Clin. exp. Immunol. (1986) **66**, 231–240

Anti-idiotypes to anti-*Lolp* I (Rye) antibodies in allergic and non-allergic individuals. Influence of immunotherapy

RATNA BOSE, D. G. MARSH* & G. DELESPESSE *MRC Group for Allergy Research, Department of Immunology, Faculty of Medicine, The University of Manitoba, Winnipeg, Manitoba, Canada R3E OW3, and *Johns Hopkins University, School of Medicine at the Good Samaritan Hospital, 5601 Loch Raven Boulevard, Baltimore, MD, USA*

(Accepted for publication 21 May 1986)

SUMMARY

Anti-idiotypes (aId) reacting with anti-*Lol* I (*Lolp* I; Rye I) antibodies were detected by their ability to bind to radioiodinated $F(ab')_2$ anti-*Lol* I. Sera were tested after removal of anti-*Lol* I and anti-heavy and light chain activity by adsorption on *Lol* I-Sepharose 4B and normal human serum Sepharose 4B. The binding of aId to Id was inhibited by affinity purified anti-*Lol* I but not by certain unrelated immunoglobulins; in some sera this binding was also inhibited by *Lol* I. The levels of aId were measured in serial bleedings collected over a 1 year period from *Lol* I-sensitive patients, allergic donors not sensitive to *Lol* I and non-allergic persons. In *Lol* I-allergic patients the levels of aId were significantly influenced by seasonal exposure to pollen and by immunotherapy with extracts of grass pollen. Moreover, in 12 out of 16 cases, there was also a significant inverse relationship between changes in serum levels of aId and of IgG or IgE anti-*Lol* I. Most interestingly, aId were also detected in non-allergic individuals; in this case, the levels of aId were not influenced by the pollen season. The data suggest that Id-aId interactions may play a role in the regulation of anti-*Lol* I antibody production.

Keywords anti-idiotype anti-*Lol* I (Rye I) antibodies non-allergic allergic-untreated immunotherapy

INTRODUCTION

Several studies in experimental animals strongly suggest that idiotype-anti-idiotypic antibody (Id-aId) interactions play a significant role in the regulation of humoral and cell-mediated immune responses (Bankert & Pressman, 1976; Blaser & de Weck, 1982; Forni *et al.*, 1980; Jerne, 1974; Nisonoff & Greene, 1980; Reth, Kelsoe & Rajewsky, 1981; Schrater *et al.*, 1979). In man, auto-aId were described in patients with selective IgA-deficiency (Cunningham-Rundles, 1981), mixed cryoglobulinaemia (Geltner, Franklin & Frangione, 1980), systemic lupus erythematosus (Abdou *et al.*, 1981) and myasthenia gravis (Dwyer *et al.*, 1983). Auto-aId were also reported in normal individuals after tetanus toxoid (TT) vaccination (Geha, 1982); and most interestingly, such auto-aId could modulate the in-vitro response of human lymphocytes to TT (Saxon & Barnett, 1984). We previously showed that the serum of one allergic patient contained auto-aId cross-reacting with Id expressed on IgE and IgG anti-*Lolp* I antibodies from several grass-allergic patients (Bose *et al.*, 1984). (*Lolp* I is the new nomenclature for the Rye I allergen isolated from *Lolium perenne* pollen (Marsh, 1975; Marsh, Milner & Johnson, 1966; Marsh *et al.*, allergen nomenclature, WHO Bulletin,

Correspondence: G. Delespesse, University of Montreal, Notre-Dame Hospital, Research Center, 1560 E. Sherbrooke Street, Montreal, Quebec, Canada H2L 4M1.

submitted). It is abbreviated to *Lol* I in this paper.) This auto-aId was paratope-induced; indeed it was detected by its ability to block the binding of radiolabelled *Lol* I to both IgE and IgG anti-*Lol* I antibodies. This assay could not be used to quantify the aId levels in a large number of sera. In the present study, aId were detected on the basis of their binding to radiolabelled, affinity-purified $F(ab')_2$ fragments of anti-*Lol* I antibodies. The results indicate that aId are present in normal individuals and that in allergic patients aId levels are influenced by natural exposure to grass pollen or by immunotherapy with grass extracts.

MATERIALS AND METHODS

Participants. A total of 80 subjects have been studied among which 26 Caucasian subjects were used for the longitudinal study. They were divided into five groups: (i) *Lol* I allergic, not receiving immunotherapy (six cases); (ii) *Lol* I allergic undergoing immunotherapy with mixed grass allergen or formaldehyde treated allergen (allergoid) (10 cases); (iii) allergic to *Lolium perenne* (Rye grass) but not allergic to *Lol* I (two cases); (iv) allergic to ragweed, cats and to house-dust but not allergic to *Lolium perenne* (two cases); and (v) non-allergic (two cases). The people in the first, third and fourth groups were part of a longitudinal study of allergic response in the employees of the Westinghouse Electric Corp. (Freidhoff *et al.*, 1981 and unpublished). The patients in the second group were followed at the allergy clinic of the Good Samaritan Hospital. The diagnosis of allergy was based on clinical data, skin testing and IgE antibody determination in all cases. The non-allergic and untreated allergic subjects were bled before and several times after the pollen season. The treated allergic patients were bled once before starting the immunotherapy and three times thereafter. Ten Rye grass non-allergic individuals were from Winnipeg, Manitoba; sera of these individuals had no IgG or IgE anti-*Lol* I antibodies. Sera from six of these individuals were used to prepare 'normal human serum' and data from five of these subjects are presented in Table 1. The sera from 44 grass-

