Seasonal Changes in IgE Antibodies and Their Relationship to IgG Antibodies during Immunotherapy for Ragweed Hay Fever

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A B S T R A C T Seasonal changes in IgE antibodies and
their relationship to IgG antibodies were studied in 52
patients with ragweed hay fever and 10 normal controls.
Allergic patients received either no immunotherapy,
preseasonal immunotherapy, or high dose perennial im-
munotherapy with aqueous-mixed ragweed extract.
Serums were collected before, during, just after, and 4
mo after the ragweed pollination season. IgE antibodies
to ragweed antigen E (AgE) were measured using the
radioallergosorbent test, and IgG antibodies were mea-
sured by radioimmunoprecipitation.

IgE antibodies to AgE were elevated in all allergic
patients, and rose during the ragweed pollination season.
The magnitude of the rise in IgE antibody was a func-
tion of the preseasonal IgE antibody level. IgE antibody
production in the treated groups was the same as in the
untreated group when patients were matched on the basis
of their preseasonal IgE antibody levels. Thus, we were
not able to confirm previous reports that immunotherapy
suppresses the seasonal rise in IgE antibodies. Further-
more, there was a close relationship between the levels
of IgE and IgG antibodies in the high dose group.
This finding is contrary to what one would expect were
IgG antibodies acting to suppress the formation of IgE
antibodies. Rather, it suggests that in certain patients
either humoral immunological reactivity to ragweed anti-
gens in the IgG and IgE classes is low to begin with or
that this reactivity may wane after treatment with high
doses of ragweed extract.

INTRODUCTION

Patients with ragweed hay fever show positive, direct,
wheal-and-flare skin reactions to extracts of ragweed
pollen and have IgE antibodies specific for ragweed anti-
gens in their serum (1, 2). These IgE antibodies may be
measured in vitro by coprecipitation with radiolabeled
antigen (3), by passive leukocyte sensitization (4) or by
the radioallergosorbent test (RAST)[1] (5). With passive
leukocyte sensitization, IgE antibodies can be readily
measured in the serums of children with ragweed hay
fever, whereas IgE antibodies are detectable in only
about 50% of adult patients with this method (6, 7).
After the ragweed pollination season, IgE antibodies in
serum increase in concentration, and in children this in-
crease appears to be partially suppressed by immunother-
apy (6). Cells of the treated subjects become less sensi-
tive or less reactive to ragweed antigen E (AgE) (6, 7),
and IgG antibodies ("blocking" antibodies) appear in
the serum (8–10). The apparent suppression of IgE
antibody has been attributed to the presence of IgG-
blocking antibody (6).

We have analyzed serums from normal individuals and
patients with ragweed hay fever to define the immuno-
logical mechanisms operating during immunotherapy.
The results indicate that rises in IgE antibodies during
the ragweed pollination season are not suppressed by im-
munotherapy. In treated patients, IgE and IgG antibod-
ies were positively correlated, a finding contrary to that
expected if IgG antibody were acting to suppress pro-
duction of IgE antibody.

METHODS

Patient groups. 62 patients were studied during 1970 and
were divided into four groups. 10 healthy nonallergic per-
sons served as controls. 52 patients with typical ragweed

[1] *Abbreviations used in this paper:* AgE, ragweed antigen
E (partially purified); AgE IV-C, ragweed antigen E
(highly purified); PNU, protein nitrogen units; RAST,
radioallergosorbent test.

hay fever were subdivided into three groups: one received no immunotherapy; a second began immunotherapy in the spring of 1970; a third group was already receiving high dose perennial immunotherapy with aqueous-mixed ragweed pollen extract. The patients in the high dose-treated group had received immunotherapy for at least 2 yr (one patient for as long as 19 yr) ; the median length of treatment was 4 yr. In 1968 and 1969 the high dose group had received an average total dose of 113,000 and 272,000 protein nitrogen units (PNU), respectively. In 1970 the range of doses for patients in this group was 170,000–381,000 PNU, and the median dose was 259,000 PNU. All of these patients continued to receive monthly injections of pollen extract during the study period.

