

Identification of anti-idiotypic antibodies in the sera of ryegrass-allergic and nonallergic individuals

David J. Valacer, MD, Mary Ellen O'Reilly, BA, Norman T. Ilowite, MD, and Vincent R. Bonagura, MD *New Hyde Park, N.Y.*

Anti-idiotypic (Id) antibodies (Abs) are generated in the humoral response to antigen (Ag). Some of these anti-Id Abs are capable of binding to the combining site or paratope of Abs that bind Ag. These Ab2 can competitively inhibit the binding of Ab1 to Ag and may trigger an Ab1 response similar to the response induced by Ag. To determine if specific Ab2 that inhibit the binding of IgE Abs to ryegrass (RG)-pollen allergens are present in the sera of RG-allergic (RGA) individuals before the initiation of allergen-specific hyposensitization with RG-pollen extract, we studied sera from five RGA and four nonallergic (NA) subjects in an IgE anti-RG RAST-inhibition assay. Ab2-enriched serum fractions were prepared from these study subjects by exhaustive mixed grass-pollen affinity chromatography to remove IgE and IgG anti-RG Ab1 from whole serum aliquots containing Ab1. Unabsorbed, twice-concentrated sera were diluted to one-time concentrated sera with equal volumes of either Ab2-enriched sera without Ab1 or borate-buffered saline absorbed by mixed grass-pollen affinity chromatography. IgE anti-RG Ab1 was determined by a standard RG RAST assay. We have detected Ab2 in the sera of the RGA patients, which inhibit the binding of autologous and allogeneic IgE anti-RG to RG Ags in solid phase. Parallel RG RAST assays with sera from NA subjects demonstrated no significant inhibition. Ab2-enriched sera from some grass-allergic and some NA subjects inhibited IgE anti-RG binding found in some RGA patients' sera. We conclude that anti-Id Abs, Ab2, specific for IgE anti-RG, Ab1, are present in some RGA patients and NA individuals. (J ALLERGY CLIN IMMUNOL 1991;88:349-55.)

Key words: Anti-idiotypic antibodies, idiotypic network, ryegrass allergy

The network of interacting idiotypes, originally hypothesized by Jerne,¹ has been implicated in the modulation of both humoral and cell-mediated immune responses in disease. This modulation has been described in the immune response to a variety of Ags.² Competitive inhibition of Ag-specific Abs (Ab1) by anti-Id Abs (Ab2) that complex with epitopes inside, or closely associated with the paratope of Ab1, have been demonstrated.³ Anti-Id Abs can also bind directly to Id determinants on immunoglobulins on the surface

Abbreviations used

Id:	Idiotypic
Ag:	Antigen
Ab:	Antibody
Ab1:	Ag-specific Ab
Ab2:	Anti-Id Ab
RW:	Ragweed
RG:	Ryegrass
GA:	Grass allergic
RGA:	Ryegrass allergic
ASH:	Allergen-specific hyposensitization
BBS:	Borate-buffered saline
MGP:	Mixed-grass pollen
SEPH:	Sephacrose
WS:	Whole serum
2XWS:	Twice concentrated WS
2XAS:	Twice absorbed serum
2XBBS:	Twice absorbed BBS
PBS:	Phosphate-buffered saline
NA:	Nonallergic
PAGE:	Polyacrylamide gel electrophoresis

From the Division of Pediatric Allergy and Immunology, Schneider Children's Hospital, Long Island Jewish Medical Center, New Hyde Park, N.Y.

Received for publication July 31, 1990.

Revised Jan. 22, 1991.

Accepted for publication April 8, 1991.

Reprint requests: David J. Valacer, MD, Division of Pediatric Allergy and Immunology, Schneider Children's Hospital, Room 235, Long Island Jewish Medical Center, 269-01 76th Ave., New Hyde Park, NY 11042.