Table 1. Inhibition of the binding of aId to ^{125}I-Id by antibody and by antigen

	% Inhibition of the binding of ^{125}I-anti-*Lol* I $F(ab')_2$ to aIdF											
Experimental conditions	1* (15)†	2 (25)	3 (16)	4 (21)	5 (27)	6 (13)	7 (11)	8 (20)	9 (24)	10 (26)	11 (12)	12 (17)
A. Inhibition by purified antibody												
aIdF + anti-*Lol* I IgG (50 μg)	100			90	85	82	72	74	86	72	91	
aIdF + anti-*Lol* I IgG (25 μg)	42			74	80	48	71	41	66	60	72	
aIdF + anti-*Lol* I IgG (4 μg)	24	47	26	26	32	8	10	18	14	33	21	21
aIdF + anti-*Lol* I IgG (2 μg)	9	25	12									10
aIdF + anti-*Lol* I IgG (1 μg)	3	9	0									0
aIdF + myeloma IgG (50 μg)	0	0	0	4	13	0	0	0	8	0	14	0
aIdF + myeloma IgG (4 μg)	0	0	0									0
aIdF + anti-TT IgG (50 μg)	1	0	1	2	9	6	0	0	0	0	7	0
aIdF + anti-TT IgG (4 μg)	0	0	0									0
B. Inhibition by antigen												
aIdF + *Lol* I (10 μg)	31	—	0	22	19	0	0	0	36	11	13	0
aIdF + *Lol* I (5 μg)	25	—	0	18	9	0	0	0	32	0	6	0
aIdF + HDM (10μg)	0	0	0	0	8	0	7	0	6	0	0	0
aIdF + HDM (2 μg)	0	0	0									0
aIdF + TT (2 Lf units)	0	0	0									0
aIdF + TT (1 Lf units)	0	0	0									0

* The aIdF used in this assay were prepared from 17 sera, sera No. 1–7 were from Rye grass allergic donors and sera No. 8–12 were from non-allergic donors.

† % of ^{125}I-anti-*Lol* I $F(ab')_2$ bound in the absence of inhibitor.

allergic donors (Fig. 1, and seven subjects in Table 1) were obtained from the Clinical Immunology Laboratory at Brussels University Hospital. The donors were selected on the basis of their RAST positivity (3 to 4+) to Rye grass pollen.

Antigens. Lol I was purified as described (Freidhoff *et al.*, 1981; Marsh, 1975; Marsh *et al.*, 1966). Tetanus toxoid (TT) was obtained from Connaught Laboratories, Willowdale, Ontario. House-dust mite (HDM) extract of *Dermatophagoides pteronyssinus* was kindly given by P. Horan, Beecham Laboratories, England (Batch 77/91 (a)).

Preparation of normal human serum. Sera from eight individuals with high levels of anti-*Lol* I IgG and IgE antibodies were pooled, precipitated with saturated ammonium sulphate (30% final concentration) three times and coupled to cyanogen bromide activated Sepharose 4B beads (Pharmacia Fine Chemicals, Uppsala, Sweden) (Axen, Porath & Ernback, 1967). Sera from six individuals with no history of allergic disease were pooled and adsorbed extensively on the above immunosorbent in order to remove any possible activity against immunoglobulin heavy chains, light chains and anti-*Lol* I idiotype. This pool of adsorbed sera was used as control human serum (NHS) in the assay for the detection of aId reacting with anti-*Lol* I antibodies. A portion of this NHS was cross-linked with Sepharose 4B beads by the same procedure as described above.

Purification of IgG F(ab')$_2$ anti-Lol I antibody. IgG antibody was purified from the plasma of one *Lol* I-allergic individual as described earlier (Bose *et al.*, 1984). The IgG was dialysed against acetate buffer, pH 4·5 and digested with pepsin at 37°C for 48 h. F(ab')$_2$ was isolated from the digested material by a combination of gel filtration on a Sephadex G200 column and affinity chromatography on Protein A-Sepharose 4B (Pharmacia). The purity of the IgG F(ab')$_2$ was tested by Ouchterlony and SDS-PAGE. IgG F(ab')$_2$ fragments were then absorbed on *Lol* I-Sepharose 4B (Bose *et al.*, 1984). After extensive washing the anti-*Lol* I IgG F(ab')$_2$ was eluted with 0·1 M glycine-HCl, pH 3·0; the eluate was neutralized immediately with 2 M Tris-HCl, pH 8·5, and dialysed against PBS. The affinity-purified material bound to *Lol* I (4 μg anti-*Lol* IgG F(ab')$_2$ bound 8% of ^{125}I-*Lol* I) but not to an unrelated antigen (TT).