Only one of the nonallergic normals had a positive direct wheal-and-flare skin reaction to ragweed extract (and this to a high concentration), whereas all of the allergic subjects reacted positively. The characteristics of these groups and the cumulative dose of ragweed pollen extract received during 1970 are listed in Table I. None of the patients received corticosteroids. 10 of the 52 allergic patients had occasional wheezing episodes during the ragweed pollination season, but asthma was a major problem in only five patients. None of the patients had atopic dermatitis.

Four serum samples were collected: one at the beginning of the study, before the onset of the ragweed pollination season, in April (the group beginning immunotherapy) or July 1970; the second at the height of the ragweed pollination season in late August or very early September; the third in mid-October; and the fourth in January 1971. Serum samples were obtained within a few minutes of the pollen extract injection in the treated groups. The serums obtained in April from the group beginning immunotherapy were drawn before the first injection of pollen extract.

IgE antibody to ragweed AgE. Serum IgE antibody to ragweed AgE was measured by means of the RAST procedure (5, 11). In this method AgE is bound to a solid phase carrier and incubated with serum. After a washing procedure the solid phase antigen is reacted with radio-labeled antibody to IgE, again washed, and counted. The radioactivity associated with the solid phase antigen is taken as a measure of the IgE antibody level. Partially purified AgE was reacted with cyanogen bromide-activated micro-crystalline cellulose to yield a solid phase ragweed antigen. The detailed description of these procedures as well as the purification of AgE is presented elsewhere (12). Briefly, ragweed pollen was defatted, extracted with distilled water, and purified by gel filtration through Sephadex G-25. The void volume peak containing AgE activity was concentrated and further purified by gel filtration on Sephadex G-100. The resulting partially purified AgE contained 4.6 mg protein/ml, of which 50% was AgE as measured by radio-immunoassay (13). In all of the analyses of IgE and IgG antibodies to AgE the same lot of partially purified AgE was utilized. In the remainder of this paper the term AgE will refer to this partially purified preparation, and AgE IV-C [2] will be used to designate the more highly purified protein of King, Norman, and Connell (14). After thorough washing to remove noncovalently bound AgE, the cellulose-AgE polymer was suspended at a concentration of 1 mg/ml in 0.1 M phosphate buffer (pH 7.4) containing 0.2% bovine serum albumin (BSA), 1% Tween 20, and 0.1% sodium azide. The AgE IV-C content of this material

[2] Kindly provided by Dr. Floyd McIntire, Abbott Laboratories, North Chicago, Ill.

TABLE I

Characteristics of Study Groups

	Nonallergic controls	Allergic		
		Untreated	Low dose	High dose
Patients	10	14	12	26
Male	8	11	6	15
Female	2	3	6	11
Age				
Range	22–55	18–48	17–46	11–58
(Mean)	(37.1)	(31.1)	(31.6)	(28.1)
Mean dose ragweed extract during 1970	—	—	50,000 PNU	270,000 PNU

was 150 ng/mg as measured by radioimmunoassay. It gave an inhibition curve not different from AgE IV-C (13).

Sheep antiserum to human IgE (ND) (Pharmacia Fine Chemicals, Inc., Piscataway, N. J., lot 7320) was purified by affinity chromatography (15). IgE protein (100 mg), purified from myeloma serum PS as described elsewhere (16), was reacted with 10 ml packed volume of cyanogen bromide-activated Sepharose 2B. No absorbance at 277 nm was recovered in the supernates from this reaction. The Sepharose-IgE complex was washed once with pH 8.0 0.2 M $H_3BO_3 - 0.04$ M NaOH, 0.16 M NaCl (borate-saline), again with pH 4.0 0.1 M sodium acetic acid, and a third time with borate-saline. Sheep anti-IgE (4 ml) was applied to a column (1.2 × 6.5 cm) of Sepharose-IgE, and, after washing with borate-saline, the anti-IgE was eluted with pH 2.9 0.05 M glycine-HCl. The 277 nm absorbance recovered after acid elution constituted 9.5% of the total applied. The purified anti-IgE (867 μg/ml) migrated in the fast gamma region and gave a single band when reacted with a potent rabbit anti-whole sheep serum in immuno-electrophoresis.

