

Measurement of the absolute levels of IgE antibodies in patients with ragweed hay fever

Effect of immunotherapy on seasonal changes and relationship to IgG antibodies

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The effect of immunotherapy with aqueous ragweed pollen extract on changes in IgE antibody was analyzed in 40 untreated patients with ragweed hay fever and compared to changes in 63 treated patients. Treated patients received cumulative doses prior to and during treatment ranging from 1.4×10^5 to 4.8×10^6 protein nitrogen units (PNU). IgG antibody to ragweed antigen E (AgE) was measured by radioimmunoprecipitation, while IgE antibody to allergens in crude ragweed extract was measured by the radioallergosorbent test (RAST). The RAST procedure was calibrated using a serum whose content of IgE antibody in nanograms per milliliter had been determined by immunoabsorption, and with this method the absolute quantities of IgE antibodies to ragweed allergens could be measured. Control experiments indicated that the IgG antibodies in the sera of the treated patients did not interfere with the measurements of IgE antibodies. The levels of IgE antibody in serum varied from less than 5 ng/ml to approximately 3,000 ng/ml. IgE antibodies decreased from October, 1973, to July, 1974, and rose sharply from July to October, 1974, following the pollination season. In both untreated and treated patient groups the magnitudes of the decreases were related ($p < 0.001$) to the levels of IgE antibody in October, and the magnitudes of the rises were related to the levels of IgE antibody in July. In the treated patients with IgE antibody less than 71 ng/ml in October, the rate of decrease (October to July) was less than in the untreated patients. Comparison of the rises in IgE antibody from July to October revealed that these were partially suppressed in the treated group ($p < 0.001$), and this effect was most marked in patients with July levels less than 36 ng/ml. Levels of IgG antibody to AgE in the treated patients were approximately 70-fold greater than in untreated patients and showed little change over the study period. The levels of IgG and IgE antibody in the treated patients were positively correlated ($p < 0.001$) at all time periods, and patients with high levels of IgG antibody also had greater declines and rises in IgE antibodies. The results indicate that immunotherapy is associated with (1) a reduction in the seasonal decrease in IgE antibody from October to July in patients with the low levels of IgE antibody, (2) a partial suppression of the rises in IgE antibody from July to October, and (3) that both these effects are related to the level of IgE antibody before the change. In many treated patients both IgE and IgG antibodies were low in spite of parenteral administration of ragweed extract. This result is consistent with the view that overall humoral immunologic reactivity to ragweed antigens is reduced in these patients.

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Supported in part by a grant and a contract from the National Institute of Allergy and Infectious Diseases, AI 11483 and AI 42546, and by the Mayo Foundation.

Received for publication April 15, 1977.

Accepted for publication June 27, 1977.

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Since the early part of the century patients with hay fever have been treated with injections of the incriminated allergens, and in the past few years many studies have examined the changes produced in patients following this treatment. These double-blind investigations have clearly demonstrated that treated patients with ragweed hay fever have less severe symptoms than untreated patients.¹⁻³ The mecha-

TABLE I. Patients with ragweed hay fever

	No.	Sex		Age (yr)		Duration of disease (yr)	
		M	F	$\bar{X} \pm \text{SD}^*$	Range	$\bar{X} \pm \text{SD}$	Range
Untreated	40	23	17	35.7 ± 13.3	15-67	19.9 ± 11.0	3-41
Treated	63	29	34	29.8 ± 15.2	9-74	14.8 ± 10.3	2-46

*Mean $\pm$ one standard deviation.

nisms responsible for the beneficial effects of treatment are likely several in number and include reductions in the ability of peripheral blood leukocytes to release histamine on challenge with ragweed allergen,⁴⁻⁷ increases in IgG antibody, and reductions in the magnitude of the seasonal rises of IgE antibodies.⁸⁻¹⁰

In this study we have established a calibrated radioallergosorbent test (RAST) to measure the absolute levels of IgE antibodies to ragweed antigens, and we have compared the seasonal changes in patients treated with aqueous extracts of ragweed pollen to those in untreated patients. We have also measured the quantity of IgG antibodies to ragweed antigen E (AgE) in order to determine whether the levels of IgG antibodies are related to the changes in IgE antibodies. Our results indicate that immunotherapy significantly alters the seasonal rises and falls of IgE antibodies, and they show that there is a positive correlation between the levels of IgG and IgE antibodies in treated patients.¹¹

MATERIALS AND METHODS

Patient groups

One hundred three patients who had ragweed hay fever, 40 untreated and 63 treated, were selected for study. The characteristics of the patients are shown in Table I. These patients all had typical histories of hay fever symptoms during the months of August and September, and all had positive skin tests to ragweed pollen extract. All of the untreated patients had elevated levels of IgE antibodies to ragweed allergens as measured by the RAST, and they were selected from a larger group of untreated hay fever subjects so that the levels of IgE in their sera spanned the range of positivity which we have encountered in prior studies of ragweed hay fever.¹¹⁻¹⁶ Initially bloods were obtained from 140 treated patients, but because many of these patients failed to keep appointments for venipunctures, the final number of treated patients was 63. These patients were receiving monthly or bimonthly injections of aqueous mixed ragweed pollen extract and had received these injections for 6.4 ± 6.6 years ($\bar{X} \pm 1 \text{ SD}$) for an average cumulative dose over the duration of therapy of $1.3 \times 10^6 \pm 7.9 \times 10^5$ and a range from 1.4×10^5 to 4.8×10^6 protein nitrogen units (PNU).¹⁷ At the time of the study most patients were receiving between 15,000 and

