IgA and IgG Anti-Ragweed Antibodies in Nasal Secretions

QUANTITATIVE MEASUREMENTS OF ANTIBODIES AND CORRELATION WITH INHIBITION OF HISTAMINE RELEASE

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ABSTRACT Total secretory IgA and specific anti-antigen E (AgE) antibodies (ab) in the IgA and IgG classes were measured in concentrated nasal washings from ragweed allergic and normal individuals by antigen binding or anti-α-radioimmunoassays. Virtually all the allergic patients had significant IgA (45/49) and IgG (46/49) ab to AgE in their nasal washings. By contrast, washings from most normal persons contained no measurable IgA (13/15) ab or IgG (13/15) ab to AgE. The total IgA levels in allergic washings were not significantly different from those in normal washings and they were used to standardize the ab measurements. Parenteral immunotherapy with ragweed extract increased specific nasal IgA ab from 10.6±2.4 (SEM) to 39.0±8.7 ng AgE bound/mg IgA and IgG ab from 17.2±2.6 to 65.1±7.4 ng AgE bound/mg IgA ($P <$ 0.001 for both classes). The ratio of IgA : IgG ab was not affected by therapy, and for treated patients, there was no correlation ($r_s + 0.32$, $P > 0.1$) between nasal IgG ab and serum IgG ab. These results suggest that at least part of the nasal IgG ab is produced locally.

Blocking activity in the nasal washings was measured by inhibition of histamine release and was found to correlate directly ($r_s + 0.85$, $P < 0.001$) with binding activity for AgE. Some washings from normal persons caused slight inhibition of histamine release but others caused enhancement. Nasal washings were fractionated by passage over Sephadex G-200. Inhibition of histamine release by dilutions of the IgA-rich and IgG-rich fractions correlated well with binding activity in these fractions. None of these results support the hypothesis that allergic individuals are deficient in secretory IgA or secretory ab responses. These results, however, are in keeping with the theory that hay fever occurs in a high-responder population which is genetically able to respond to low doses of inhalant antigens.

INTRODUCTION

It is known that the serum of patients with ragweed hay fever contains IgG (1, 2) and IgA (2) anti-ragweed antibodies (ab) [1] as well as IgE ab (3). In addition, it has been shown that the serum of patients with ragweed hay fever can 'block' antigen E (AgE)-induced release of histamine from the leukocytes of allergic persons (4), and that this blocking activity increases with immunotherapy (4). By contrast, serum from most normal people contains neither blocking activity (4) nor detectable anti-ragweed IgG (1) nor IgE (3) ab. For many years it has been known that there is an increased incidence of allergy in some families (5) and recently it has been reported that there is some association between ragweed hay fever and HL-A type (6, 7). This pattern of a subpopulation of humans who respond to very small amounts of environmental ragweed antigens (8) with IgE (3) and IgG (1) ab suggests that allergic people are a high-responder population analogous to a high-responder animal strain (9, 10). If this analogy is correct, nonallergic people must be low responders and they would not be expected to produce anti-ragweed ab of any class in response to environmental exposure.

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[1] *Abbreviations used in this paper:* ab, antibody; AgE, antigen E of ragweed pollen; BA, binding activity for AgE; BBS, borate-buffered saline, pH 8.0; Ra IgG, normal rabbit IgG; SP, secretory piece.

On the other hand, Turk et al. (11) reported that nasal washings from both allergic and nonallergic persons would inhibit AgE-induced histamine release and they presented some evidence that this blocking activity was mediated by IgA ab. Although subsequently, some nonquantitative data suggested that anti-ragweed ab was not present in nasal washings from nonallergic individuals (12), more recent results appeared to show that nonallergic individuals have more IgA ab than allergic persons in both their serum (13) and in their nasal washings (14). These data (13, 14) support the totally different hypothesis that IgA ab protects nonallergic individuals from developing IgE ab, and that the underlying defect in allergic persons is IgA (15) or IgA ab (13–16) deficiency.

We report here the use of a quantitative radioimmunoassay for IgA- and IgG-AgE binding activity (BA) in nasal washings. Total IgA in the washings was measured using a radioimmunoassay for IgA heavy chain determinants (17) and the BA was then expressed relative to the total IgA. This standardization has made it possible to compare allergic and nonallergic washings and also to quantitate changes in ab during the pollen season and in response to parenteral immunotherapy. In addition, measurements of the blocking of AgE-induced histamine release by nasal washings have been correlated with BA and we have investigated the class distribution of nasal-blocking ab.

METHODS

Monospecific antisera. The preparation of rabbit antisera has been described in detail previously (17). Briefly, rabbits were immunized with human IgA or IgG in complete Freund's adjuvant (Difco Laboratories, Detroit, Mich.). The anti-IgA was made monospecific by repeated passage over an IgG immunosorbent column and the anti-IgG was made specific for IgG by repeated passage over a Fab immunosorbent column. Human IgG and its Fab component were linked to Sepharose (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) by the cyanogen bromide technique (18). The gamma-globulin fraction of rabbit antiserum to human secretory piece (SP) was kindly provided by Dr. Richard Newcomb (Pritzker School of Medicine, Chicago, Ill.). Goat antiserum to rabbit IgG (Ra IgG) (Antibodies Inc., Davis, Calif.) was passed over a human IgG immunosorbent column to remove antibodies cross-reacting with human IgG. Immunodiffusion was carried out using 1% agarose in 0.05 M barbital buffer, pH 8.6, and 0.01 M EDTA.