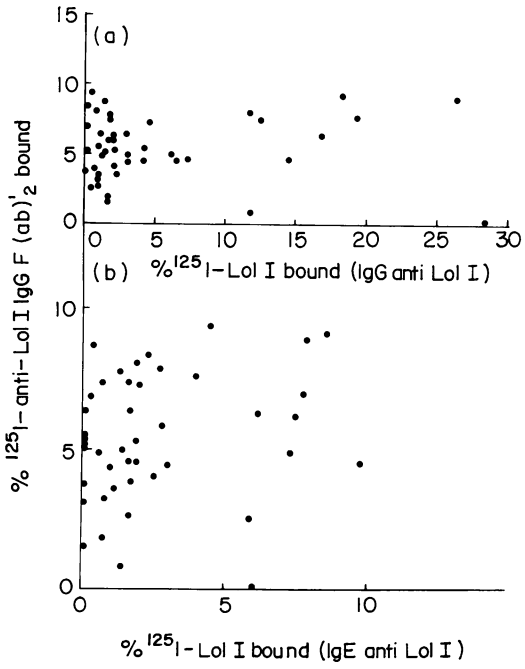


Fig. 1. Comparison of aId levels and IgG or IgE anti-*Lol* I antibodies in sera from 44 patients allergic to Rye grass. No significant relationships were found. Total radioactivity was 20,000 ct/min for ^{125}I-anti-*Lol* I F(ab')$_2$ and 50,000 ct/min for ^{125}-*Lol* I.

Radioiodination. IgG F(ab′)$_2$ anti-*Lol* I was radiolabelled with carrier-free ^{125}I by the chloramine-T method (Klinman & Taylor, 1969) at a specific activity of 2000 to 4000 ct/min/ng; more than 90% of labelled material was precipitated by trichloroacetic acid. The ^{125}I-anti-*Lol* I F(ab′)$_2$ was diluted in assay buffer containing 1% normal sheep serum (NHS-Sepharose 4B adsorbed) and filtered through a 0·22 μ filter before use.

Preparation of test sera for the determination of aId. Before being tested for aId, sera were adsorbed once with NHS-Sepharose 4B (prepared as described above) and twice with *Lol* I-Sepharose 4B. In each case, 1 volume serum and 1 volume packed gel were allowed to rotate overnight at 4°C; as a result of these adsorptions, test sera were diluted six times. The adsorbed sera are referred to hereafter as aId fractions (aIdF).

RIA for anti-Lol I IgG F(ab′)$_2$ specific IgG. The presence of anti-idiotype in the aIdF was tested by double antibody RIA (Geha, 1982) using ^{125}I-anti-*Lol* I F(ab′)$_2$. Briefly, 60 μl of aIdF was incubated overnight at room temperature with 25 μl of ^{125} I-anti-*Lol* I F(ab′)$_2$ (10 ng and 20,000 ct/min). An excess of Fc-specific sheep anti-human IgG was then added and the incubation was continued for 5 h at room temperature and overnight at 4°C. The precipitate was washed five times with assay buffer, transferred to fresh tubes and the radioactivity was counted in a gamma counter. For inhibition experiments the inhibitor and the aIdF were incubated for 2 h at room temperature before the addition of ^{125}I-anti-*Lol* I F(ab′)$_2$.

Assay of serum IgE antibody to Lol I in treated patients. IgE anti-*Lol* I for *Lol* I-allergic, treated patients was assayed using solid phase radioimmunoassay because in double antibody assay, competition with high levels of IgG anti-*Lol* I antibodies was expected. All steps were done at room temperature in a humid chamber. Briefly, wells of polystyrene microtitre plates were coated with 250 μl of mouse monoclonal anti-IgE (clone No. 89; 1 μg/ml in 0·01 M carbonate buffer, pH 9). The plates were washed with PBS and blocked for 3 h with PBS containing 10% fetal calf serum. After washing, 200 μl of the human serum to be tested was added to each well and incubated overnight at room temperature. The plates were washed and supplemented with ^{125}I-*Lol* I (PBS containing 0·1% BSA and 0·05% Tween 20) 50,000 ct/min/200 μl. After overnight incubation the plates were washed and the individual wells were separated and counted in a gamma counter.

Assay of anti-Lol I serum IgG antibody and of IgE antibody in untreated patients. IgG antibody to *Lol* I was measured using a double-antibody radioimmunoassay analogous to that previously described for determining anti-*Amb* V (Ra5) antibody (Marsh *et al.*, 1982). This assay employs highly purified *Lol* I radiolabelled with ^{125}I and goat anti-human IgG (Fc-specific) as the second antibody. All analyses were performed in triplicate in at least two experiments. Serum IgE antibody in untreated patients was measured by a similar assay, except that goat antihuman IgE (Fc-specific) was used as the second antibody (Marsh *et al.*, 1982). In this case, a small amount of a myeloma serum was added as a carrier to facilitate immunoprecipitation. Results are expressed in ng/ml of IgG or IgE antibody to *Lol* I, based on titration against a reference human serum of known high anti-*Lol* I IgG antibody content (Freidhoff *et al.*, submitted for publication).

Statistical analysis. Pearson's correlation coefficient was calculated for each case between the slopes of the three variables: IgE anti-*Lol* I, IgG anti-*Lol* I and aId, using the sign of the correlation coefficient as an indicator of the direction of the relationship for the individuals within a group. A Binomial non-parametric test was used when comparing the proportion of positive correlations versus negative correlations. To determine whether the changes of aId levels over time were significantly different, a repeated measure analysis of variance was performed.

RESULTS

Assay for the detection of aId. Anti-idiotype binding to anti-*Lol* I antibodies were detected by their binding to radiolabelled F(ab′)$_2$ fragments of affinity-purified anti-*Lol* I IgG antibodies [anti-*Lol* I F(ab′)$_2$] of one particular patient. In preliminary assays it was found that the sera from 10 non-allergic individuals (only NHIgG-Sepharose (Bose *et al.*, 1984) absorbed) displayed a significant binding activity to anti-*Lol* I F(ab′)$_2$, in spite of the fact that they contained no detectable IgG nor IgE anti-*Lol* I antibodies. These sera were pooled and extensively adsorbed on anti-*Lol* I IgG-

Sepharose 4B in order to remove aId as well as anti-Fab activity. This pool of adsorbed non-allergic sera was used to determine the nonspecific binding of ^{125}I-anti-*Lol* I F(ab′)$_2$ to human sera; such background, ranging from 3 to 5% of the total radioactivity, was deducted from the values of each experimental serum.