1/1/30102

of B cells and thereby regulate specific B cell expression.² In addition, anti-Id Abs specific for clonotypic determinant(s) of the T cell receptor have been detected.⁴ These Abs have been demonstrated to interrupt the network of anti-idiotypes that are generated in the normal immune response in certain disease states.^{5,6}

Contrary to Dr. Jerne's original hypothesis, the Id network does not appear to be open ended.² Although the Ab1 response to foreign Ags is characterized by the production of several hundred different Ag-specific Ab molecules, the Ab2 response to Ab1 appears to be much more restricted.² This restriction may be secondary to shared amino acid sequences, idiotopes, within, or closely associated with, the Ab-combining sites of different Ab1s that bind Ag.⁷ This finding would explain cross-reactivity between several Ab1s that is frequently observed in the anti-Id response to Ag.⁸

Although many investigations of the Id network have focused on IgG Abs, human IgE Abs, which may share Id determinants with IgG Abs in both animals and humans, have been infrequently studied.⁸⁻¹² Anti-Id Abs have been demonstrated to inhibit synthesis of Ag-specific IgE as well as IgG in human B cell cultures.¹⁰ Because repetitive exposure to Ag-Ab complexes predisposes to the production of Ab2,² induction of Ab2 formation during routine ASH might be expected. In fact, anti-Id IgG Abs to IgE and IgG anti-RG I Ab1 have been detected in the serum of one RGA patient who had received RG ASH.⁸ Similarly, anti-Id Abs, capable of binding RW Ag E-specific Ab1, have been isolated from the plasmapheresed serum of one RW-allergic patient with "high-titer anti-RW IgG and IgE" after 5 years of ASH.¹² Circulating human anti-Id Abs have also been demonstrated to bind murine monoclonal Abs of the gamma class specific for RG I allergens.¹³ Higher levels of anti-Id Abs capable of binding these allergen-specific murine monoclonal antibodies were detected in the sera of nonallergic control subjects and ASH-treated allergic patients than in the sera of allergic patients before ASH therapy.¹³ Thus, the Ab2 generated in response to pollen-specific Ab1 of both the epsilon and gamma isotypes has been detected in the sera of ASH-treated allergic patients.¹¹⁻¹³ However, the annual seasonal pollen exposure of allergic individuals results in a postseasonal rise in allergen-specific IgE that may also induce formation of an Ab2 response even before ASH. The purpose of this study was to determine if anti-Id Abs specific for polyclonal anti-RG IgE were present in the sera of RGA patients before ASH therapy and to determine whether such anti-Id Abs bind allogeneic human RG-specific IgE.

MATERIAL AND METHODS

Patients

Serum specimens from RGA patients were obtained from the Clinical Allergy/Immunology Laboratory of Schneider Children's Hospital in which they were stored at -70°C before use. Patients were selected on the bases of their clinical history and positive skin test and/or RAST to RG pollen. Five RGA patients (two male and three female patients, aged 27 to 42 years; mean, 32.5 years) were studied. None had received ASH within the previous 7 years. The five control subjects (two male and three female subjects, aged 32 to 45 years; mean, 37.5 years) did not have grass-pollen allergy by history or by specific IgE anti-RG RAST. Serum specimens with IgG anti-epsilon Ab from patients with the hyper-IgE syndrome (the generous gift of Dr. Rebecca Buckley) and patients with chronic urticaria and elevated total serum IgE levels were used for positive control subjects in an IgG anti-epsilon RAST assay. These sera were stored at -70°C before use.

Immunosorbents

Thirty milligrams of MGP protein (Center Laboratories, Port Washington, N.Y.) was exhaustively dialyzed into 0.15 mol/L of NaCl, pH 6.8, and then coupled to 5 gm of cyanogen bromide-activated SEPH 4B beads (Pharmacia Fine Chemicals, Uppsala, Sweden) by standard methods.¹⁴ The MGP-SEPH beads were packed in 1.5 by 20 cm columns (Bio Rad Laboratories, Richmond, Calif.) and washed extensively with BBS, pH 8.3.

Serum preparations

A 2 ml aliquot of WS from each subject was concentrated to 1 ml (2XWS) by vacuum extraction in BBS with a UH100/25 nitrocellulose collodion bag, molecular weight exclusion 25,000 daltons (Schleicher & Schuell Inc., Keene, N.H.). These 2XWS aliquots, diluted with fresh BBS, maintained the same IgE RG RAST activity as the aliquots of WS from which they were prepared (data not presented). A second 2 ml aliquot of serum was dialyzed into BBS and cycled over the MGP-SEPH column for 24 hours at 25°C . The absorbed serum fraction that did not bind to the MGP-SEPH was collected from the column in BBS. The nonadherent serum fraction from the first cycling was again cycled over the MGP-SEPH column in BBS for 24 hours at 25°C to ensure a complete removal of RG-specific IgE and IgG Ab (Ab1). The serum fraction that did not bind to MGP-SEPH after the second cycling was concentrated to 1 ml by vacuum extraction as described above and is hereafter referred to as 2XAS. This 2XAS was free of RG-specific Ab1, as demonstrated by IgE and IgG RG RAST (described below). These absorbed serum fractions are hereafter referred to as Ab2 enriched.