Ragweed AgE. Ragweed AgE research agent was obtained from the National Institutes of Health and passed through a Sephadex G-25 column (Pharmacia Fine Chemicals). The glycerine-free AgE peak was concentrated with a UM-2 filter (Amicon Corp., Lexington, Mass.) to 4 mg/ml. This AgE was radiolabeled with ^{125}I (high specific activity, Cambridge Nuclear Corp., Cambridge, Mass.) using a modification of the technique of Klinman and Taylor (19). To 40 μg AgE was added 50 μl borate buffer, pH 8.9, 2.5 mCi ^{125}I-Na, and 100 μg chloramine-T. After 60 s, 100 μg sodium metabisulphate was added, pH was restored with 0.15 M citrate buffer, pH 5.4, and the AgE was dialyzed against borate-buffered saline, pH 8.0 (BBS). The ^{125}I-labeled AgE gave 34,000 cpm/ng; 62% could be bound by hyperimmune human serum, and 88% was precipitated by trichloroacetic acid.

Patients and nasal washings. Patients with ragweed hay fever were diagnosed by history and skin testing. 12 of the allergic people had never received immunotherapy and are designated 'untreated'. The other 37 'treated' patients were currently undergoing parenteral immunotherapy with ragweed extract, and 33 of these treated patients had received parenteral immunotherapy in previous years. Of the 15 nonallergic controls, 10 were long-term residents of ragweed areas, 3 had been in Baltimore for 2 yr, and the other 2 had only recently arrived from a nonendemic area. Nasal washings were obtained as described in a previous paper (11); 10 ml of Tris-buffered saline, pH 7.3, was instilled into each nostril twice and allowed to drain out. The washings were centrifuged and concentrated by negative pressure using a collodion bag-apparatus which would exclude 70,000 daltons. The washings were concentrated to approximately 3–4 ml and stored at −20°C. All of the washings were inspected for erythrocytes or free hemoglobin; in addition, 30 washings were checked for occult blood using the benzidine test, and they were all found to be negative. Serial specimens were obtained from 19 allergic and 7 nonallergic persons.

Fractionation of nasal washings. Nasal washings from allergic individuals were chosen for their high ab content and pooled; a parallel pool was made of washings from nonallergic individuals. To reduce the possibility of enzymic damage to IgA (20) and reduce adherence of mucus, the pools of nasal washings were chelated with 0.01 M EDTA; they were then concentrated a further 10-fold before fractionation. 0.4 ml of pooled washings was placed on a continuous sucrose gradient, 5–20% in BBS. After centrifugation at 80,000 *g* for 20 h in a model L 3-50 Beckman preparative ultracentrifuge (Beckman Instruments Inc., Spinco Div., Palo Alto, Calif.), 0.25-ml fractions were collected through a hole pierced in the bottom of the tube. 5-ml samples of pooled nasal washings were applied to a 2.5 × 100-cm Sephadex G-200 column and equilibrated with 0.01 M EDTA. The protein eluted from the column in three peaks, and fractions from these peaks were pooled, concentrated, and dialyzed for 18 h against three changes of Tris buffer with calcium and magnesium, and are referred to as fractions I, II, and III.

AgE-binding radioimmunoassay. IgA and IgG antibodies to AgE were measured by a double-antibody technique using ^{125}I-labeled AgE, carrier immunoglobulin, and anti-IgA or anti-IgG (17, 21). 0.2% bovine serum albumin in BBS was used as diluent, and for carrier immunoglobulin, the serum of a nonallergic person who had not been exposed to ragweed was used. This serum contained no detectable BA at a dilution of 1/20 and was used at that dilution for IgA ab assays and at 1/40 for IgG ab assays. The assay was carried out as follows: 0.2 ml of nasal washings was added to 0.05 ml of ^{125}I-AgE. After 3 h at room temperature anti-IgA or anti-IgG was added. The amount of antiserum necessary was estimated by two-drop precipitation with the carrier Ig. Individual washings were not tested for equivalence, but the amount of antiserum added was arranged to be sufficient to precipitate the carrier IgA or IgG, plus the IgA or IgG in the nasal washings. The precipitate was allowed to form overnight at 4°C and was then washed three times with BBS; after the second wash, the precipitates were transferred to new tubes. Radioactivity in the precipitate was measured with an automatic gamma-counter

(Nuclear-Chicago, Searle Analytic Inc., Des Plaines, Ill.). The assays were arranged so that at least 80% of the radiolabeled AgE was not bound by any class of ab so that there was always an excess of AgE (21). The nasal washings were diluted where necessary.