Purified anti-IgE (17 μg) was radiolabeled with 2 mCi ^{125}I or ^{131}I by a modification of the technique of Greenwood, Hunter, and Glover (17) as described previously (16). The radiolabeled purified antibody was separated from unreacted radioiodine by gel filtration on Sephadex G-100 columns. Between 96–99% of the radioactivity in the labeled anti-serum was precipitated by 10% phosphotungstic acid. A portion of radiolabeled anti-IgE was reapplied to a small Sepharose-IgE column; 65% of counts bound and were eluted with pH 2.9 0.05 M glycine-HCl.

The RAST procedure was carried out by mixing 0.5 mg solid phase AgE in 0.5 ml buffer, 0.05 ml test serum, and 0.2 ml diluent in 10 × 44-mm tubes (Fisher Scientific Co., Pittsburgh, Pa.). The tubes were capped and were slowly rotated overnight at room temperature, after which they were centrifuged at 3,420 g for 15 min and the supernate aspirated. The solid phase AgE was washed thrice with 1 ml of 0.1 M phosphate buffer (pH 7.4) containing 1% Tween 20. Approximately 1.4 ng of the radio-labeled sheep anti-IgE was added to each tube and the volume adjusted to 0.75 ml. The tubes were rotated overnight and washed again. Radioactive counts associated with the final washed polymer were measured in a gamma spectrometer, and the results were expressed as counts bound divided by counts added, × 100. In all of these studies the same batch of cellulose-AgE polymer was used. In preliminary experiments the specificity of the RAST for IgE antibody was

TABLE II

Effect of IgG Antibody to Ragweed AgE on Binding of IgE Antibody

Serum	IgG added*	IgE antibody
	mg	% of total counts
W. W., undiluted	—	24.4
W. W., 1:10	—	13.7
W. W., 1:100	—	5.0
W. W., undiluted	1.0	22.6
W. W., 1:10	1.0	11.2
W. W., 1:100	1.0	4.5
—	1.0	2.9
Normal serum	—	0.7
Fetal calf serum	—	0.8

* IgG was purified from the serum of patient, D. E., who was receiving high dose immunotherapy and whose serum had the highest quantity of IgG antibody among patients in this group. Serum and an equal volume of 28% sodium sulfate were mixed and the resulting precipitate washed, dissolved in distilled water, and dialyzed. At a concentration of 8 mg/ml, this preparation was analyzed by immunoelectrophoresis with potent antiserum to whole human serum and produced a single band in the gamma region. The preparation was heated for 2 h at 56°C to destroy IgE antigenic determinants, and after this treatment it contained less than 6 ng IgE/ml. Analysis by radioimmunoprecipitation showed that 1 mg of the IgG preparation bound 0.5 μg partially purified AgE. In the experiment above 100 μl of a solution containing 10 mg/ml was added in the first step of the RAST. This quantity of IgG antibody to AgE approximates the levels found in serums with the highest IgG-binding capacities.

determined by incubation of nonradioactive proteins of the five immunoglobulin classes with radio-labeled antibody to IgE during the second step of the RAST. Addition of IgE protein reduced binding by 93%, whereas addition of other immunoglobulins reduced the counts associated with the washed polymer by a maximum of 9%. All four serum samples from the same individual were run in duplicate in the same assay. Serums from two ragweed-sensitive patients, one with a high level of binding and another with an intermediate level, plus serum from a third nonallergic person were included as controls. The coefficient of variation for these controls was 13.3, 6.0, and 16.9%, respectively. The coefficient of variation for replicate samples in the same assay was 2.9%.

The possibility that IgG antibody in the serums of treated patients (*vide infra*) might interfere with the measurement of IgE antibodies was also tested. IgG antibodies, by competing for antigenic determinants on the cellulose-AgE polymers, might prevent or reduce binding of IgE antibodies and thus produce spuriously low values. The results of an experiment to determine the effect of IgG antibody are shown in Table II. Only a slight reduction in binding of IgE antibody was seen, even when IgG antibody was added in an amount approximately equal to the binding capacity of the highest serum.