To document that MGP protein was not being shed from MGP-SEPH during cycling of serum aliquots, a volume of BBS equivalent to the average final volume of a serum specimen after two passes over a MGP-SEPH column was concentrated to 1 ml as described above (2XBBS). This 2XBBS contained no detectable protein bands in 7% poly-

acrylamide gel electrophoresis (PAGE) (data not presented). This reagent was used as a control in the RAST assays described below.

Serum incubations

Equal volumes of 2XWS and 2XAS were mixed and incubated at -20°C for 16 hours in microcentrifuge tubes and then centrifuged at 1260 g for 20 minutes. Supernatants were removed, and RG-specific IgE was measured by RAST as described below. Equal volumes of 2XWS and 2XBBS were mixed, incubated, and centrifuged in parallel, as stated above, and these supernatants were tested as controls in the RG-specific IgE RAST described below.

RAST for RG-specific IgG and IgE Abs

IgE Abs to RG were measured by the modified, double, overnight RAST procedure¹⁵ with the Phadebas RAST kit with *Lolium perenne* G5 Phadebas RAST disks as the solid-phase RG allergen (Pharmacia Diagnostics AB, Uppsala, Sweden). Briefly, 100 μl of supernatant from each 2XWS-2XAS or 2XWS-2XBBS serum incubation was added to a 12 by 75 mm polystyrene-capped tube containing a single RG allergen-coated disk. After a 16 hour incubation at 25°C , tubes were washed three times with 2.5 ml of RAST kit washing solution. Fifty microliters of RAST kit anti-IgE-¹²⁵I solution was added to each tube. After 16 hours at 25°C , tubes were washed again three times, and the total bound ¹²⁵I counts were measured. Counting time was determined by a parallel IgE PRIST standard assay that used a 25 kU/L Phadebas IgE standard and anti-IgE disks, as previously described.¹⁴ RAST activity of each supernatant was assayed in triplicate, and the results are expressed as mean total ¹²⁵I counts.

IgG Abs to RG pollen were measured with a Pharmacia IgG RAST kit and 12 by 75 *L. perenne*-coated Phadebas Allertubes (Pharmacia Diagnostics AB). Serum samples were diluted 1:50 with IgG RAST kit sample diluent, and 100 μl of each sample was added to capped tubes in triplicate. After 3 hours at 25°C , tubes were washed as described above, and 100 μl of RAST kit anti-IgG-¹²⁵I Ab solution was added to each tube for 16 hours at 25°C . Tubes were washed three times as described above, and the total bound ¹²⁵I counts were measured for 60 seconds for each tube. Results of triplicate IgG RAST assays are expressed as mean total ¹²⁵I counts bound.

IgG antiepsilon RAST

To determine if IgG Abs capable of binding epsilon were present in patients' sera, as previously described,¹⁶ we tested all sera in an antiepsilon RAST. Human myeloma IgE was purified from PS myeloma serum (the generous gift of Dr. Shaul Kochwa), as previously described.¹⁷ Purity was established by 7% PAGE, Ouchterlony analysis, and standard nephelometric analysis for immunoglobulin isotypes other than IgE. Myeloma IgE was diluted in 0.01 mol/L of PBS, pH 7.2, to 10 $\mu\text{g}/\text{ml}$. Flat-bottom wells of Immulon 3 Removawell strip microtiter plates (Dynatech Laboratories, Chantilly, Va.) were coated with myeloma IgE by the ad-