A control serum was obtained from a patient who had received very high-dose immunotherapy for several years. The AgE bound by twofold dilutions of this serum from 1/1,920 to 1/480,000 was measured using the assay with anti-IgG and freshly radiolabeled AgE. From the radioactivity bound and the specific activity of the AgE, IgG BA was calculated for each dilution of the serum. This curve was used for all subsequent assays to obtain values for the BA of a given dilution of the control serum. A control curve was set up in parallel with each assay so that the radioactivity bound by nasal washings was first expressed as an equivalent dilution of the control serum, and from this as nanograms of AgE bound per milliliter. The variation between repeated assays of the BA in a given nasal washing was less than ±4%. Two nasal washings were found to have high levels of IgA and IgG ab, respectively. Serial twofold dilutions of these washings were assayed for IgA and IgG-BA in parallel with the control serum. The curves were found to be exactly superimposable over the range 0.04–2.0 ng AgE bound/ml, so the serum IgG control curve was used for both IgA- and IgG-nasal ab. From the linear portion of the control serum BA curve it was estimated that the control serum would bind 20.5 μg AgE/ml. In addition, the BA of the control serum was compared with the BA of a rabbit antiserum to AgE. The antibody activity of the rabbit antiserum had been measured by quantitative precipitation at equivalence, so that the antibody activity of the human control serum could be estimated. From this estimate it was calculated that in antigen excess, the human control serum would bind approximately 24 μg AgE/ml.

Serum IgG anti-AgE ab was measured by a similar technique using carrier Ig, ^{125}I-labeled AgE, and precipitating with anti-IgG. However, the sera were assayed at several dilutions and the result was given as the reciprocal of the dilution of the serum which bound 50% of a fixed amount of AgE.

For measurement of the AgE binding associated with secretory piece, two modifications of the antigen-binding assay were used. In the Sephadex fractions I and II, sufficient SP was present to give a direct precipitate with anti-SP, equivalence being estimated by immunodiffusion. For the dilution of nasal washings, a triple-antibody technique was used with rabbit (Ra) anti-SP diluted in Ra IgG and finally precipitated by goat anti-Ra IgG. Details of this procedure were described in (17). In each case, IgA BA was measured in parallel by a similar technique.

Radioimmunoassay for total IgA and IgG. The inhibition radioimmunoassay for human IgA (and IgG) has been fully described elsewhere (17). Briefly, samples were incubated with anti-IgA (or anti-IgG), diluted in Ra IgG; after 3 h, ^{125}I-labeled IgA (or IgG) was added, and after another 3 h, goat anti-Ra IgG was added to precipitate all the Ra IgG. The precipitate was allowed to form overnight, washed three times with BBS, and the radioactivity in the precipitate was measured with an automatic gamma counter. This assay for IgA avoids the problems of radial immunodiffusion because it detects only IgA heavy chain-determinants and is not affected by variations in diffusion coefficients. When nasal washings or serum IgA were separated on sucrose density gradients, the distribution of IgA detected by the assay showed that the assay could detect monomer or polymer IgA with or without SP (17). A serum IgA standard was used for this assay, but serial dilutions of nasal washings gave parallel curves over the range 0.2–0.02 μg IgA/ml. All the washings were diluted so as to fall within this range and assays were repeated where necessary.

Assay for blocking of histamine release. The techniques used for in vitro histamine release from human peripheral blood leukocytes were as previously described (11, 22, 23). The AgE used to stimulate histamine release was kindly provided by Dr. T. P. King (The Rockefeller University, New York) (24). Two donors were chosen whose cells each released 60–80% of their histamine after challenge with quantities of AgE which we estimated could be bound by the ab in allergic nasal washings. The assay was performed by incubating 0.2 ml of nasal washings at 4°C with 0.2 ml of AgE (1 ng/ml), in tris buffer with albumin, calcium, and magnesium as described previously. After 18 h, 1.0-ml aliquots ($\sim 10^7$ cells) of washed human leukocytes were added. The histamine released was measured spectrophotometrically. Results were derived from the percent of histamine release caused by AgE alone (y) and the percent release in the presence of nasal washings (x) using the formula (reference 11):

$$\% \text{ inhibition} = (y - x/y) \times 100.$$

Statistical methods. The χ^2 test was used to compare the incidence of significant BA in nasal washings from allergic or nonallergic individuals.

Student's t test was used to compare the total IgA in nasal washings, and also to compare IgA (and IgG) BA per milligram of IgA in nasal washings from nonallergic and allergic individuals both treated and untreated.

The Spearman rank-correlation coefficient was used to compare BA/ml with blocking activity in individual washings, to compare IgG BA with IgA BA, and also to compare serum IgG BA with the nasal IgG BA.

The Spearman rank-correlation coefficient was also used to compare BA per milligram of IgA in washings both with BA at other times of the year and with clinical symptom scores.

RESULTS

Nasal washings were obtained from 49 persons with ragweed hay fever and from 15 nonallergic controls; in many cases specimens were obtained before, during, and after the ragweed season (mid-August to the end of September). Specific IgA and IgG BA were measured in the nasal washings by antigen-binding radioimmunoassay. With this assay a 1/480,000 dilution of the control serum, equivalent to the binding of 0.04 ng AgE/ml (see Methods) increased the radioactivity in the precipitate by more than 2 SD above background. This amount of AgE binding was regarded as significant. The results show that significant IgA and IgG BA were found in at least one specimen from almost all the allergic persons (Table I). Both IgA and IgG BA in allergic nasal washings varied widely (from < 0.1 to > 50 ng/ml). On the average, IgG BA was 1.6 times higher than IgA BA, but the ratio of IgG BA to IgA BA was also very variable.