20,000 PNU per injection and they received an average of $2.9 \times 10^5 \pm 1.2 \times 10^5$ PNU containing an average of 41 μg AgE during the 13-month study period. Serum samples were obtained every three months beginning in July, 1973, for the untreated group and in October, 1973, for the treated group. The sera were stored at -20°C .

Ragweed pollen extract

Mixed ragweed extract (40,000 PNU per milliliter) containing equal portions of giant and short ragweed was obtained from Center Laboratories, Port Washington, N. Y. (Lot No. 315901L). The extract had an AgE content of 5.6 $\mu\text{g}/\text{ml}$ by radioimmunoassay.¹⁸ Analysis of this extract by RAST inhibition showed that it yielded an inhibition response (slope of the inhibition curve)¹⁹ not statistically different from the United States Reference Standard kindly provided by Dr. Harold Baer of the Food and Drug Administration, Bureau of Biologics.

Measurement of IgE antibody to ragweed allergens by the radioallergosorbent test

IgE antibodies to ragweed allergens were measured by the RAST as described previously.^{11, 20} A short ragweed extract was prepared for coupling to microcrystalline cellulose by exposure of 157 gm of undefatted pollen (Greer Laboratories, Lenoir, N. C., Lot No. 18-56-72) to 1,050 ml of distilled water for 48 hr. Ten drops of toluene were added as a preservative. The pH was initially adjusted to 7.0 with 3 M NH_4OH and additional NH_4OH was added as needed (total of 50 ml). The suspension was filtered and the extracts centrifuged for 30 min at 5,000 rpm and for 1 hr at 45,000 rpm. The ragweed extract separated into three layers and the clear, middle layer, 725 ml, was recovered. After concentration to 140 ml using a UM-2 membrane (Amicon Corp., Lexington, Mass.), the extract was centrifuged at 15,000 rpm for 30 min. The resulting clear solution was fractionated on a Sephadex G-25 column, $4 \times 86 \text{ cm}$ (Pharmacia Fine Chemicals, Piscataway, N. J.), and the fractions eluting from the void volume (blue dextran) to the elution volume of bacitracin, 1411 d, were pooled, concentrated, dialyzed against 0.05 M NH_4HCO_3 , and finally lyophilized (yield of 7.7 gm of dry material). This extract contained AgE, AgK, Ra3, Ra4, and Ra5 activity as measured by RAST inhibition.*

*Adolphson, C., Goodfriend, L., and Gleich, G. J.: Unpublished observations.

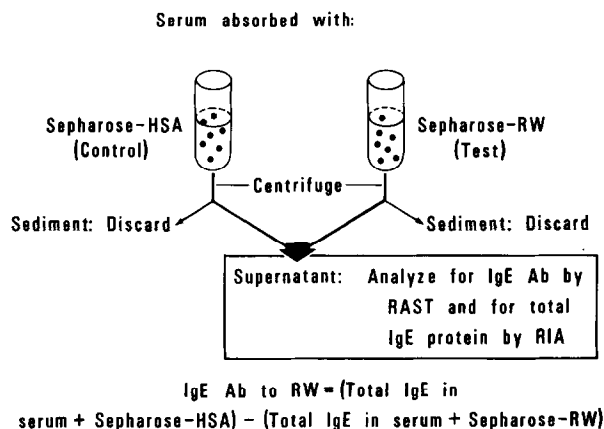


FIG. 1. Measurement of IgE antibody to ragweed by immunoabsorption.

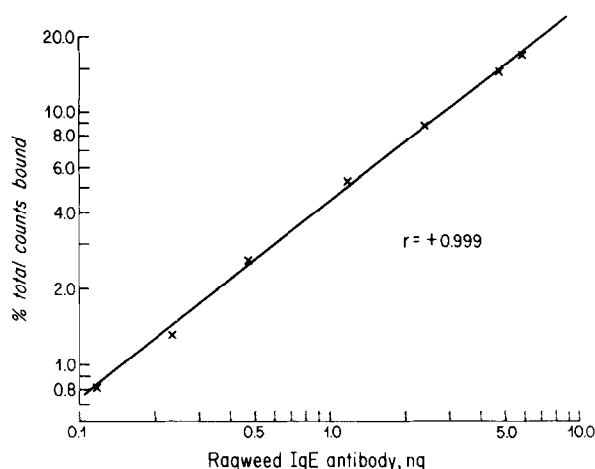


FIG. 2. Calibration of the radioallergosorbent test in terms of mass units of IgE antibody to ragweed allergens. Normal serum, 50 μ l, yielded between 0.1% and 0.3% counts bound. Beyond approximately 18% total counts bound the dose-response curve became nonlinear.