Measurement of IgG antibodies. IgG-blocking antibodies were measured in preseasonal and peak seasonal serums by radioimmunoprecipitation (3, 18, 19). Partially purified AgE (4.6 mg) was radiolabeled with 2 mCi ^{131}I by the chloramine-T method (17), and free ^{131}I was removed by dialysis against pH 7.3 0.01 M K$_2$HPO$_4$-KH$_2$PO$_4$ 0.13 M NaCl phosphate-buffered saline (PBS) until the radioactive counts in the dialysate were negligible. About 98% of the total radioactivity in the retentate was precipitable by 10% phosphotungstic acid. Radiolabeled AgE was added to 0.01 ml patient serum and diluted with 0.2 ml of 1% BSA in pH 7.4 0.1 M phosphate buffer in 10 × 75-mm glass tubes. The tubes were incubated for 1 h at 37°C, then overnight at 4°C, and 0.25 ml of goat antihuman IgG was added. After a second incubation for 1 h at 37°C, and overnight at 4°C, the resulting precipitates were centrifuged at 3,420 g for 30 min at 4°C and washed thrice with 3 ml aliquots of pH 7.4 0.1 M phosphate buffer. The radioactivity associated with the washed precipitates from test serum was measured in a gamma spectrometer. As shown in Fig. 1a, addition of increasing amounts of radiolabeled antigen resulted in the formation of plateau or maximum binding regions, and as shown in Fig. 1b, the quantity of AgE bound was linearly related to the quantity of serum analyzed. In the assay of serum from the study groups 1000 ng of radio-labeled AgE was added to 0.01 ml of patient serum, and results were recorded as nanograms of AgE bound per 0.01 ml of patient serum. As controls in these determinations a serum from a treated patient with a high level of binding (100 ng/0.01 ml) and another from a normal subject with low binding (1 ng/0.01 ml) were analyzed. The coefficients of variation for these serums were 8.1 and 20.8, respectively.

Statistical evaluation of data. Numerical results were compared using nonparametric statistical methods (20), such as the sign test, the signed rank test, the rank sum test, and the rank correlation test. For samples with *n* greater than 10 we applied the normal approximation of the rank sum test. For comparison of rises in IgE antibody, data were tested by analysis of covariance.

RESULTS

The results of measurement of IgE antibodies to partially purified ragweed AgE are shown in Figs. 2 and 3. IgE antibody levels were elevated in all allergic groups during all four sampling periods as compared with nonallergic controls, and there was essentially no overlap between the allergic and nonallergic patients. As shown in Fig. 2, IgE antibodies rose after the ragweed pollination season in the allergic group not treated with immunotherapy. Also shown for comparison in this figure are the values for the nonallergic controls, which did not change appreciably during the study period. The IgE antibody levels began to rise by late August and reached maximal values in October, with gradual declines by January. The January values were greater than the July values in 13 of the 14 patients in the untreated group, whereas among patients beginning hyposensitization, only 6 of 11 had antibody levels in January greater than those in April. Among the group receiving high dose immunotherapy only 12 of 26 had higher levels in January compared with the preceding July. The initial anti-

body levels for the individuals in all groups are shown in Fig. 3. Although the levels in the high dose group tended to be lower than those in the untreated group, this difference was not significant (Rank sum test; $z = + 1.52, P = 0.14$).

In Fig. 4 the changes in IgE antibody from the initial value to the October value ($\Delta 1$–3) are shown. In all of the allergic groups the mean IgE antibody level increased during this period. In the untreated and low dose groups the change was significant ($P < 0.01$ by the sign test), and this was also the case with the high dose group ($P < 0.01$ by the signed rank test). These results indicate that immunotherapy did not completely suppress the rises in IgE antibody after the ragweed pollination season, even in the most intensively treated patients. Inspection of the individual rises in IgE antibody suggested that patients with high preseasonal levels had rises greater than patients with low preseasonal levels. Analysis of the relationship between the initial IgE antibody levels and the increases from July to October in the untreated group indicated that these were positively correlated, as shown in Fig. 5. The relationship between the initial IgE antibody levels and the seasonal rises was even more striking when the high dose-treated group was analyzed ($r_s = + 0.65, P < 0.001$).