TABLE I
*Incidence of Significant AgE-Binding Activity in Concentrated Nasal Washings**

	ppt. by antisera to	
	IgA	IgG
Allergic		
Individuals‡	45/49	46/49
Specimens (2.9)§	114/142	135/142
Nonallergic		
Individuals	2/15	2/15
	$P < 0.001$‖	$P < 0.001$
Specimens (3.1)§	2/47	3/47

* Measured by antigen-binding radioimmunoassay using ^{125}I-labeled AgE and anti-IgA or anti-IgG. Significant binding was considered to be present if the washing bound >0.05 ng AgE/ml.
‡ Number of individuals where at least one washing contained significant BA related to the number of individuals tested.
§ Average number of specimens per individual.
‖ P values were calculated by χ^2 test and refer to the incidence in normals relative to that in allergic persons.

Total IgA was measured by radioimmunoassay in washings from 28 of the allergic and all of the nonallergic individuals. The total IgA was higher in washings from allergic 25.7±22.5 SD μg/ml, than from nonallergic individuals, 18.7±23.7 μg/ml, but the difference was not significant ($P > 0.05$ by Student's t test). The BA was then expressed relative to the IgA concentration in each washing so that different washings could be compared. On the average, treated patients had a fourfold higher BA per milligram of IgA than untreated patients and this difference was very similar for both IgA and IgG (Table II). That is, treatment does not appear to alter the ratio of IgG BA to IgA BA in nasal secretions.

It has previously been reported that environmental exposure during the ragweed season leads to an increase in both serum IgE ab (25) and total IgE in nasal washings (26). For some of our patients nasal washings were obtained on five different occasions in 1 yr. The IgA BA in these washings are shown in Fig. 1. Out of six untreated patients, five showed a rise in IgA BA after the season (Fig. 1), but in three of these five, IgA BA during the season was significantly lower than preseasonal values. For treated patients the rise after the season was less consistent but was again matched in several cases by a fall in IgA BA during the season (Fig. 1). The dip in nasal ab during the season was not expected and the explanation for it is not clear. For IgG ab (data not shown) changes were less marked during the season but there was a correlation between the two classes. Of all the washings showing a change in IgA BA during or after the season, in 32 cases out of 37, the change in IgG BA was in the same direction. Particularly, in 9 cases out of 10 in which IgA BA fell during the season, there was a simultaneous fall in IgG BA. The different specimens obtained from each patient preseasonally did not show the marked variation observed during the season. Thus, the three preseasonal values for each treated pa-

TABLE II
*Effect of Immunotherapy on IgA and IgG BA for AgE in Nasal Secretions**

	ppt. by antisera to		Ratio of
	IgA	IgG	IgG/IgA
Untreated (45/13)‡	10.6±2.4	17.2±2.6	1.62/1
Treated (75/19)‡	39.0±8.7	65.1±7.1	1.66/1
	($P < 0.001$)§	($P < 0.001$)	

* Calculated from the results of the AgE-binding radioimmunoassay and the radioimmunoassay for total IgA. Values are nanograms of AgE bound by IgA (or IgG)/milligrams of IgA±SEM.
‡ Number of specimens measured and the number of individuals.
§ P values refer to the effect of treatment on each class of antibody, determined by Student's t test.

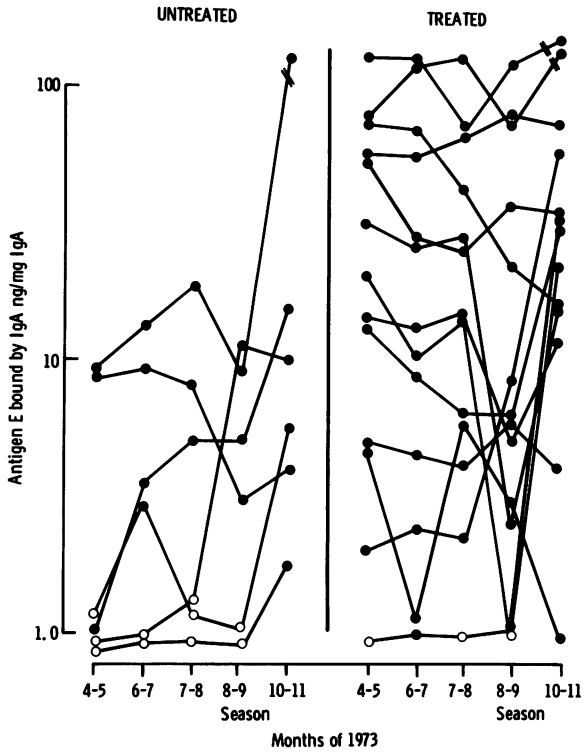

FIGURE 1 Seasonal variation in nasal IgA antibody. IgA BA in nanograms per milligram of IgA was calculated from the results of the AgE-binding radioimmunoassay and the total IgA measured by radioimmunoassay. Specimens where significant IgA BA was present are represented by (●), when no significant binding was detected the maximum level that could have been present is indicated by (○).

tient in Fig. 1 when compared with each other gave Spearman rank-correlation coefficients of 0.90–0.94 ($P < 0.001$), while the postseasonal values did not appear to correlate with any groups of the preseasonal values (r_s 0.43–0.46, $P > 0.05$). It seems clear that IgA ab was affected by the ragweed season but the pattern of changes observed was very variable.