Solid phase ragweed antigen was prepared by activation of microcrystalline cellulose (Avicel, Brinkmann Instruments, Inc., Westbury, N. Y.) and reaction with ragweed extract essentially as described previously.^{20, 21} Briefly, microcrystalline cellulose, 3.0 gm, was washed once with 30 ml of 0.1 M HCl, thrice with distilled water, and reacted at pH 11 with cyanogen bromide, 6 gm dissolved in 6 ml of N, N-dimethylformamide. After washing, the cellulose was exposed to 750 mg of ragweed extract in 30 ml 0.2 M H_3BO_3 -0.04 M NaOH-0.16 M NaCl, pH 8.0, for 4 days, washed again, and suspended in 3 L of RAST BSA diluent (0.1 M KH_2PO_4 - K_2HPO_4 , pH 7.5, containing 0.1% NaN_3 , 1.0% Tween-20, and 0.2% bovine serum albumin).

In the actual performance of the RAST, 500 μ l of cellulose-ragweed suspension was added to 10 $\times$ 60 mm plastic tubes (Luckham Ltd., Burgess Hill, Sussex, England), and after addition of the test serum the volume was

brought to 750 μ l with RAST bovine serum albumin (BSA) diluent. The tubes were rotated overnight at room temperature and washed twice with RAST wash solution (0.1 M KH_2PO_4 - K_2HPO_4 , pH 7.5, 1% Tween-20). In the second step of the reaction, 650 μ l of RAST fetal calf serum (FCS) diluent (same composition as RAST BSA diluent except that FCS 4% is substituted for BSA) and 100 μ l of ^{131}I affinity chromatography purified rabbit anti-IgE containing 20 ng antibody protein were added to the cellulose-ragweed.²⁰ The tubes were rotated overnight and washed twice with the RAST wash solution. Radioactivity was determined in a Nuclear-Chicago automatic gamma scintillation counter (Searle Analytic Inc., Des Plaines, Ill.) and the results were expressed as counts bound divided by counts added $\times$ 100.

RAST results as ordinarily obtained yield relative estimates of the quantity of IgE antibody in sera. In this study we determined whether the RAST could be standardized so that the quantities of IgE antibody to ragweed allergens could be measured in mass units. A pool of five sera from untreated patients with relatively high levels of IgE antibody was prepared and the IgE antibodies removed by immunoabsorption with Sepharose 2B ragweed (Pharmacia Fine Chemicals, Piscataway, N. J.), as shown in Fig. 1. The content of total IgE protein in the supernatants from these absorptions was determined by the double-antibody radioimmunoassay,²² and the difference represented the quantity of IgE antibody to ragweed allergens. The serum contained 2,370 ng/ml IgE antibody to ragweed antigens and was used to construct a dose-response relating nanograms IgE antibody to percent counts bound (Fig. 2). As a control, a sample of the same pool was treated with human serum albumin (HSA) coupled to Sepharose 2B.¹⁵ In a prior study we had shown that the use of Sepharose-HSA is a valid control for nonspecific adsorption of IgE protein in the immunoabsorption procedure.¹⁵

Measurement of IgG antibodies to ragweed antigen E

The levels of IgG antibody to ragweed AgE were determined by radioimmunoprecipitation essentially as described previously.¹¹ Purified AgE in 50% glycerol was provided by the Research Resources Branch of the National Institute of Allergy and Infectious Diseases through the courtesy of Dr. John Nutter. In initial experiments we found that AgE could not be labeled with ^{131}I by the chloramine T procedure²³ unless glycerol was removed by gel filtration on Sephadex G-25. After radioiodination, 97% of the radioactivity was precipitable by 10% phosphotungstic acid. In the performance of the radioimmunoprecipitation procedure, 0.1 ml of a 1:20 dilution of the patient's serum in 0.1 M pH 7.5 K_2HPO_4 - KH_2PO_4 containing 1% BSA (phosphate-albumin) was mixed with 1.0 μ g of ^{131}I -AgE in phosphate-albumin. After incubation at 37° C for 1 hr and 4° C overnight, the IgG protein was precipitated by addition of an excess of burro antiserum specific for human IgG, after which the tubes were again incubated 1 hr at 37° C and overnight at 4° C. In each experiment a serum with a high content of IgG

antibody, 1,000 arbitrary U/ml, was analyzed and a standard curve constructed by plotting the percent of AgE bound and microliters serum tested. The resulting curves were linear when plotted on log-log paper and the results were expressed in units of IgG antibody. The nonspecific binding of AgE to glass, $0.65 \pm 0.39\%$ of counts added, was subtracted from the serum values. The coefficient of variation of a positive serum ($\bar{X} = 413$ U/ml) tested in every assay was 2.4%. One unit of IgG antibody corresponds to 0.21 ng AgE bound per milliliter of serum. With this procedure normal serum bound an average of 3.2 U per milliliter.