Inhibition of ^{125}I-anti-*Lol* I F(ab′)$_2$ binding to aId was tested by employing affinity-purified anti-*Lol* IgG; the results were compared to those observed with an unrelated antibody anti-TT, or myeloma IgG. These studies were performed on 12 sera selected for their relatively high binding (10 to 25%) to ^{125}I-F(ab′)$_2$ anti-*Lol* I. Anti-*Lol* I IgG inhibited in a dose-dependent manner the binding of ^{125}I-anti-*Lol* I F(ab′)$_2$ to IgG from the serum of *Lol* I-allergic (No. 1 to No. 7) and *Lol* I-non-allergic (Nos 8–12) individuals (Table 1), whereas anti-TT or myeloma IgG had no or very little effect. It is interesting to note that *Lol* I blocked the binding of anti-*Lol* I F(ab′)$_2$ to aId in six out of 12 (No. 1,4,5,9,10 and 11) sera only, whereas unrelated antigens such as TT or HDM had no effect. Finally, it should be mentioned that the positive sera did not bind to F(ab′)$_2$ fragments of IgG anti-TT or IgG anti-thyroglobulin indicating that the assay was not detecting pepsin agglutinators.

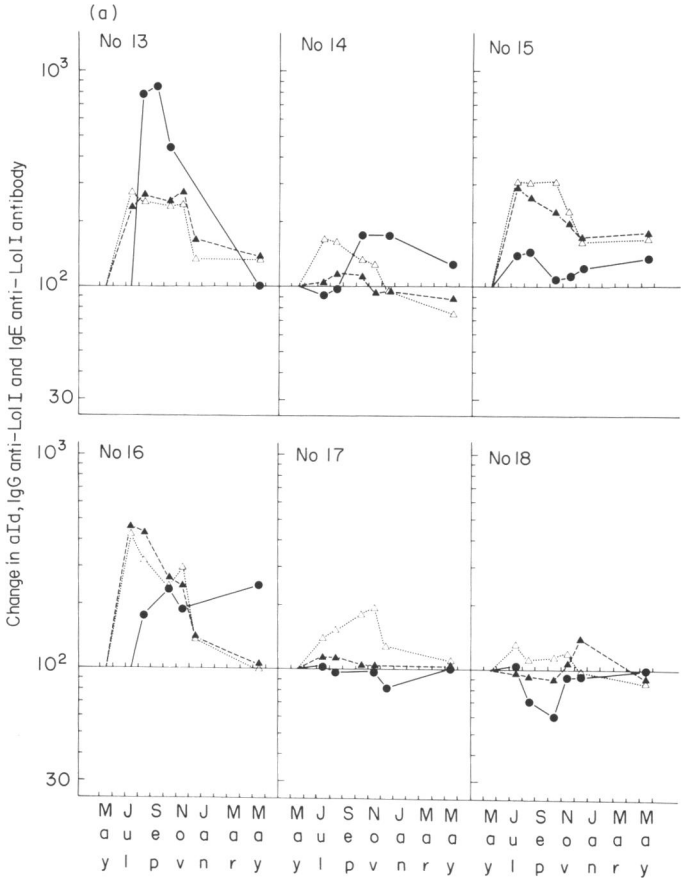
Relationship between the levels of aId and Lol I-specific IgG or IgE antibodies in Lol I-allergic individuals. Sera from 44 *Lol* I-allergic patients were assayed for the presence of aId, anti-*Lol* I IgG and anti-*Lol* I IgE antibodies. As shown in Fig. 1, the levels of aId were not correlated with those of anti-*Lol* I antibodies. Since this study included testing sera collected from allergic subjects at various times during the year, irrespective of their receiving immunotherapy, the following two questions were addressed: (i) is there a seasonal variation in aId level in *Lol* I-allergic and non-allergic individuals; and (ii) does immunotherapy affect the level of aId?

Influence of seasonal exposure to grass pollen on the levels of aId in untreated Lol I-allergic and Lol I-non-allergic individuals. Twelve participants were tested before (early May) and several times after the grass pollen season for their serum levels of aId, as well as IgG and IgE anti-*Lol* I antibodies. Six participants were allergic to Rye grass pollen and were *Lol* I responders (No. 13 to No. 18), two were allergic to Rye grass pollen but were not sensitive to *Lol* I (No. 19 and No. 20), two were atopic but not sensitive to Rye grass (No. 21 and No. 22) and two were normal controls (No 23 and No. 24).

The level of aId in untreated *Lol* I-allergic individuals changed significantly throughout the year ($P = 0{\cdot}04$) (see Fig. 2a). In four out of six cases (No. 13–No. 16) the aId levels (solid lines) rose during or soon after the grass pollen season. One individual (No. 17) did not show any significant change, whereas another (No. 18) showed a reverse pattern, that is a slight drop in aId between mid-July and mid-August which stayed essentially unchanged until mid-October. As expected, the levels of IgG and IgE anti-*Lol* I antibodies increased following pollen exposure in most of the patients; but, notably, subjects No. 17 and No. 18 exhibited little change in either IgG or IgE antibodies across the grass pollen season.