Correlation of AgE binding by nasal washings and blocking of AgE-induced histamine release. Using the quantitative measurements of BA in nasal washings, we decided to re-examine the ability of nasal washings to block AgE-induced histamine release (11). The results (Fig. 2) show a direct correlation (Spearman rank-correlation coefficient = 0.85, $P < 0.001$) between inhibition of histamine release and BA, expressed as the sum of IgA and IgG BA in nanograms per milliliter. The inhibition experiments showed considerable intrinsic variation. While variation between duplicates in a single experiment was less than ±5%, the variation between repeat determinations was generally ±15–30%, even when using the same donor. This is certainly more than is seen in measuring serum-blocking activity (4) and all the values are the mean of duplicate measurements in two separate experiments. In these experiments we incubated 1 ng AgE/ml with the nasal washings and under these conditions some nonallergic washings caused inhibition but most caused enhancement (Fig. 2).

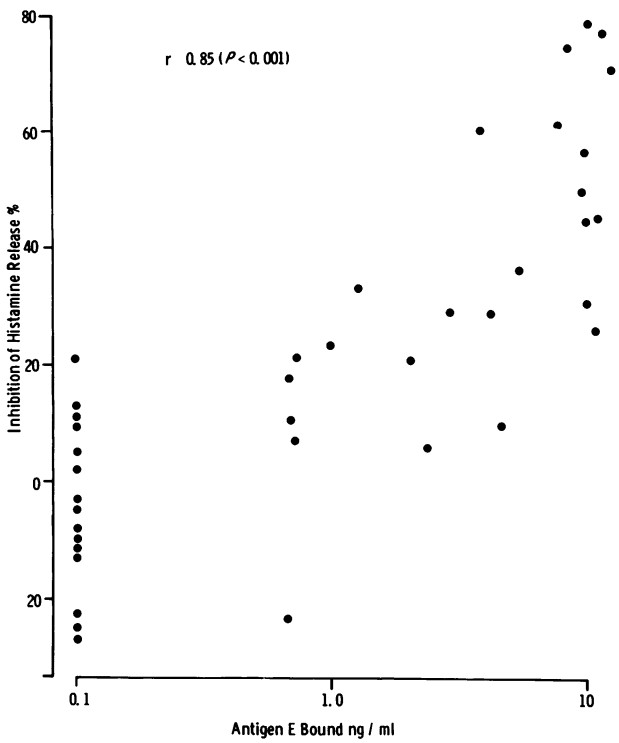

FIGURE 2 Correlation of BA and inhibition of histamine release. BA in nasal washings was measured by antigen-binding radioimmunoassay and values are the sum of IgG and IgA BA in nanograms per milliliter. AgE was incubated with nasal washings at 4°C for 18 h before adding peripheral blood leukocytes from an allergic patient; histamine release was measured spectrophotometrically. Inhibition (or enhancement) was calculated relative to the histamine release seen using AgE incubated in buffer only. Spearman rank-correlation coefficient: $r_s = 0.85$ ($P < 0.001$).

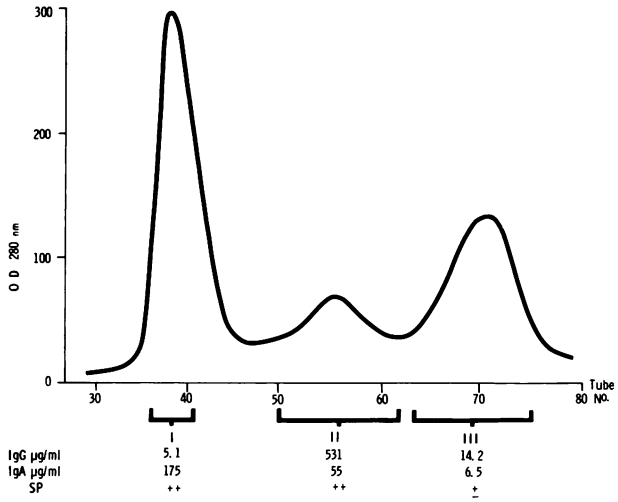

FIGURE 3 Sephadex G-200 separation of nasal washings. Highly concentrated washings were chelated with 0.1 M EDTA and passed over Sephadex G-200. Total protein in the eluate was measured by OD at 280 nm; the fractions were pooled and reconcentrated. Total IgA and IgG in the fractions was measured by radioimmunoassay; the presence of SP was detected by immunodiffusion.

Class distribution of 'blocking' ab in nasal washings. Some of the nasal washings used for blocking studies (Fig. 2) contained predominantly one class of BA. For example, one washing bound 10 ng AgE/ml as IgG, 0.4 ng AgE/ml as IgA, and caused 47% inhibition of histamine release, while another washing at a dilution of 1:25 bound 8.9 ng AgE/ml as IgA, 1.5 ng AgE/ml as IgG, and caused 56% inhibition of histamine release. These results suggest very strongly that both IgA and IgG nasal ab can inhibit histamine release. This was confirmed by experiments with partially purified nasal washings. Since preliminary experiments with sucrose density gradient separation showed that the bulk of the IgA appeared in a high mol wt peak relatively free of IgG, separation of IgA was attempted using Sephadex G-200. A pool of allergic nasal washings with high BA was chelated with 0.01 M EDTA, centrifuged at 10,000 *g* for 30 min, concentrated, and passed through a Sephadex G-200 column (Fig. 3). Only the early part of the first peak was used (fraction I) to reduce IgG contamination; the second peak (fraction II) coincided with the elution volume for ^{125}I-labeled IgG, and the third peak (fraction III) was