dition of 100 μl of myeloma IgE (10 $\mu\text{g}/\text{ml}$) for 16 hours at 4°C . IgE was aspirated, and the remaining binding sites were blocked by the addition of 250 μl of 0.1% gelatin in PBS to each well for 2 hours at 25°C . The gelatin solution was aspirated, and wells were washed four times with PBS containing 0.05% Tween (PBS-Tween). Wells without myeloma IgE coating were also blocked with gelatin and run in parallel to measure nonspecific background binding. One hundred microliters of serum diluted 1:10 in PBS was added to each well. After 16 hours at 37°C , diluted sera were aspirated, wells were washed three times with PBS-Tween, and 100 μl of ¹²⁵I-labeled rabbit antihuman IgG (Pharmacia Diagnostics AB), diluted 1:10 in PBS-Tween, was added to each well. After 1 hour at 37°C , ¹²⁵I-labeled Abs were aspirated, and the wells were washed five times with PBS-Tween. Wells were separated and placed in individual 12 by 75 mm borosilicate glass tubes. The ¹²⁵I counts bound were measured for 60 seconds. Serum specimens were assayed in quadruplicate, and the results for each serum specimen are presented as the mean total counts bound to IgE-coated wells minus the mean total counts bound to gelatin-blocked wells without myeloma IgE.

Statistical methods

Mean total ¹²⁵I counts bound in the RG RAST assay from aliquots of 2XWS mixed with 2XBBS were compared to mean total ¹²⁵I counts bound from aliquots of 2XWS absorbed with 2XAS for sera from both RGA and NA patients with the two-tailed Student's paired *t* test. Mean total ¹²⁵I counts bound in the antiepsilon RAST assay for sera from RGA and NA patients were compared to mean total ¹²⁵I counts bound obtained for sera from patients with IgG antiepsilon Abs by the one-tailed Wilcoxon's Rank-sum test.

RESULTS

Demonstration of autologous anti-Id Ab

Serum aliquots absorbed twice over an MGP-SEPH affinity column were concentrated to one half original volume (2XAS). Serum had no significant RG-specific Ab1 detected by IgE RAST assays with *L. perenne* G5 Phadebas RAST disks or in IgG RAST assays with *L. perenne*-coated tubes (data not presented). The presence of anti-Id Ab in serum from patients and control subjects was determined by the ability of 2XAS to prevent binding of autologous RG-specific IgE (Ab1) to *L. perenne* G5 RAST disks. In Table I are presented the mean percent total ¹²⁵I counts bound, obtained from aliquots of each study subject's 2XWS absorbed with their 2XAS (Ab2 enriched) serum fraction and their 2XWS absorbed with 2XBBS. For the five RGA-subjects studied, the RG-specific IgE binding was reduced by 17.1% to 44.9% (two-tailed Student's paired *t* test, $p < 0.005$). Of the sera from four NA subjects studied, no significant change in mean percent total counts bound (two-tailed Student's paired *t* test, $p < 0.4546$) occurred when 2XWS was preincubated with 2XAS.

TABLE I. Inhibition of IgE anti-RG binding by absorption of 2×WS plus 2×BBS aliquots with Ab2-enriched autologous sera

	% Binding of total ¹²⁵ I counts added*		
	2×WS + 2×BBS†	Ab2-enriched serum added†	% Chg
RGA Pts			
S. B.	15.8 ± 1.3	8.7 ± 0.4‡	-44.9
J. S.	22.5 ± 0.2	13.4 ± 0.2‡	-40.4
C. L.	26.6 ± 1.3	17.7 ± 0.9‡	-33.5
D. V.	21.7 ± 1.1	18.0 ± 0.8‡	-17.1
B. E.	18.7 ± 0.2	14.9 ± 0.5‡	-20.3
NA Pts			
L. H.	0.6 ± 0.05	0.6 ± 0.01§	0
P. K.	0.5 ± 0.03	0.5 ± 0.04§	0
Z. S.	0.4 ± 0.02	0.3 ± 0.02§	-0.1
J. W.	0.4 ± 0.05	0.3 ± 0.03§	-0.1

Pts, Patients; Chg, change.

*Percent ¹²⁵I-labeled anti-IgE bound = $\frac{\text{Mean total counts bound/sample}}{\text{Mean total counts added/sample}} \times 100$

†Mean ± SD for assays performed in triplicate.

‡Student's two-tailed paired *t* test, *p* = 0.005; statistical comparison made between ws **only** and Ab2-enriched serum **added**.

§Student's two-tailed paired *t* test, *p* = 0.182; statistical comparison made between ws **only** and Ab2-enriched serum **added**.