The correlation between the aId and the anti-*Lol* I profile as a function of seasonal variation in untreated allergic patients was tested by regression analysis using the slope values of every two adjacent bleedings. A significant negative correlation with auto-aId was observed in two out of six cases (No. 13 and No. 16) for the IgG and four out of six cases (No. 13, 14, 16 and No. 17) for the IgE anti-*Lol* I antibodies. However, when the whole group of six patients was analysed by non-parametric's test, there was no significant correlation. In the *Lol* I-non-allergic subjects (Fig. 2b), no distinct patterns were discernable and statistical analysis revealed no significant overall changes throughout the year.

Anti-idiotype in patients treated by immunotherapy. Ten patients were tested before and three times after initiation of hyposensitization with either native or formaldehyde-treated mixed grass pollen extracts. The results (Fig. 3) indicated that immunotherapy influenced not only the levels of IgG and IgE anti-*Lol* I, as expected, but also the levels of aId ($P = 0{\cdot}03$). In the whole group of patients, the inverse relationship between the changes in aId and anti-*Lol* I antibodies was significant at $P = 0{\cdot}05$. In five cases (No. 25 to No. 29) (Fig. 3a) there was an initial drop of aId levels contrasting with a rise of IgG anti-*Lol* I; the aId then rose to (or above) the initial values whereas the IgG anti-*Lol* I either decreased or reached a plateau. Three patients (No. 30, 31 and No. 32) displayed a reverse pattern (Fig. 3b), i.e. there was an initial elevation of aId levels followed by a return to or below the initial values; again the IgG anti-*Lol* I (and to some extent the IgE anti-*Lol* I) changed in opposite direction to that of the aId. In one patient (No. 34, Fig. 3c) there was no change



in aId levels, although the IgG and IgE anti-*Lol* I significantly increased. In case No. 33, the initial rise of IgG and IgE anti-*Lol* I was not associated with a modification of the aId levels, whereas the drop in IgG anti-*Lol* I between May and July was parallel to the change of aId.

DISCUSSION

By convention, aId was defined in the present study as IgG antibodies binding to $F(ab')_2$ fragments of affinity purified anti-*Lol* I antibodies. The same definition was employed in earlier studies documenting the presence of aId specific to anti-TT antibodies in the serum of TT-immunized individuals (Geha, 1982). This assay detects a variety of aId reacting with public idiotype determinants; indeed the $F(ab')_2$ fragments are polyclonal and are derived from one single allergic patient. There are two major difficulties to demonstrate the specificity of such an assay: first, the IgG binding to $F(ab')_2$ anti-*Lol* I might well react with the same idiotypic determinants expressed on antibodies of unrelated specificities, i.e. aId may react with parallel sets of idiotypes (Oudin & Cazenave, 1971). Hence, it is impossible to conclude that the aId detected in the present study are exclusively reacting with anti-*Lol* I antibodies. The second difficulty is due to the fact that only some aId are paratope induced and inhibitable by the homologous antigen. With these restrictions in mind the specificity of the present assay was documented by showing that the binding of aId could be inhibited by unlabelled anti-*Lol* I antibody but not by an unrelated affinity purified antibody (anti-TT) or human myeloma IgG. Indeed, such inhibition was observed in 12 consecutive sera

