb displayed internal image properties as previously demonstrated [13].

The purpose of this work was to confirm that this aId mAb mimics the stereospecificity and functionality of Lol p I. Its ability to trigger histamine release was assessed and compared to that obtained with Ag. Our aId mAb induced histamine release by leukocytes obtained from atopic patients in a dose-response fashion. Percentages of histamine release obtained with the aId mAb were clearly higher than those previously reported by us and using rabbit polyclonal aId Ab [12]. Indeed, with aId mAb, basophils from 4/6 grass-sensitive patients released more than 50% of their histamine content while, using polyclonal aId, basophils from only 1/19 patient had 50% histamine release. Lol p I induced a significant histamine release by basophils of all patients. However, some subjects (2/6) demonstrated a weak aId-induced histamine release. These data suggested that in these patients other Id could dominate confirming previous findings observed with polyclonal aId Ab [12].

The aId-induced histamine release was inhibited by pre-incubation with the Id to insure the specificity of the assay. The variability of inhibition by the Id to block the aId-induced histamine release could reflect the different cellular sensitivity of the different patients allergic to grasses. For these patients, it might well be that higher concentrations of Id (< 0.4 µg) will be required to obtain complete inhibition of aId-induced histamine release.

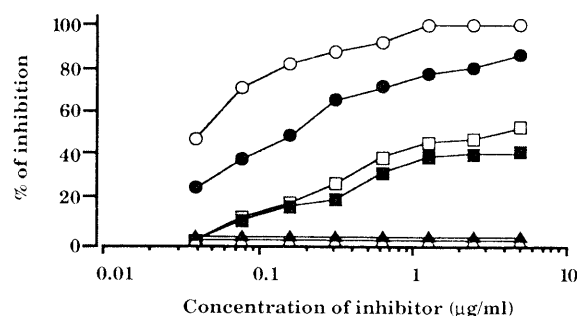


Fig. 1. Binding-inhibition of pooled human IgE Ab to Lol p I by Lol p I (○); anti-Lol p I mAb, 290A-167 (□); aId mAb, A7H2 (●); anti-aId mAb (■), 1H4 and control mAb 13A-12 (▲) and 3E12 (△).

Table 3. Concentration of aId mAb required to inhibit 50% of the binding of human IgE to Lol p I

Patients	Concentration of A7H2 ng/ml ± SEM
C.P.	63 ± 7 ^a
C.L.	92 ± 13
A.V.	117 ± 21
C.M.	> 250
M.B.	81 ± 36
D.S.	77 ± 6

^a Data represent the means of three experiments.

However, these results suggest that aId mAb is a better tool than polyclonal Ab to insure the sensibility and specificity for the analysis of immunoregulation with aId Ab. These data also suggested that the idiotope recognized by A7H2 might be strongly expressed on human IgE.

Biological assays such as the histamine release experiments prove in this system that our aId mAb recognizes idiotopes expressed by IgE by shortcutting the possible interference with IgG Ab. However, they do not provide information whether the idiotope is located in or outside the Ag combination site of the IgE molecule. Using binding inhibition of specific IgE to Lol p I, we report that our aId mAb inhibited up to 80% of the binding of human IgE to Lol p I while for the same concentration of Ab, the Id (290A-167) inhibited no more than 40%. A difference in the affinity between the two mAb and/or by steric factors in the inhibition of the binding of IgE by aId can explain the better inhibition capacity of aId.

Although the experiments were performed on a relatively small group of patients, these data and previous

findings support that the Id recognized by 290A-167 is an important public Id and it may represent a preferential target for selective immunoregulation. Also, they confirm that our aId mAb represents the internal image of the Lol p I and it can mimic Lol p I in its ability to induce histamine release. Therefore, A7H2 will be a useful tool to study the role of idiotypic interaction in the modulation of immune response to Lol p I.

Acknowledgments

Yvan Boutin is recipient of a studentship of the Medical Research Council of Canada.

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