TABLE III

*AgE BA and Inhibition of Histamine Release by Fractions of a Nasal Washing Pool from Allergic Patients**

		Total BA	IgG BA‡	IgA BA‡	Inhibition of histamine release§ Exp. 1	Exp. 2
			ng/ml		%	
	1/1	9.9	0.75	9.2	55	—
Fraction I	1/2	4.95	0.37	4.6	—	73
	1/4	2.5	0.18	2.3	26	52
	1/4	21	20.5	0.6	53	—
Fraction II	1/16	5.2	5.1	0.15	25	56
	1/32	2.6	2.5	0.04	—	21
	1/1	2.6	2.6	<0.2	6	10
Fraction III	1/2	1.3	1.3	<0.1	—	8
	1/4	0.6	0.6	<0.1	0	2

* Fractions from a Sephadex G-200 column, see Fig. 3.

‡ IgA and IgG BA were measured by radioimmunoassay.

§ Dilutions were incubated with AgE and then allergic leukocytes were added. % of inhibition of histamine release was calculated relative to the release seen with equal dilutions of fractions from a pool of washings from nonallergic individuals.

presumed to be mainly albumin. The fractions were concentrated and dialyzed against three changes of Tris CM buffer. Measurements of total IgA and IgG in the fractions (Fig. 3) showed good separation of IgA in fraction I and a 10-fold excess of IgG in fraction II. A pool of nasal washings from eight nonallergic people was treated similarly; the fractions were adjusted to comparable total protein concentrations and were found to have similar concentration of IgA and IgG. Dilutions of the fractions were then assayed for BA and tested for blocking activity. The results (Table III) showed that dilutions of both the IgA-rich fraction I and the IgG-rich fraction II from the allergic pool inhibited histamine release. Dilutions of the fractions obtained from nonallergic donors enhanced histamine release and were used as controls for calculating inhibition by the allergic fractions. There was a clear correlation between the total BA in the fractions and the highest dilution giving significant inhibition of histamine release (Table III).

Furthermore, neither the IgG ab in a 1/4 dilution of fraction I (0.18 ng/ml) nor the IgA ab in a 1/16 dilution of fraction II (0.15 ng/ml) would be expected to cause significant inhibition of histamine release on their own (Table II and Fig. 2). It seems clear from these results that both IgA and IgG nasal ab can inhibit histamine release.

The fractions of the pool of nonallergic washings contained no detectable BA, i.e., < 0.05 ng AgE bound/ml. In the nonallergic fraction I, the IgA BA was less than 0.2 ng AgE/mg IgA; this value is at least 50-fold less than the average IgA BA in washings from untreated allergic patients (10.6±2.4 ng AgE bound/mg IgA, Table II).

Presence of secretory piece on nasal ab. It is known that secretory but not serum IgA is linked to SP. When concentrated nasal washings were tested by immunodiffusion with anti-IgA and anti-SP there was a strong line of identity between IgA and SP with only small spurs, suggesting that the majority of the IgA in nasal washings was linked to SP as has been previously reported (27). When the fraction of allergic nasal washing-pool were tested by immunodiffusion, the lines seen with anti-IgA and anti-IgG were in keeping with the radioimmunoassay results (Fig. 3). Testing fraction I with anti-SP and anti-IgA showed a line of identity between IgA and SP with no visible spurs. By contrast, testing fraction II with anti-SP gave a stronger line than with anti-IgA and there was a spur showing that a large part of the SP in fraction II was not associated with IgA (or IgG). It has previously been reported that "free" SP in human secretions elutes from Sephadex G-200 in a fraction before albumin (28) and our results are in keeping with this.

The immunodiffusion results suggest that the bulk of the IgA in nasal washings is associated with SP; to be sure that the nasal IgA BA we have measured is indeed a secretory IgA antibody, we tried to precipitate BA with anti-SP. The results (Table IV) showed a good correlation between BA precipitated by anti-SP and that precipitated by anti-IgA, whereas there was no correlation with the BA precipitated by anti-IgG. In fraction II (Table IV) there appears to be more BA associated with IgA than with SP. This suggests that some 7S IgA ab does not bear SP, which is in keeping with the results of immunodiffusion. However, fraction II contained a large amount of IgG BA and the results

TABLE IV

*Association of SP with AgE BA in Nasal Washings**

	ppt. by antiserum to IgA	SP	IgG
Allergic pool			
Fraction I	9.2	7.1	0.75
Fraction II	2.3	1.2	82
Allergic washing‡	1.5§	1.5§	0.25
Normal pool	<0.1	<0.1	<0.1

* Measured by antigen-binding radioimmunoassay using anti-IgA, anti-SP, or anti-IgG values are nanograms of AgE bound per milliliter.

‡ pH 10/3/73, diluted 1/135.

§ Measured by modified triple-antibody technique using goat anti-RaIgG.

are less reliable than those for fraction I or the unseparated nasal washing.