Statistical evaluation of data

Results were evaluated by regression analysis, analysis of covariance, and the paired *t* test²⁴ using a programmable Hewlett-Packard (Loveland, Colo.) 9810 A calculator.

RESULTS

Measurement of the absolute quantities of IgE antibody to ragweed by the RAST

We have previously described an immunoabsorption procedure for measurement of the quantities of IgE protein to ragweed allergens.¹⁵ Although this procedure yields unambiguous measurements of the quantity of IgE antibody to pollen allergens, it is time-consuming and not suited for analysis of large numbers of sera. Therefore, we determined whether the quantities of IgE antibodies could be measured by the calibrated RAST procedure. A typical standard curve obtained by analysis of varying quantities of IgE antibodies is shown in Fig. 2, and between 0.8% and approximately 18% counts bound there is a linear relationship between log percent counts bound and log ng IgE antibody. For measurement of the IgE antibody content of an unknown sample, the volume of test serum was adjusted so that its response in the RAST, in terms of percent of total counts bound, fell within the linear range of the calibrated standard. The quantity of IgE antibody in the test serum was determined from the regression line. The coefficient of variation for this assay was 7.2%. The values of IgE antibody obtained by this procedure were compared to those determined by immunoabsorption (Fig. 1) in 24 sera from untreated patients, and the results are shown in Fig. 3. The correlation coefficient was +0.97 ($p < 0.001$), encouraging belief that the levels of IgE antibody to ragweed allergens in mass units could be measured by the calibrated RAST. However, because we desired to measure IgE antibody in sera from individuals receiving immunotherapy and because these sera contain substantial quantities of IgG antibodies, we determined whether the IgG antibodies interfered with the RAST

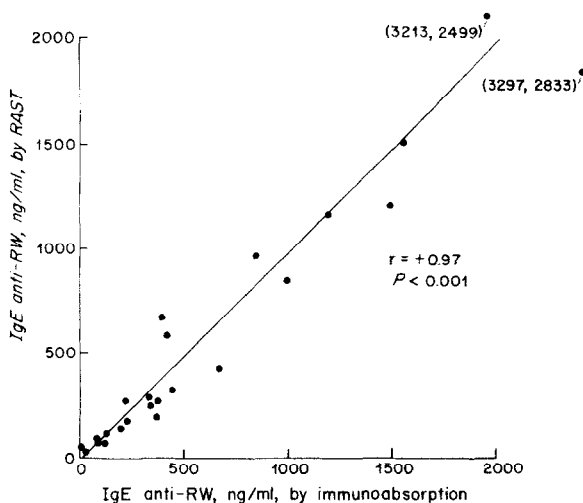


FIG. 3. Comparison of the quantities of IgE antibodies in 24 sera from ragweed-allergic subjects measured by immunoabsorption and by the calibrated RAST.

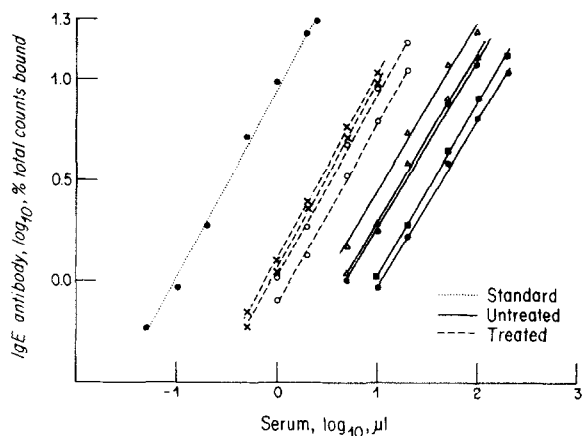


FIG. 4. Comparison of dose-response curves of sera from treated and untreated subjects in the radioallergosorbent test. The standard here is the pool of sera from ragweed-sensitive subjects containing 2,370 ng/ml IgE antibody. Although there is a significant difference in the slopes of the regression lines [$F = 3.02$; $DF(9, 32)$ $p < 0.025$], it is evident on inspection that the slopes are very similar.

by competing with IgE antibodies for the solid-phase allergens. To answer this question, we first examined the slopes of the dose-response curves in the RAST relating the percent of counts bound and the dose of serum tested. We compared sera from patients with and without IgG antibodies and we assumed that the slopes of the dose-response curves must be similar to the slope of the IgE antibody standard if the measurements of IgE antibodies are valid. Inspection of the results shown in Fig. 4 reveals that the slopes of the dose-response curves of both treated and untreated patients are very similar to the IgE antibody standard.