Comparison of the maximal rises in IgE antibodies from the preseasonal to the October levels ($\Delta 1$–3) indicated that the increase in IgE antibodies was less in the high dose-treated group than in the untreated group

FIGURE 2 Seasonal changes in IgE antibodies to AgE in untreated and control groups.

(Rank sum test, $z = + 2.57, P = 0.01$). However, when patients in these two groups were matched on the basis of their initial IgE antibody levels, the difference was not significant (Rank sum test, $n = 12, P < 0.16$). Moreover, comparison of rises in the allergic untreated group and the high dose-treated group by analysis of covariance showed no statistically significant differences between the groups. Thus, we could not demonstrate even a reduction in the magnitude of the rises in IgE antibody between the untreated and high dose-treated patients.

The levels of IgG antibody to ragweed AgE before and at the peak of the ragweed pollination season are shown in Fig. 6. The nonallergic and allergic untreated groups had low levels of AgE binding, and neither group showed any rise in binding during the pollination season. The low dose group had binding levels comparable with both the nonallergic and untreated allergic groups before beginning hyposensitization in April. By August the IgG antibody level of the low dose group had risen significantly ($P < 0.01$). The high dose-treated group showed initial IgG antibody levels in July which were higher than those of the other three groups ($P < 0.001$), and even in August the IgG levels of the high dose group were greater ($P < 0.01$) than those of the low dose-treated group.
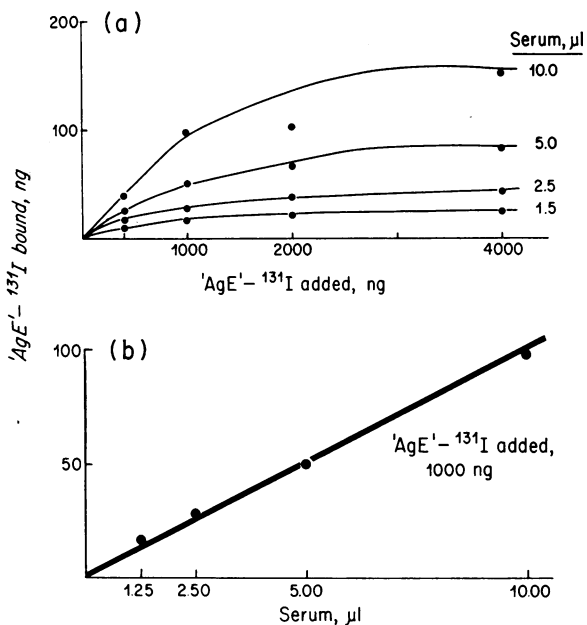