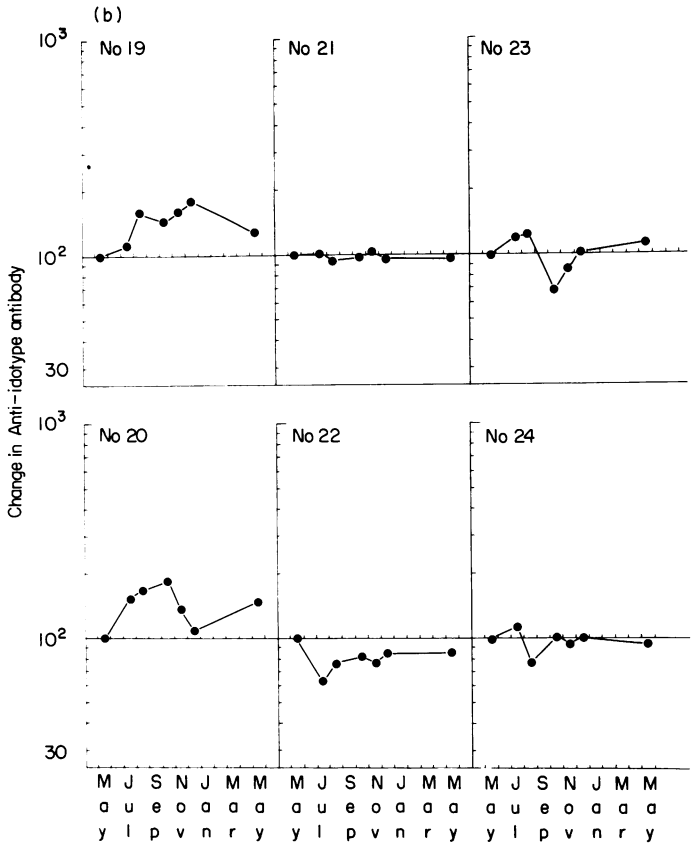


Fig. 2.a. Percent change of aId (●) IgG anti-*Lol* I (▲) and IgE anti-*Lol* I (△) antibodies during a 1-year period in untreated *Lol* I-allergic subjects. Results are expressed in percent of the initial value considered to be 100%. Values of the first bleeding for the six patients (No. 13–No. 18) are given in the following order: aId (% ^{125}I-anti-*Lol* I IgG F(ab′)$_2$ bound), IgG anti-*Lol* I (ng/ml) and IgE anti-*Lol* I (ng/ml). No. 13: 1·0, 96, 27; No. 14: 9·3, 59, 19; No. 15: 10·6, 175, 19·1; No. 16: 6·5, 49, 2·75; No. 17: 17·3, 72, 15; No. 18: 17·2, 338, 124. The grass pollen season in Baltimore (the home of the study patients) is from mid-May through the end of June. **b.** Percent change of aId (●) antibodies during 1-year period in *Lol* I non-allergic individuals. Values are given for two patients allergic to Rye grass pollen but not sensitive to *Lol* I (No. 19 and No. 20), two patients allergic to ragweed, cats and HDM (No. 21 and No. 22) and two non-allergic individuals (No. 23 and No. 24). Values for all the patients are given as percent change from the value of the first bleeding which is considered as 100%. Values for the level of aId of the first bleeding for these six subjects are given as % ^{125}I-anti-*Lol* I IgG F(ab′)$_2$ bound. No. 19: 9·0; No. 20: 9·7; No. 21: 21·8; No. 22: 20·7; No. 23: 14·7 and No. 24: 16·8.

derived both from allergic (No. 1 to No. 7) and non-allergic (No. 8 to No. 12) individuals. Moreover, small but consistant inhibition of binding of aId to anti-*Lol* I F(ab′)$_2$ by *Lol* I was also detected in some sera. In the latter case, the inhibition with *Lol* I was rather small but was repeatedly detected in three consecutive assays and, in addition, no inhibition was detected by unrelated antigens. These data suggest that at least some sera contain paratope-induced aId. Such aId were recently reported in the serum of one allergic individual with a previous history of hyposensitization therapy (Bose *et al.*, 1984).

The aim of the present study was to analyse the influence of pollen exposure on the levels of aId and to correlate the change of aId to those of anti-*Lol* I antibodies. Two conclusions emerged from this study, (i) aid to anti-*Lol* I antibodies were detected not only in *Lol* I allergic, but also in *Lol* I non-allergic individuals, and (ii) in *Lol* I allergic patients the levels of aId were influenced either by

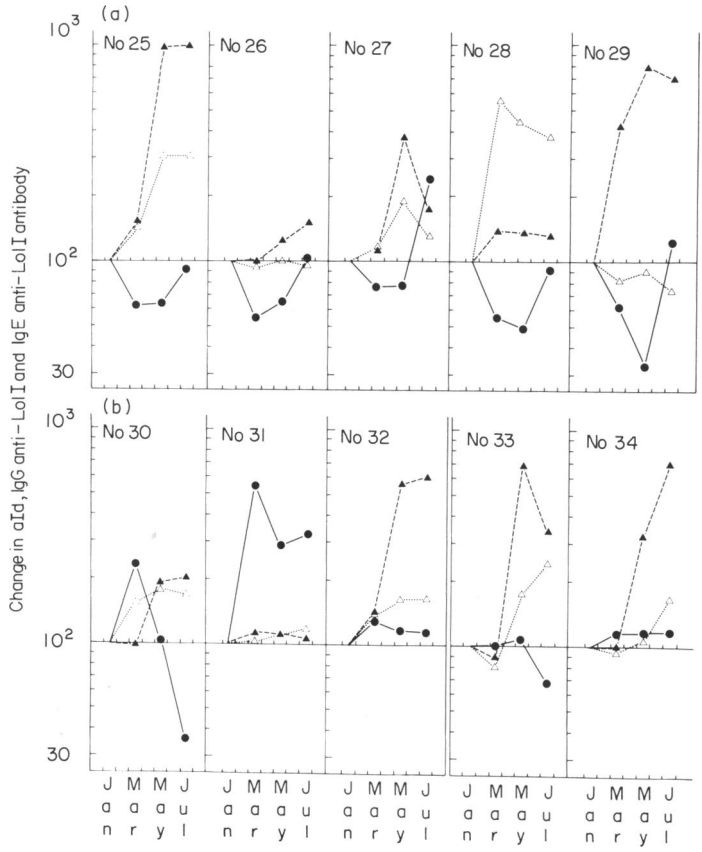


Fig. 3. Percent change of aId (●), IgG anti-*Lol* I (▲) and IgE anti-*Lol* I (△) antibodies in Rye grass allergic patients treated wth Rye grass immunotherapy. The results in the 10 patients are shown in three subgroups (A, B and C). Values for all the patients are given as a percent change from the value of the first bleeding (pretreatment) which is considered as 100%. Values of the first bleeding for these 10 patients are given in the following order: aId (% ^{125}I-anti-*Lol* I IgG F(ab′)$_2$ bound), IgG anti-*Lol* I (ng/ml) and IgE anti-*Lol* I ct/min of ^{125}I-*Lol* I bound). No. 25: 23·3, 171, 84; No. 26: 19·1, 235, 1500; No. 27: 10·4, 201, 1875; No. 28: 11·8, 155; No. 29: 6·3, 390, 327; No. 30: 2·5, 719, 920; No. 31: 0·9, 268, 608; No. 32: 5·9, 36, 235; No. 33: 11·6, 1290, 699; No. 34: 9·9, 66, 532. The pretreatment bleeding is January, mid-treatment bleeding is March and the two post-treatment bleedings are May and July.

exposure to pollen, or by immunotherapy and in some cases there was an inverse relationship between changes in serum levels of aId and of IgG/IgE anti-*Lol* I antibodies.