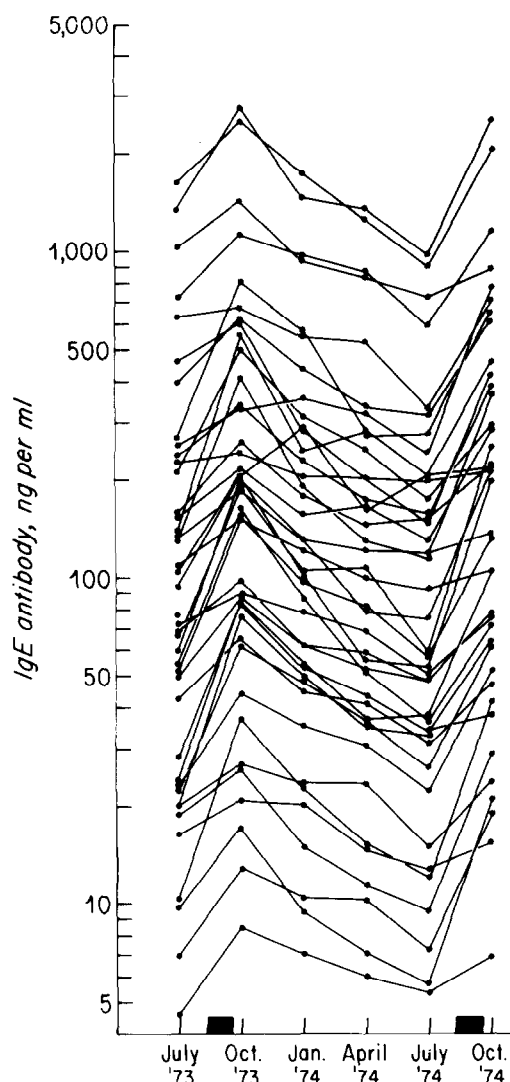


FIG. 5. Levels and changes of IgE antibodies to ragweed allergens in 40 untreated allergic patients. The ragweed pollination season is indicated by the black bar on the abscissa.

Statistical comparison did not show a difference between the slopes of the standard and the treated patients, while a borderline difference ($p < 0.05$) was found between the standard and the untreated patients.* Second, we analyzed 11 sera from patients

*In additional experiments (not shown) we determined the dose-response curves using 59 sera from untreated and treated patients containing a range of levels of IgE antibody. In order to determine whether sera containing the low levels of IgE antibody yielded dose-response curves parallel to the standard, we were forced to compare these responses in the range of 0.3% to 2.0% counts bound. Statistical analysis revealed significant differences in the slopes of the dose-response curves, but even with sera containing very low quantities of IgE antibodies the slopes did not differ markedly from that of the IgE antibody standard.

TABLE II. Relationships between measurements of IgE antibody by immunoabsorption and RAST in patients with elevated levels of IgG antibody to ragweed AgE*

	IgE antibody (ng/ml)		IgG antibody (U/ml)
	Immuno- absorption	RAST	
1	963	989	2,438
2	544	534	764
3	249	369	614
4	213	306	1,116
5	102	200	487
6	39	153	72
7	93	135	1,553
8	72	98	739
9	36	67	306
10	58	47	480
11	20	26	450

*The correlation coefficient for the two measurements of IgE was $r = +0.99$ ($p < 0.0005$).

receiving immunotherapy by immunoabsorption and compared the levels of IgE antibody by this method with those determined by the calibrated RAST procedure. The results are shown in Table II and reveal a close agreement between the levels determined by the two procedures. These findings indicate that IgG antibodies do not significantly interfere with the measurement of IgE antibodies in the sera of the treated patients using the calibrated RAST procedure.

Levels of IgE antibodies in patients

The levels of IgE antibody and the changes over a 15-mo period in the 40 untreated patients with ragweed hay fever were measured by the calibrated RAST. The results shown in Fig. 5 reveal several points of interest. First, the levels of IgE antibodies in July, 1973, range from about 5 to approximately 2,000 ng per ml, or about a 400-fold difference between the highest and lowest patients. Second, the levels of IgE antibody rose abruptly from July to October, 1973, following the ragweed pollination season, and gradually decreased from October, 1973, to July, 1974, after which they rose again. Third, the rises in IgE antibody from July to October in both 1973 and 1974 were related to levels of IgE antibody in July before the rise. Similarly, decreases were related to the levels of IgE antibody in October, 1973. Thus patients with low levels of IgE antibody in October tended to have smaller decreases than patients with high levels of IgE antibody. The relationship between

TABLE III. Relationship between changes in IgE antibodies and the levels before the change

Initial levels vs change	Untreated patients (40)		Treated patients (63)	
	r	Significance	r	Significance
October vs decrease, October to July	+0.99	p < 0.001	+0.97	p < 0.001
July vs increase, July to October	+0.85	p < 0.001	+0.73	p < 0.001

TABLE IV. Statistical comparison of changes of IgE antibodies in treated and untreated patients

Changes in IgE antibodies	Patient groups		Statistical test	Significance
	Untreated (No.)	Treated (No.)		
<i>Decreases</i>				
October levels greater than 71 ng/ml	30	30	ACV*	NS†
October levels less than 71 ng/ml	10	10	Paired t‡	p < 0.001
<i>Increases</i>				
July levels greater than 36 ng/ml	28	33	ACV	p < 0.001
July levels less than 36 ng/ml	12	30	ACV	p < 0.001

*Analysis of covariance.

†Not significant.

‡In this case the slopes of regression lines relating the decreases of IgE antibody as a function of the October levels were not parallel and thus ACV could not be applied. See Reference 34 for a detailed explanation of the application of ACV to this type of problem.

the initial levels and the changes in IgE antibodies is shown in Table III and Fig. 6.