Inhibition of RG RAST activity by autologous or allogeneic Ab2-enriched sera

In Table II are the mean total bound ¹²⁵I counts obtained in the RG IgE RAST from aliquots of sera from four RGA subjects. Their 2XWS absorbed with autologous or allogeneic 2XAS Ab2-enriched fraction is compared to their 2XWS absorbed with 2XBBS. Cross-absorptions of the sera from the four RGA subjects with 2XAS from the sera of the remaining three RGA subjects inhibited specific IgE binding to the extent of 15% or more in eight of 12 instances (Table II). The maximum percent inhibition observed was 41%. All four RGA subjects' 2XAS inhibited by >15% the RG-specific IgE binding of at least one other RGA subject's serum, although only S. B.'s serum was capable of inhibiting the RG-specific IgE binding of all other GA patients' sera studied (Table II).

The percent binding of total ¹²⁵I counts added in the RG IgE RAST, when aliquots of each GA subject's 2XWS were absorbed with allogeneic NA subject's 2XAS, Ab2 enriched, fraction compared to their 2XWS absorbed with 2XBBS, is presented in Table III. There were 16 cross-absorption experiments performed with the same four GA subjects' 2XWS presented in Table II and the 2XAS, Ab2 enriched, serum fractions from four NA control subjects as absorbants. In four of 16 instances, >15% inhibition of the GA

subjects' specific IgE binding in RG IgE RAST was observed when 2XWS from GA subjects was preincubated with 2XAS from NA subjects (Table III). Fifteen percent inhibition represents that value that is 2 SD more than the mean value of all percent values of sera of NA subjects that were less than the lowest autologous percent-inhibition value of sera of GA subjects obtained. The maximum percent inhibition observed was 30%. The 2XAS of one NA subject, L. H., failed to significantly inhibit the RG IgE binding of any of the four GA patients' sera tested. The 2XAS of another NA study subject, Z. S., inhibited specific IgE binding in three of four experiments by 15% or more.

Detection of IgG anti-epsilon activity

PAGE failed to detect MGP protein being shed from the MGP immunosorbent. Therefore, shed MGP was not responsible for the inhibition of RG-specific IgE binding presented in Tables I to III (data not presented). Because epsilon-specific IgG could competitively inhibit ¹²⁵I-labeled antihuman IgE binding to allergen-bound IgE, causing a decrease in the total ¹²⁵I counts bound, and because epsilon-specific IgG in serum specimens would not be completely removed with RG-specific IgE by MGP-SEPH immunoadsorption, all serum specimens were assayed for possible IgG anti-epsilon Ab. In Table IV are the mean total ¹²⁵I

TABLE II. Inhibition of IgE anti-RG binding by absorption of GA patient's serum aliquots with Ab2-enriched, autologous, or allogeneic RGA patients' sera

% Binding of total ¹²⁵ I counts* added for RGA 2XWS† plus 2X Ab2-enriched serum added‡									
Pt	WS§	D. V.§	% Chg	S. B.§	% Chg	J. S.§	% Chg	C. L.§	% Chg
S. B.	15.8 ± 1.3¶	16.9 ± 0.7	+6.9	8.7 ± 0.4	-44.9¶	9.3 ± 0.7	-41.1	12.2 ± 1.3	-22.8
J. S.	22.5 ± 0.2¶	18.2 ± 0.5	-19.1	17.0 ± 0.6	-24.4	13.4 ± 0.2	-40.4¶	19.0 ± 0.2	-15.6
C. L.	26.6 ± 1.3¶	26.9 ± 0.5	+1.1	17.8 ± 0.5	-33.1	15.6 ± 1.6	-41.3	17.7 ± 0.9	-33.5¶
D. V.	21.7 ± 1.1¶	18.0 ± 0.8	-17.1¶	16.8 ± 1.4	-26.6	20.9 ± 1.1	-3.7	23.9 ± 0.7	+10.1

Pt, Patient; Chg, change.

*Percent ¹²⁵I-labeled anti-IgE bound = $\frac{\text{Mean total counts bound/sample}}{\text{Mean total counts added/sample}} \times 100$

†RGA patient's 2XWS made 1XWS, vol/vol, with 2XBBS passed over the MGP-SEPH column.

‡RGA patient's 2XWS made 1XWS, vol/vol, with 2X Ab2-enriched sera.