The finding of aId in *Lol* I non-allergic individuals was unexpected and merits some comments. First, in our preliminary assays the normal sera were tested after the removal of anti-heavy and light-chain activity by adsorption on NHIgG-Sepharose (see detection of aId in Results section). Moreover, the binding of ^{125}I-anti-*Lol* I F(ab′)2 could be abrogated either by preadsorption of the sera on anti-*Lol* I IgG-Sepharose 4B (see preparation of NHS in Methods section) or as mentioned above by competition with unlabelled affinity purified anti-*Lol* I IgG antibodies. It can be speculated that the aId detected in non-allergic individuals are directed to unrelated antibodies sharing idiotypic determinants with anti-*Lol* I antibodies. Indeed, the non-allergic individuals had no detectable IgG or IgE anti-*Lol* I antibodies. However, it should be reiterated that all the sera were preadsorbed on NHS-Sepharose 4B before being tested for the presence of aId; hence, it is possible that after such adsorption the aId reacting with the parallel sets of idiotype had been removed. Alternatively, the presence of aId in these non-allergic individuals may be associated with a suppressor mechanism which prevents the development of an antibody response to *Lol* I. In

keeping with this view, it was suggested that the major difference between the allergic and non-allergic individuals is not that the former produce IgE antibodies to airborne allergens but rather that the latter produce neither IgG nor IgE antibodies to the same allergens (Platts-Mills, 1979).

A possible role of aId in the regulation of anti-*Lol* I antibodies, and/or vice-versa, is suggested by the longitudinal study of untreated allergic individuals. In four out of six cases, aId levels rose during or soon after the pollen season and then returned to preseasonal values. Most interestingly, the changes of aId levels were negatively correlated with those of IgE or IgG anti-*Lol* I antibodies in four and two sera, respectively. A similar inverse relationship between the changes of Id and aId levels has been reported in longitudinal studies of patients with auto-immune diseases such as myasthenia gravis (Dwyer *et al.*, 1984) or systemic lupus erythematosus (Abdou *et al.*, 1981). In these conditions, aId tended to be higher in remission than in the evolutive phases of the disease. Another example documenting the inverse association between aId and Id was provided by Geha (1982) who showed that after immunization of normal individuals with tetanus toxoid, aId appeared when the levels of the corresponding idiotype decreased. Also, in the T-15/phosphoryl-choline system in BALB/c mice the periodic expansion of an antigen-activated idiotypic clone associated with a reciprocal expansion and diminution of cell-associated, anti-idiotypic activity has been shown (Kelsoe & Cerny, 1979).

Although the exact mode of action of hyposensitization therapy remains to be established, it is known that immunotherapy induces both an elevation of serum IgG antibody and inhibits the post-seasonal rise of IgE antibody levels (Gleich *et al.*, 1982). The present results not only confirm these findings but further indicate that in eight out of 10 cases treated by immunotherapy, the time-course profiles of auto-aId and IgG/IgE anti-*Lol* I antibodies are negatively correlated ($P = 0{\cdot}05$). Among these 10 individuals, five displayed an initial drop in aId level followed by a rise or plateau, whereas the idiotype level first increased and then either decreased or stayed unchanged (Fig. 3a). Three individuals (Fig. 3b) had a reverse pattern, i.e. the aId level rose initially and then returned to (or below) the initial level; again, the idiotype changed in a reverse direction to that of the aId. Taken collectively, these data suggest that immunotherapy has modified the equilibrium between Id and aId interactions, but due to the small number of cases no other conclusion can be drawn at this stage.

There are several obstacles in the investigation of the network theory of antibody regulation in man when compared to inbred strains of rodents. The major one is the extreme heterogeneity of the genetic background and thus probably also of the idiotypic repertoire. The possibility of producing human monoclonal antibody specific to well-defined antigens should be exploited for a more accurate but more restricted evaluation of the role played by Id-aId interactions in the control of antibody production by allergic patients. With the above restrictions in mind, the data suggest that Id-aId interactions play a role in the regulation of antibody response to airborne allergens and that these interactions might be manipulated by immunotherapy.

The excellent secretarial assistance of Mrs J. Gilmour and Mrs L. Van Kooten and excellent technical help of Mrs C. Fonteyn and Eva Kautzky is gratefully acknowledged. We thank Mrs Mary Cheang for providing us the statistical help. We wish to thank the employees and management of the Westinghouse Electric Corp. for their participation in this study. Finally, we wish to thank Dr Phillip S. Norman for providing the sera from treated patients. This work was supported by NIH Grant No. AI 19727 to Dr D. G. Marsh.

REFERENCES

ABDOU, N.I., WALL, H., LINDSLEY, H.B., HALSEY, J.F. & SUZUKI, T. (1981) Network theory in autoimmunity. In vitro suppression of serum anti-DNA antibody binding to DNA by anti-idiotypic antibody in systemic lupus erythematosus. *J. clin. Invest.* **67,** 1297.

AXEN, R., PORATH, J. & ERNBACK, S.S. (1967) Chemical coupling of peptides and proteins to polysaccharides by means of cyanogen halides. *Nature* **214,** 1302.