The seasonal changes of IgE antibody over a 13-mo period in the 63 treated patients are shown in Fig. 7. In the case of the treated patients we were unable to obtain blood samples for July, 1973, so that we have only the samples for the period October, 1973, to October, 1974. IgE antibodies decreased from October to July and rose from July to October. As shown in Table III, rises and falls in the treated patients were a function of the initial levels in October and July, as with the untreated patients. Analysis of the results in the treated and untreated patients revealed that the decreases of IgE antibody in treated patients with levels less than 71 ng/ml in October, 1973, tended to be relatively slight. In contrast, comparison of the decreases from October to July in the treated and untreated patients with levels greater than 71 ng/ml in October does not reveal any difference. Comparison of the rises in IgE antibody from July to October indicates that there is a clear-cut suppression of the seasonal rise in the treated patients with low levels of IgE antibody, less than 36 ng/ml. In the case of patients with IgE antibody levels above 36 ng/ml, the

difference between the two groups is less striking, but the rises in the treated group are partially suppressed. The statistical evaluation of these results is shown in Table IV.

Comparison of levels of IgE and IgG antibodies

The levels of IgG antibody to AgE for the two allergic groups as well as a group of 21 normal subjects not sensitive to ragweed pollen are shown in Fig. 8. The mean value for the IgG antibody in the allergic untreated group was only slightly higher than that of the 21 normal sera. In contrast, the IgG levels in the treated patients were markedly elevated, ranging from about 80 U/ml to 4,000 U/ml, approximately a 50-fold range, and no overlap was present between the allergic-treated and allergic-untreated groups. The changes of IgG antibody over the one year period were analyzed for the treated and untreated groups, and the levels in both groups remained essentially the same during the year of observation (data not shown). In a previous study of the relationship of IgG and IgE antibodies to AgE, we had found that there was a positive relationship between their concentrations.¹¹

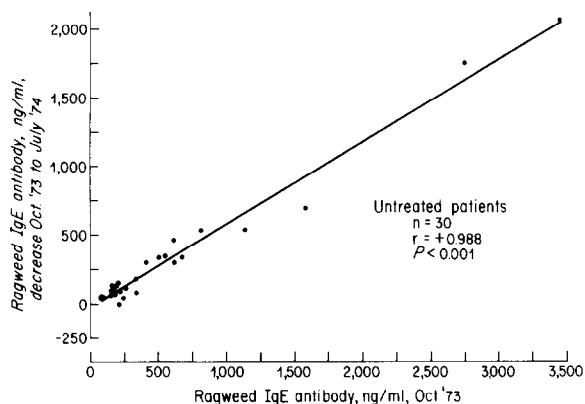


FIG. 6. Relationship of decreases of IgE antibody and the levels of IgE antibody in October. The decreases occurred from October, 1973, to July, 1974, and were compared to the IgE antibody levels in October, 1973, before the decrease. Only patients with levels of IgE antibody greater than 71 ng per milliliter in October, 1973, are shown here.

In this study we again examined this relationship and compared IgE antibody to ragweed allergens and IgG antibody to AgE. The results are shown in Fig. 9. For the 63 treated patients the levels of IgG and IgE antibodies were significantly correlated at all times, and for the October, 1974, results shown in Fig. 9 the correlation coefficient of +0.67 is remarkably close to the value of +0.73 we had found previously.

DISCUSSION

In this report we present results (1) on the development of a procedure for ready measurement of the absolute amounts of IgE antibodies to ragweed allergens, (2) on the seasonal changes of the levels of IgE antibodies in treated and untreated patients with ragweed hay fever, and (3) on the relationship of IgE antibodies to ragweed allergens and IgG antibodies to ragweed AgE. Earlier we had shown that IgE antibodies account for remarkably high percentages of the total IgE protein in the sera of individuals with hay fever due to grass or ragweed pollen allergens using an immunoabsorbent procedure for the measurement of the total IgE antibody in mass units per milliliter.¹⁵ While the immunoabsorption procedure is useful and unambiguously yields these measurements, the procedure is tedious and time-consuming and would be difficult to apply to large numbers of sera. Therefore, we determined whether the RAST could be standardized in terms of mass units using a calibrated serum containing a known quantity of IgE antibody. The results shown in Fig. 2 indicate that log-log plots of the percent of counts bound versus the quantity of IgE antibody in the range of 0.1 and 6 ng yield a linear relationship. Further-