FIGURE 1 Measurement of IgG antibody to AgE. (a) Determination of maximal binding values. (b) Relationship between maximal binding levels and quantity of serum.
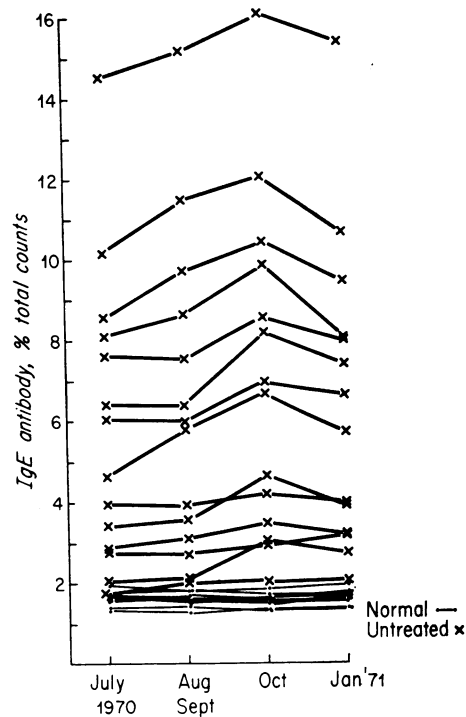
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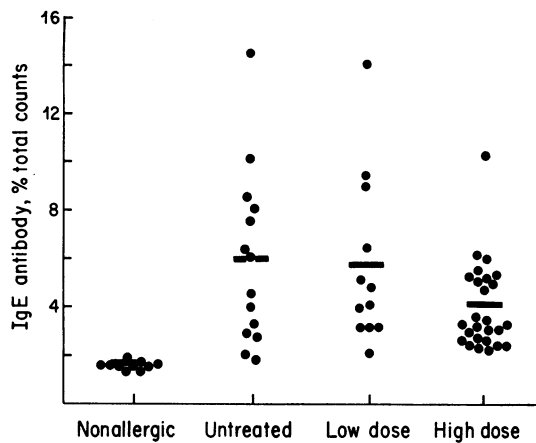
FIGURE 3 Preseasonal IgE antibody levels in the study groups. Mean values are indicated by the bars.

Because IgG antibodies might influence the quantity of IgE antibody being produced, we compared the levels of IgG and IgE antibodies in the high dose group. As shown in Fig. 7 the antibody levels in these classes were positively related in serum obtained before the ragweed pollination season and also during the height of the pollination season. Furthermore, in the high dose group there was a tendency for the rises in IgE to parallel the level of IgG antibodies ($r_s = +0.36$; $0.05 < P < 0.1$). In the low dose-treated group IgE and IgG antibody levels in August–September samples also tended to be related ($r_s = +0.56$; $0.05 < P < 0.1$). IgE and IgG antibody levels were not related in the allergic untreated group at any time.
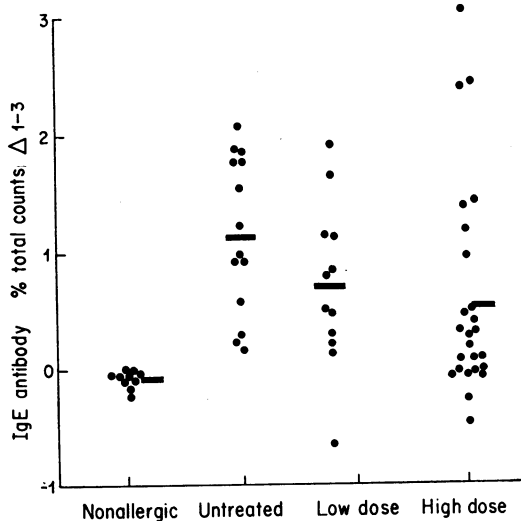

FIGURE 4 Changes in IgE antibody levels from preseasonal values to those in October 1970 ($\Delta 1$–3). Mean values are indicated by the bars.

In this study we have analyzed changes in IgE antibodies to ragweed antigens following the pollination season and the relationship of these changes to IgG antibodies. In normal subjects neither IgE antibody nor IgE protein changed appreciably during the study period (21). IgE antibodies to AgE increased in the allergic subjects during the ragweed pollination season, a finding in keeping with prior measurements of IgE antibody to ragweed AgE by passive leukocyte sensitization (4, 6, 7). Also, Berg and Johansson have found seasonal increases in IgE antibodies to timothy and birch pollen (22). In the present study the maximal changes in IgE antibodies to AgE were usually found in October, and by January the levels had declined. The decrease in IgE antibody occurred in all of the allergic patients, even those receiving monthly injections of pollen extract. In a study of untreated patients in 1971 we found that no change in IgE antibody occurred from July 15 to August 20, but by early September 50% of the levels had increased significantly.[3] By mid-September the IgE antibody level had increased in almost all patients. In five of seven patients a further increase, usually slight, was seen between mid-September and mid-October. Thus, the level of IgE antibody likely reaches a maximum in October, although additional studies are needed to define this with precision.