§Mean ± SD for assays performed in triplicate.

||Percent change = $\frac{\% \text{ }^{125}\text{I counts bound 2XWS} + 2\text{X Ab2-enriched serum} - \% \text{ }^{125}\text{I counts bound 2XWS} + 2\text{XBBS}}{\% \text{ }^{125}\text{I counts bound 2XWS} + 2\text{XBBS}} \times 100$

¶Results repeated from Table I for comparison.

TABLE III. Inhibition of IgE anti-RG binding by absorption of GA patients' sera with Ab2-enriched sera from NA control subjects

% Binding of total ¹²⁵ I counts* added for RGA 2XWS† plus Ab2-enriched 2X serum added‡							
RGA	WS§	Z. S.§	% Chg¶	L. H.§	% Chg	M. R.§	% Chg
S. B.	16.5 ± 0.0	13.5 ± 1.5	-18.2¶	15.4 ± 0.7	-6.7	15.3 ± 0.9	-7.3
J. S.	21.5 ± 2.3	15.1 ± 0.36	-29.8¶	20.3 ± 0.5	-5.6	16.7 ± 2.1	-22.5¶
C. L.	23.3 ± 2.1	16.7 ± 0.04	-28.3¶	21.9 ± 0.3	-6.0	20.4 ± 1.4	-12.4
D. V.	19.4 ± 1.1	17.4 ± 0.3	-10.3	17.8 ± 0.2	-8.2	19.1 ± 0.5	-1.5

Chg, Change.

*Percent ¹²⁵I-labeled anti-IgE bound = $\frac{\text{Mean total counts bound/sample}}{\text{Mean total counts added/sample}} \times 100$

†RGA patients' 2XWS concentrated made 1XWS, vol/vol, with 2XBBS passed over MGP-SEPH column.

‡RGA patients' 2XWS made 1XWS, vol/vol, with Ab2-enriched sera from NA subjects.

§Mean ± SD for assays performed in triplicate.

||Percent change = $\frac{\% \text{ }^{125}\text{I counts bound 2XWS} + 2\text{X Ab2-enriched serum} - \% \text{ }^{125}\text{I counts bound 2XWS} + 2\text{XBBS}}{\% \text{ }^{125}\text{I counts bound 2XWS} + 2\text{XBBS}} \times 100$

¶Samples with 15% or more reduction in mean total ¹²⁵I counts bound. In all other cross absorptions, Ab2-enriched sera from the NA control subjects did not significantly inhibit GA patients' sera RG RAST activity ($p = 0.5859$, two-tailed Wilcoxon's rank-sum test).

counts bound, when each of the sera from GA and NA subjects and known antiepsilon-positive control subjects were studied. Only one NA control subject, P. K., demonstrated epsilon-specific IgG binding comparable to levels attained from sera of positive control subjects (Table IV). Serum from P. K. was not used in the cross-absorption experiments of sera from NA subjects. The sera of the remaining GA and NA subjects failed to reveal significant epsilon-specific IgG binding in this RAST when the sera were

compared to sera from the positive control subjects ($p = 0.002$, one-tailed Wilcoxon's rank-sum test).

DISCUSSION

We have demonstrated the presence of anti-Id Ab to RG-specific IgE Ab in the sera of both NA subjects and grass pollen-allergic patients before ASH therapy. All five of the RGA patients' Ab2-enriched sera had anti-Id Abs capable of inhibiting autologous, spe-

TABLE IV. Occurrence of IgG Abs directed against IgE in sera of GA and NA subjects and control subjects known to have IgG antiepsilon Abs

	Total counts*
RGA Pts	
D. V.	1659 $\pm$ 288
S. B.	1296 $\pm$ 150
J. S.	1947 $\pm$ 353
C. L.	1550 $\pm$ 296
B. E.	1638 $\pm$ 365
NA subjects	
L. H.	1750 $\pm$ 199
M. R.	1865 $\pm$ 271
J. W.	1868 $\pm$ 231
Z. S.	1796 $\pm$ 213
P. K.	2975 $\pm$ 237†
IgG antiepsilon control subjects	
G. M.	4602 $\pm$ 433
P. Z.	4277 $\pm$ 512
N. G.	3438 $\pm$ 674
P. P.	3110 $\pm$ 546
J. M.	3086 $\pm$ 336
P. R.	2942 $\pm$ 722
J. F.	2752 $\pm$ 562
J. S.	2683 $\pm$ 378
A. S.	2619 $\pm$ 194

Pts, Patients.