Serum IgG ab and hay fever symptoms in relation to nasal ab. For a group of 14 patients, clinical symptom scores were obtained using previously described techniques (25). Serum was obtained from this group of patients during the ragweed season, and IgG BA was measured in these sera by a 50% binding technique (25). For these patients the data on nasal BA, serum IgG BA, and clinical symptoms were further analyzed using Spearman rank-correlation. Direct comparison of serum IgG BA with nasal IgG BA (Fig. 4) showed no correlation (r_s 0. 32, $P > 0.1$). Furthermore, neither preseasonal, seasonal, nor postseasonal nasal BA was related to the clinical severity of the patient's ragweed hay fever. Comparing IgA and IgG BA in individual nasal washings revealed a modest correlation (r_s 0.56, $P < 0.05$) and this correlation was similar in preseasonal and seasonal specimens.

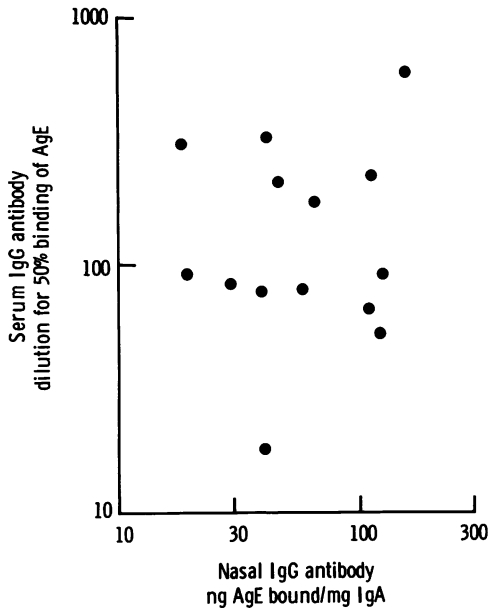

FIGURE 4 Serum IgG BA and nasal IgG in treated patients were each measured by radioimmunoassay. No correlation was found ($r_s = 0.32$, $P > 0.05$).

DISCUSSION

Our results leave no doubt that patients with ragweed hay fever have more IgA and IgG nasal ab to AgE than nonallergic individuals. Not only was the incidence of detectable BA in nasal washings from nonallergic individuals very low (Table I) but we estimate that the average IgA and IgG BA in nasal washings from untreated allergic patients (Table II) was at least 50 times greater than in the fractions of pooled nonallergic nasal washings (Table III). Only 3 (out of 47) washings from nonallergic individuals contained detectable BA, in 1 case this was IgG alone, in the others both classes were present. No nasal washings from the five normal people who had previously lived in nonragweed infested areas contained detectable BA, but the significance of the data was not affected ($P < 0.001$) if these washings were excluded. Finding detectable ab in nasal washings from a small proportion of nonallergic individuals is not surprising, as some nonallergic individuals have IgG anti-AgE in their serum.[2] The very high incidence of IgG and IgA ab to ragweed in the nasal washings of allergic people (Table I) has been reported before (12) and is in keeping with the finding that all patients with ragweed hay fever have IgG ab to ragweed in their serum (1, 2).

IgA in the washings was measured by radioimmunoassay; this assay was specific for IgA heavy chain determinants, was much more sensitive than radial immunodiffusion, and has been shown to detect both monomeric and polymeric IgA (17) in secretions or serum. Expressing nasal BA relative to the total IgA made it possible to compare different washings quantitatively (29) and several conclusions became clear. There is, on average, more IgG than IgA BA per milligrams of IgA (Table II). The average concentration of IgA in nasal secretions is, however, five times that of the IgG so that IgA is still quantitatively the most important ab (29). Immunotherapy increases IgA and IgG BA equally (Table II). This was surprising as the serum response to immunotherapy is much larger (1) and is predominantly IgG (30). In addition, we could find no correlation between nasal IgG BA and serum IgG BA in a group of treated patients (Fig. 4). These findings taken together suggest that nasal IgG BA (as well as IgA BA) does not reflect serum ab and is presumably produced locally; however, our results do not exclude some contribution by transudation of serum ab. The failure of a quantitative correlation between serum and secretory IgG levels may be attributed to individual differences in response. Alternatively, it is possible that immunotherapy might have enhanced the local ab response to environmental antigen. Comparing specimens showed that repeated washings from the same patient before the season have rather consistent levels of IgA BA (Fig. 1). However, during the season the IgA BA varied widely, including in several cases a sharp dip (Fig. 1). A rise in BA during or after the season due to environmental exposure was expected and is known to occur with serum IgE ab (25) but these falls are less easy to explain. In many cases IgG BA fell simultaneously which would suggest the possibility that environmental ragweed pollen was neutralizing antiragweed ab, but calculations of the number of pollen grains and their

[2] Marsh, D. G. Personal communication.

AgE content (nanograms per season) makes direct neutralization unlikely (8). It is also difficult to see why increasing symptoms should reduce the proportion of a specific ab unless rhinitis leads to increased transudation of serum IgA which dilutes the local ab. However, transudation in treated patients would be expected to cause an increase in IgG BA which was not observed.