Analysis of the changes in serum IgE antibodies after the ragweed pollination season revealed that the magnitudes of the rises were related to the preseasonal IgE antibody levels. This relationship was seen in the untreated patients with hay fever as shown in Fig. 5 and was even more strikingly seen in the treated patients. Therefore, to compare IgE antibody responses in treated and untreated patients it was necessary to match them on the basis of preseasonal IgE antibody levels. When patients in the untreated and high dose-treated groups were so matched, no difference was found in the magnitude of the rises of IgE antibody. Furthermore, comparison of rises between the groups by analysis of covariance failed to show any difference between them. Thus, we were not able to confirm earlier studies showing that immunotherapy results in a suppresion of IgE antibody synthesis (6, 7, 23). The difference in our results and those in earlier studies may be due to differences in the methods used to measure IgE antibody activity, or possibly to differences in the study groups; most of the subjects studied earlier were children (6, 7).

Because IgG antibody can reduce production of IgE antibody in experimental animals (24, 25) and because a similar role for IgG in treated patients has been sug-

[3] Gleich, G. J., and J. W. Yunginger. Manuscript in preparation.

gested (6), we compared the levels of IgG and IgE antibodies to AgE in the high dose-treated group. Were IgG to suppress IgE antibody production in these patients, one would expect to find an inverse relationship between the levels of these antibodies. Surprisingly, we found a strong positive correlation between the levels of IgG and IgE antibodies in the high dose group both in the July and in peak-season serum samples. Earlier studies had demonstrated a gradual waning of the level of IgE antibody to ragweed antigen as measured in vivo by passive sensitization (26, 27). Although blocking antibody (IgG) was measured in these earlier studies, the authors did not correlate blocking (IgG) and skin-sensitizing (IgE) antibodies. However, Sherman, Stull, and Cooke in 1940 found that serums from patients treated for 10 or more yr had low levels of blocking (IgG) antibody, and these serums also transferred skin-sensitizing activity (IgE) poorly (28). Moreover there was a rough positive cor-
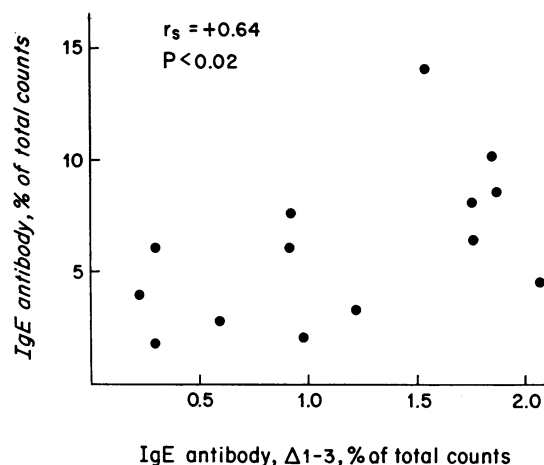

FIGURE 5 Relationship between changes in IgE antibody levels (Δ1-3) and preseasonal IgE antibody values in the untreated allergic group.