*Mean total ^{125} I counts bound $\pm$ SD of quadruplicate values minus the mean total of nonspecific background counts bound.†IgG antiepsilon Ab detected in one NA study subject. IgG antiepsilon Ab was not detected in the sera of any other study subject ($p = 0.002$, one-tailed Wilcoxon's rank-sum test).

cific IgE binding in an RG RAST assay. Of the four RGA patients' sera studied in cross-absorption experiments, each patient's sera had anti-Id Abs capable of inhibiting the binding of the specific IgE in some of the other RGA patients' sera. The inhibitory activity of the anti-Id Abs that prevent anti-RG Ab1 from binding RG Ag indicates that these Ab2 bind to Id determinants expressed in, or closely associated with, the Ag-combining site of Ab1. Alternate explanations for the observed Ag-specific RAST inhibition were ruled out by the following experiments: First, the Ab2-enriched sera did not contain free MGP protein Ag that could account for the inhibition in the RG RAST. The absence of shed MGP proteins from the SEPH-MGP immunosorbent and the fact that Ab2-enriched sera reduce RG-specific Ab1 RAST activity only in some cross-absorption experiments rule out free Ag as a cause for the inhibition observed in the IgE anti-RG RAST. Second, the Ab2-enriched sera used did

not contain residual IgG anti-RG Ab1. The absence of IgG RG-specific Ab in Ab2-enriched sera and the selective nature of the inhibition of the Ab2-enriched sera of IgE binding to solid-phase *L. perenne* Ags in cross-absorption experiments rule out IgG Ab1 as a competitive inhibitor in the IgE anti-RG RAST.

Finally, the absence of IgG antiepsilon Abs in the Ab2-enriched sera of the RGA study subjects eliminates this possible cause for significant inhibition of the IgE anti-RG RAST. In addition, the selective nature of the inhibition caused by the Ab2-enriched sera in cross-absorption experiments rules out a nonspecific cause for the RG RAST inhibition we have observed.

Although one of the five RGA patients, C. L., had been treated with ASH therapy 7 years before the study, the remaining patients had never received ASH. Although it is possible that the anti-Id Ab detected in C. L.'s serum was induced by the previous ASH, the generation of anti-Id Ab in the sera of the remaining four RGA patients must have occurred in response to the spontaneous IgG and/or the IgE Ab1 response of these untreated allergic individuals to RG seasonal challenge.

As has been previously described in the anti-Id response to RW Ag E Abs,¹¹ we have also detected anti-Id Abs capable of selectively inhibiting binding of RGA patients' specific IgE to *L. perenne* Ags in the sera of all four NA patients. The ability of the anti-Id Abs in sera of both NA and RGA subjects to inhibit IgE anti-RG-specific Ab1 implies that some NA individuals make Ab2 that may down regulate autologous RG-specific Ab1 expression and thereby prevents clinical symptoms. In addition, these results suggest that the anti-Id Ab response to RG pollen Ags may be derived from germ-line V genes that are expressed in RGA and NA individuals. If this were the case, IgE anti-RG Ab1 may share common idiotopes within their hypervariable regions and express public or cross-reactive idiotypes, as has been demonstrated in the network responses to other Ags.¹²

The role these naturally occurring anti-Id Abs play in the regulation of the humoral response to RG-pollen allergy and whether these anti-Ids change in specificity or concentration during ASH remains to be determined. Anti-Id Abs have been demonstrated to down regulate specific IgE response to tetanus toxoid.⁶ Such regulatory idiotypes would most probably not be mirror images of the Ag and not induce an Ab1 response. However, anti-Id Abs that bear structural mimicry with the original Ag, so-called "internal image anti-Ids" or "network antigens,"³ may behave as surrogate Ags, resulting in an augmented Ab1 response. It is possible that the anti-Id Abs expressed in the GA

individuals function as "network antigens" in vivo, whereas the anti-Ids expressed in NA control subjects are regulatory anti-idiotypes that down regulate the RG-specific IgE response. Future studies designed to identify the effect of anti-Id Abs from RGA and NA subjects on Ag-specific autologous and allogeneic B cells in vitro will determine the role of these anti-idiotypes in the network of idiotypes induced by RG pollen.