The data in Fig. 2 shows a direct correlation between AgE BA in nasal washings and inhibition of histamine release. Most washings from nonallergic individuals caused enhancement or only slight inhibition (Fig. 2). On the other hand, a previous report from this laboratory showed that nasal washings from allergic and nonallergic individuals could inhibit AgE-induced histamine release (11). These results were obtained using leukocytes from highly sensitive patients and very low concentrations of AgE (< 0.2 ng/ml). In preliminary experiments using similar conditions, we found that most washings from nonallergic individuals inhibit histamine release. On the other hand, when using > 4.0 ng of AgE/ml almost all washings from nonallergic individuals enhanced histamine release and only washings with high BA caused inhibition of histamine release. The concentration of AgE used in the present experiments (Fig. 2, Table III) was chosen to avoid nonspecific inhibition and yet give sufficient sensitivity with low levels of BA. We believe that the inhibition of histamine release previously observed with nasal washings from normal persons (11) was not due to the presence of anti-AgE ab. Since AgE is easily denatured (31) some of the inhibition seen could well represent damage to AgE by enzymes in the nasal washings (32). With the conditions used in these experiments, most washings from nonallergic individuals enhanced AgE-induced histamine release. A similar enhancement was seen with fractions of the pooled washings from nonallergic individuals. It seems likely that this enhancing effect is similar to the complex enhancing effects of normal serum (33). And it seems reasonable to assume that the fractions of the pooled allergic washings contain enhancing factors as well as the anti-AgE ab which inhibit histamine release (Table III). Clearly, nasal washings affect histamine release in multiple ways and it is not surprising that the results of separate experiments show considerable scatter.

Our present results make it clear that nasal washings from allergic patients contain not only IgA and IgG ab, but that both classes of ab can inhibit histamine release. The data suggest that IgA ab inhibits histamine release more effectively than IgG ab (Table III); this may reflect higher avidity of polymeric IgA ab. It has previously been reported that the IgA fraction of hyperimmune allergic serum contains very little 'blocking' activity (30). We reinvestigated the IgA-rich fraction IIa of Lichtenstein et al. (30) and found that it contained little BA of either class but it did contain 140 μg/ml of IgA. From those fractions of hyperimmune serum (30) we estimate that serum IgA BA was less than 2% of the IgG BA. Thus, the failure of serum IgA to inhibit histamine release reflects the poor serum IgA ab response to parenteral immunization; and for both serum and nasal washings the ability of fractions to block AgE-induced histamine release correlates well with their BA as measured by radioimmunoassay.

We have shown that the nasal secretions of allergic patients contain more ab than the secretions of nonallergic individuals (1, 2, 4). However, two recent reports have suggested that allergic patients have less IgA ab in nasal washings (14) and serum (13) than nonallergic individuals. We believe that these results (13, 14) are attributable to an artifact of the immunosorbent system used to measure IgA ab. In our hands it has not proved possible to prepare a ragweed immunosorbent (25) which binds IgA (or IgG) antiragweed ab without binding normal human Ig as well. While it is true that those studies (13, 14) used different antigens, it seems very unlikely that the situation is completely different for grass than for ragweed. Also it is known that rye-grass allergic patients have serum anti-Rye I ab while most nonallergic persons do not.[2]

Immunotherapy for hay fever is usually less than completely effective at reducing symptoms despite the production of high titers of serum-blocking ab (1, 25, 34). A possible explanation for this is that the antigen reaches sensitized mast cells in the nose before being exposed to serum ab. That nasal ab would be more effective in controlling hay fever was also suggested by the report (35) that autologous serum-blocking ab applied locally in the nose will reduce symptoms. However, more recent attempts to confirm this finding were not successful (36). The present study was designed to investigate the role of nasal ab in hay fever and the possible relevance of nasal immunization. We have shown that nasal ab can inhibit histamine release in vitro and that parenteral immunotherapy produces modest increases in nasal ab. It was disappointing that there was no correlation between nasal ab and symptoms of hay fever. However, if nasal ab affects symptoms this would depend on other factors including IgE ab and the sensitivity of basophils or mast cells so that a correlation with nasal ab might be obscured. Further studies are planned to investigate methods for increasing nasal ab and to relate changes in nasal ab to symptoms.

Several theories about the pathogenesis of allergic diseases in man (16) revolve around two possibilities: One, that nonallergic people lack the genetic ability to recognize and respond to allergens at low doses (6, 7,

37), and two, that in nonallergic people, allergens are prevented from reaching the relevant sites for stimulating the production of IgE ab. It has been postulated that the entry of allergens is prevented by nasal impermeability (16, 38) or by specific nasal IgA ab (13–16). Our own data make it most unlikely that nonallergic persons are prevented from becoming allergic by the presence of nasal ab. Although some experiments had suggested that nasal permeability was lower in nonallergic individuals (38, 39), this has not been confirmed by direct experiments (40). Animal experiments generally show that optimal antigen doses for IgE ab formation will also produce other classes of ab in responding strains (9, 41) but will produce no response of any class in nonresponding strains (9). Allergic patients produce nasal IgA and IgG ab as well as serum IgG and IgE ab, while no ab is detectable in serum or secretions from most nonallergic individuals. In addition, the doses of pollen antigen encountered environmentally are very low. It therefore seems likely that allergic patients are analogous to a high responder strain (9, 10) and that it is not necessary to propose a nasal block in nonallergic individuals.

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