relation between the blocking and skin-sensitizing antibody activities. Their results from in vivo studies using less precise techniques than those employed here nonetheless are consistent with our findings of a relationship between IgE and IgG antibodies. That patients with low levels of IgE antibody also tended to have low levels of IgG antibody suggests that their overall humoral immunological responsiveness to ragweed antigens in both IgE and IgG classes was either low to begin with or declined during immunotherapy. Whether patients treated with pollen injections over a period of years lose humoral reactivity in both IgE and IgG classes cannot be determined from our results. It would appear that a longitudinal study of immunotherapy is necessary to determine whether some patients develop partial im-
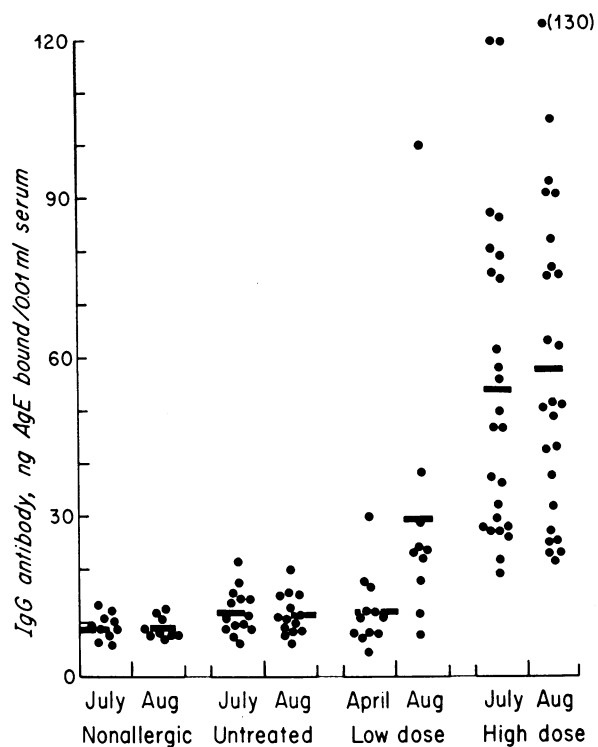

FIGURE 6 Changes in IgG antibodies to AgE in the study groups. Mean values are indicated by the bars.

munological tolerance to ragweed antigens in the IgG and IgE classes.

We have expressed RAST results as the ratio of counts bound divided by total counts added. These ratios, expressed as percentages, are related to the quantity of IgE antibodies in the serum, but they are not
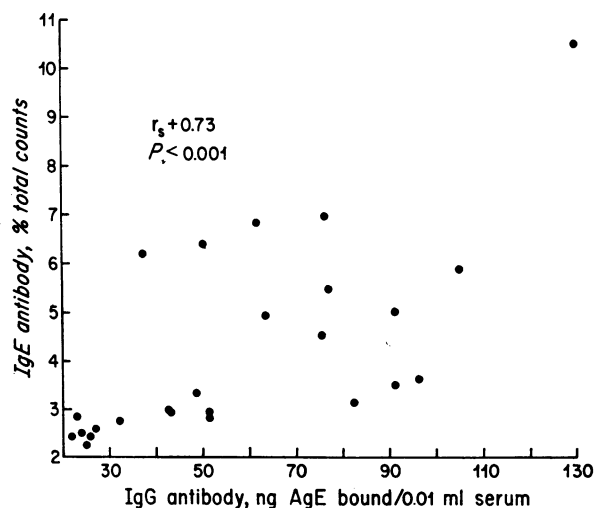

FIGURE 7 Relationship between preseasonal IgG and IgE antibodies to AgE in high dose-treated group.

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linearly proportional to the amount of serum added. When the RAST result in percent of total counts bound is plotted against the quantity of serum tested on log-log graph paper, the resultant curves approximate straight lines. Thus the response of a given patient could be expressed in terms of a reference serum, as is done by Johansson, Bennich, and Berg (11). Because our results have been analyzed by nonparametric statistical methods, expression of the results in terms of percent of total counts bound should not influence the comparisons between the various groups.

Measurement of IgE antibodies to partially purified ragweed AgE by RAST clearly distinguished the allergic serums from those of normal controls. Only once in 248 determinations was a serum misclassified, and after exposure to ragweed pollen this patient's IgE antibody level increased to a value greater than any of the normal subjects. Berg and Johansson have also noted that samples obtained long after exposure to antigen may not contain IgE antibody detectable by RAST (22). Clearly, if RAST is used as a diagnostic test (5), serum samples should be obtained shortly after exposure to antigen, when levels of IgE antibody should be high. In addition to its relative technical simplicity, RAST would appear to be more sensitive than passive leukocyte sensitization for detection of IgE antibodies (6), in that IgE antibody can be measured by the latter method in the serum of only half of adult patients with ragweed hay fever. Finally, the solid-phase ragweed antigen was remarkably stable, and even after storage for 1 yr at 4°C in the RAST buffer, its reactivity with a standard serum was essentially unchanged.[4]

ACKNOWLEDGMENTS

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