REFERENCES

1. Jerne NK. Towards a network theory of the immune system. *Ann Immunol* 1974;125C:373-89.
2. Geha RS. Idiotypic interactions in the treatment of human diseases. *Adv Immunol* 1986;39:255-97.
3. Kohler H, Kiebler-Emmons T, Srinivasan A, et al. Revised immune network concepts. *Clin Immunol Immunopathol* 1989;52:104-16.
4. Bonagura VR, Ma A, McDowell J, Lewison A, King DW, Suciu-Foca N. Anti-clonotypic autoantibodies in pregnancy. *Cell Immunol* 1987;108:356-65.
5. Bonagura VR, Rohowsky-Kochan C, Reed E, et al. Perturbation of the immune network in herpes gestationis. *Human Immunol* 1986;15:211-9.
6. Geha RS. Presence of circulating anti-idiotypic-bearing cells after booster immunization with tetanus toxoid (TT) and inhibition of anti-TT antibody synthesis by auto-anti-idiotypic antibody. *J Immunol* 1983;130:1634-9.
7. Bonilla FA, Bona CA. Idiotypic immunoregulation: interclonal connections based on a special category of idiotopes. In: Reichlin M, Capra JD, eds. *Idiotypes*. Orlando: Academic, 1986:229-47.
8. Bose R, Marsh DG, Duchateau J, Schon AH, Delespesse G. Demonstration of auto-anti-idiotypic antibody cross-reacting with public idiotypic determinants in the serum of rye-sensitive-allergic patients. *J Immunol* 1984;135:2474-8.
9. Mourad W, Pelletier G, Herbert J. Characterization of anti-idiotypic antibodies and their use as probes for determination of shared idiotopes expressed on murine and human IgE anti-rye I antibodies. *Immunology* 1988;63:397-401.
10. Geha RS, Comunale M. Regulation of immunoglobulin E antibody synthesis in man by antiidiotypic antibodies. *J Clin Invest* 1983;71:46-54.
11. Bose R, Marsh DG, Delespesse G. Anti-idiotypes to anti-*Lol p I* (rye) antibodies in allergic and non-allergic individuals: influence of immunotherapy. *Clin Exp Immunol* 1986;66:231-40.
12. Castracane JM, Rocklin RE. Detection of human auto-anti-idiotypic antibodies (Ab2). Isolation and characterization of Ab2 in the serum of a ragweed immunotherapy-treated patient. *Int Arch Allergy Appl Immunol* 1988;86:288-94.
13. Hebert J, Bernier D, Mourad W. Detection of auto-anti-idiotypic antibodies to *Lol p I* (Rye I) IgE antibodies in human sera by the use of murine idiotypes: levels in atopic and non-atopic subjects and effects of immunotherapy. *Clin Exp Immunol* 1990;80:413-9.
14. Axen R, Porath J, Ernback S. Chemical coupling of peptides and proteins to polysaccharides by means of cyanogen halides. *Nature* 1967;214:1302-4.
15. Hoffman DR. Comparison of methods of performing the radioallergosorbent test: Phadebas, Fadal-Nalebuff, and Hoffman protocols. *Ann Allergy* 1980;45:343-6.
16. Quinti I, Brozek C, Wood N, Geha RS, Leung DYM. Circulating IgG autoantibodies to IgE in atopic syndromes. *J ALLERGY CLIN IMMUNOL* 1986;77:586-94.
17. Ogawa M, McIntyre OR, Ishizaka K, Ishizaka T, Terry WD, Waldmann TA. Biologic properties of E myeloma proteins. *Am J Med* 1971;51:193-9.

Bound volumes available to subscribers

Bound volumes of THE JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY are available to subscribers (only) for the 1991 issues from the Publisher, at a cost of \$49.00 for domestic, \$70.43 for Canadian, and \$67.00 for international subscribers for Vol. 87 (January-June) and Vol. 88 (July-December). Shipping charges are included. Each bound volume contains a subject and author index, and all advertising is removed. Copies are shipped within 30 days after publication of the last issue in the volume. The binding is durable buckram with the journal name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact Mosby-Year Book, Inc., Subscription Services, 11830 Westline Industrial Dr., St. Louis, MO 63146-3318; phone (800) 325-4177, ext. 4351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular journal subscription.