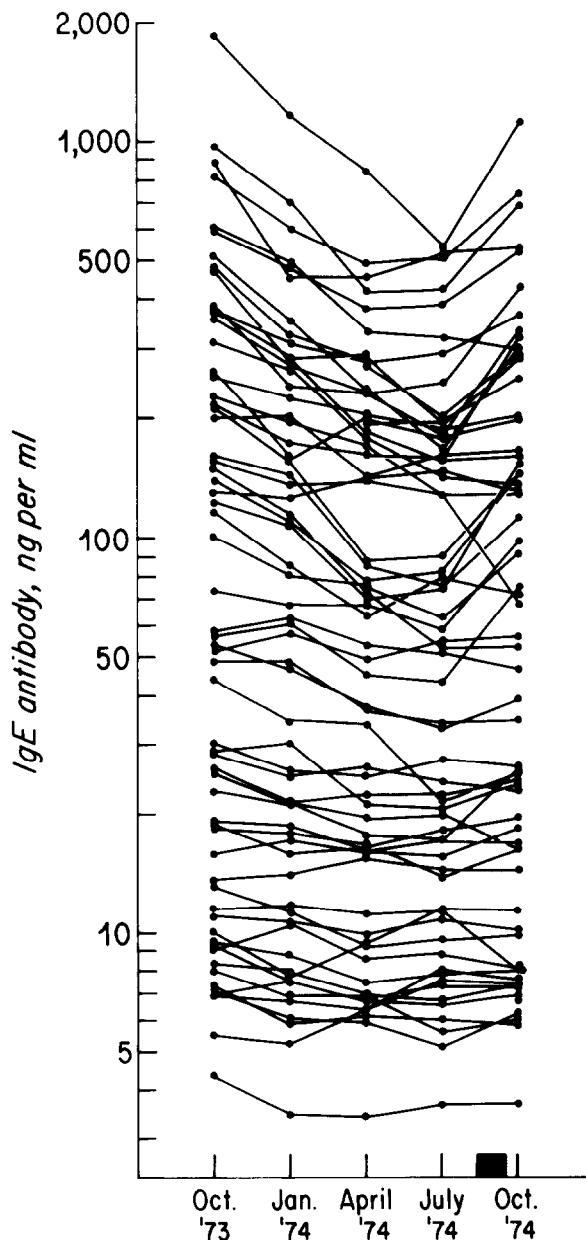


FIG. 7. Levels and changes of IgE antibodies to ragweed allergens in 63 patients treated with injections of ragweed extract. The ragweed pollination season is indicated by the black bar on the abscissa.

more, as shown in Fig. 3, levels of IgE antibody measured by the calibrated RAST and by the immunoabsorption procedure were in good agreement. That this procedure can also be applied to quantitation of IgE antibodies in sera containing IgG antibodies is supported by the results shown in Fig. 4 and Table II. In Fig. 4 the slopes of the dose-response curves relating the percent of counts bound and the dose of sera tested were nearly parallel for sera from untreated and

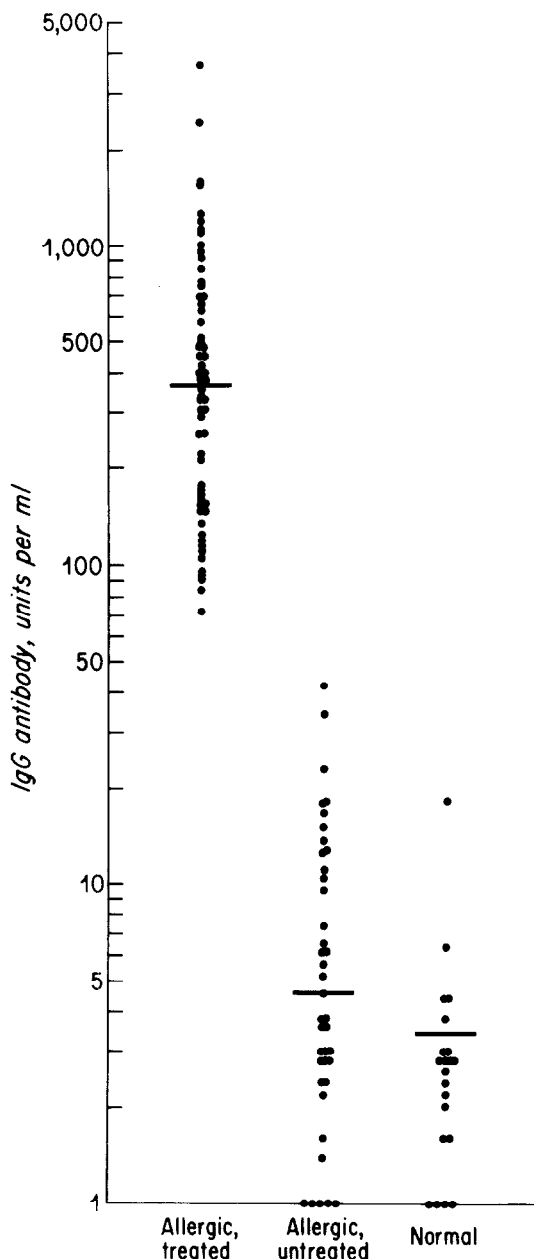


FIG. 8. IgG antibody to ragweed AgE in the study subjects. One unit of IgG antibody corresponds to 0.21 ng AgE bound per milliliter serum. The results shown here were from sera obtained in October, 1974. The geometric mean IgG antibody in each group is indicated by the horizontal line.