BANKERT, R.B. & PRESSMAN, D. (1976) Receptor-blocking factor present in immune serum resembling auto-anti-idiotype antibody. *J. Immunol.* **117,** 457.

BLASER, K. & DE WECK, A.L. (1982) Regulation of the IgE antibody response by idiotype anti-idiotype network. *Prog. Allergy*, **32,** 203.

BOSE, R., MARSH, D.G., DUCHATEAU, J., SEHON, A.H. & DELESPESSE, G. (1984) Demonstration of auto-anti-idiotypic antibody cross-reacting with public idiotypic determinants in the serum of Rye-sensitive allergic patients. *J. Immunol.* **135,** 2474.

CUNNINGHAM-RUNDLES, C. (1981) Demonstration of naturally-occurring autologous anti-idiotypic antibodies in human sera: participation in immune complex formations in selective IgA deficiency. *Clin. Res.* **29,** 375A.

DWYER, D.S., BRADLEY, R.J., URQUHART, C.K. & KEARNEY, J.F. (1983) Naturally occurring anti-idiotypic antibodies in myasthenia gravis patients. *Nature (London)* **301,** 611.

DWYER, D.S., BRADLEY, R.J., OH, S.J. & KEARNEY, J.F. (1984) Idiotypes in myasthenia gravis. In: *Idiotype in Biology and Medicine.* (ed. by H. Köhler, J. Urbain & P. A. Cazenave) p. 347. Academic Press, New York.

FORNI, L., COUTINHO, A., KOHLER, C. & JERNE, N.K. (1980) IgM antibodies induce the production of antibodies of the same specificity. *Proc. natn Acad. Sci. USA.* **77,** 1125.

FREIDHOFF, L.R., MEYERS, D.A., BIAS, L.B., CHASE, G.A., HUSSAIN, R. & MARSH, D.G. (1981) A genetic-epidemiologic study of human immune responsiveness to allergens in an industrial population. I. Epidemiology of reported allergy and skin-test positivity. *Amer. J. med. Genet.* **72,** 274.

GEHA, R.S. (1982) Presence of auto-anti-idiotypic antibody during the normal human immune response to tetanus toxoid antigen. *J. Immunol.* **129,** 139.

GELTNER, D., FRANKLIN, E.C. & FRANGIONE, B. (1980) Anti-idiotypic activity in the IgM fraction of mixed cryoglobulins. *J. Immunol.* **125,** 1530.

GLEICH, G.J., ZIMMERMAN, E.M., HENDERSON L.L. & YUNGINGER, J.W. (1982) Effect of immunotherapy on immunoglobulin E and immunoglobulin G antibodies to ragweed antigens: a six-year prospective study. *J. Allergy clin. Immunol.* **70,** 261.

JERNE, N.K. (1974) Towards a network theory of the immune system. *Ann. Immunol. (Paris)* **125,** 373.

KELSOE, G. & CERNY, J. (1979) Reciprocal expansions of idiotypic and anti-idiotypic clones following antigen stimulation. *Nature* **279,** 333.

KLINMAN, N.R. & TAYLOR, R.B. (1969) General methods for the study of cells and serum during the immune response: the response to dinitrophenyl in mice. *Clin. exp. Immunol.* **4,** 473.

MARSH, D.G. (1975) Allergens and the genetics of allergy. In: *The Antigens*, Vol. III, (ed. by M. Sela) p. 271. Academic Press, New York.

MARSH, D.G., HSU, S.H., ROEBBER, M., KAUTZKY, E.E., FREIDHOF, L.R., MEYERS, D.A., POLLARD, M.K. & BIAS, W.B. (1982) HLA-DW2: a genetic marker for human immune response to short ragweed pollen allergen Ra5. I. Response resulting primarily from natural antigenic exposure. *J. exp. Med.* **155,** 1439.

MARSH, D.G., MILNER, F.H. & JOHNSON, P. (1966) The allergenic activity and stability of purified allergens from the pollen of common Rye grass (*Lolium perenne*). *Int. Arch, Allergy appl. Immunol.* **29,** 521.

NISONOFF, A. & GREENE, M.I. (1980) Regulation through idiotypic determinants of the immune response to the p-Azophenyl arsonate hapten in strain A mice. In: *Immunology* 80. (ed. by M. Fougereau & J. Dausset) p. 57. Academic Press, London.

OUDIN, J. & CAZENAVE, P.A. (1971) Similar idiotypic specificities in immunoglobulin fractions with different functions or even without detectable antibody function. *Proc. Natn Acad. Sci. USA.* **68,** 2616.

PLATTS-MILLS, T.A.E. (1979) Local production of IgG, IgA and IgE antibodies in grass pollen hay fever. *J. Immunol.* **122,** 2218.

RETH, M., KELSOE, G. & RAJEWSKY, K. (1981) Idiotypic regulation by isologous monoclonal anti-idiotope antibodies. *Nature* **290,** 257.

SAXON, A. & BARNETT, E (1984) Human auto-anti-idiotypes regulating T cell-mediated reactivity to tetanus toxoid. *J. clin. Invest.* **73,** 342.

SCHRATER, A.F., GOIDL, E.A., THORBECKE, G.J. & SISKIND, G.W. (1979) Production of auto-anti-idiotypic antibody during the normal immune response to TNP-Ficoll. I. Occurrence of AKR/J and Balb/c mice to hapten-augmentable, anti-TNP plaque-forming cells and their accelerated appearance in recipients of immune spleen cells. *J. exp. Med.* **150,** 138.