treated subjects. If these slopes were markedly different from that of the IgE antibody standard, the assay would not be suitable for determination of the content of IgE antibody. In Table II the levels of IgE antibody in the sera of 11 treated patients measured by the calibrated RAST were compared to the levels of IgE antibody found by immunoabsorption. Inspection of

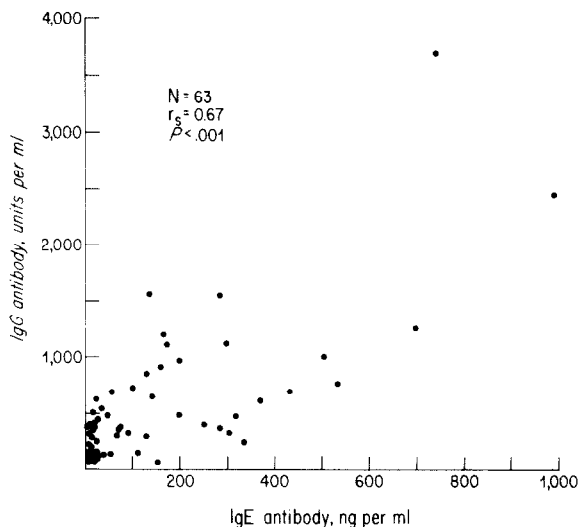


FIG. 9. Relationship between IgE antibody to ragweed allergens and IgG antibody to AgE in the treated patients in October, 1974.

the results in Table II indicates that in most cases the levels of IgE antibody by RAST were somewhat greater than by immunoabsorption. Since our initial presentation of these findings,²⁵ Schellenberg and Adkinson²⁶ have independently shown an excellent agreement ($r = +0.99$) between the levels of IgE antibody to AgE measured by the calibrated RAST procedure and by an elution procedure. For measurement of IgE antibodies to crude ragweed extract by the calibrated RAST, one assumes that all of the relevant antigenic determinants are present and sterically available on the solid-phase allergen. An alternative method for measurement of IgE antibody is the antigen-binding procedure described by Zeiss and his associates.²⁷ This procedure requires radiolabeled purified allergen and appreciable quantities of IgE protein. At the present time all of the procedures available for measurement of IgE antibody to ragweed possess shortcomings. We believe that the use of the calibrated RAST is presently the best compromise in that the procedure is simple, rapid, and agrees well with the results of the more rigorous immunoabsorption experiments.

In the sera of patients with ragweed hay fever, measurement of the levels of IgE antibodies to ragweed allergens revealed about a 600-fold difference. This difference is similar to the 1,000-fold range of skin test sensitivity that we had found using a battery of crude ragweed extracts.¹⁹ The seasonal changes in the levels of IgE antibody shown in Figs. 5 and 6 are in agreement with our prior findings^{11, 13} and those of others^{8, 9} that IgE antibody levels peak in late Sep-

tember or October and decline until the following ragweed pollination season, and they extend the earlier reports by providing the actual quantities of IgE antibody to ragweed in mass units at these intervals. In our previous study we could not demonstrate a significant reduction in the rises of IgE antibodies in the treated group.¹¹ Because rises are a function of the IgE antibody levels before the rise, the results must be analyzed either by matching patients on the basis of their levels of antibody or by using a procedure, such as analysis of covariance, which allows comparison of results in patients with differing initial levels of IgE antibody. In our earlier study we may have failed to find a difference in rises because many of the treated patients had quite low levels of IgE antibody initially; hence only a slight rise would be expected. In this study with a larger number of patients having both high and low levels of IgE antibody, the results show a clear-cut suppression of the rises in the treated patients, and this was especially striking in the patients with low levels of IgE antibody below 36 ng/ml. Among these patients there was virtually no rise in IgE antibodies following the ragweed pollination season. Similarly, there was a partial suppression of the rises of IgE antibody in treated patients, with July levels greater than 36 ng, although here the effect was not nearly so dramatic as in the patients with levels less than 36 ng/ml. Concerning declines of IgE antibody from October to July, we were not able to demonstrate a difference between the treated and untreated groups in patients with IgE antibodies greater than 71 ng/ml in October. In contrast among patients with October levels less than 71 ng, the usual decay seen in the untreated patient was blunted and the IgE value remained essentially the same from October to July. Thus in this subgroup of treated patients the treatment actually seemed to maintain the level of IgE antibody. Our results in treated patients are in general agreement with those of Lichtenstein and co-workers⁹ and Evans and co-workers,¹⁰ who claim that immunotherapy decreases the levels of IgE antibodies to AgE. However, these studies did not investigate the possible inhibition of the binding of IgE antibodies to solid-phase allergens by IgG antibodies. That such inhibition may occur is supported by the reports of Lynch and his associates²⁸ and Vervloet and co-workers.²⁹ The results presented here and earlier¹¹ discourage belief that IgG antibodies interfere in measurement of IgE antibodies to ragweed, at least using the solid-phase allergens and anti-IgE employed in these studies. Finally, a remarkable finding is the ability of the ragweed-sensitive subjects to maintain serum IgE antibody at high levels for long periods of

time. For example, the levels of IgE antibody shown in Figs. 5 and 6 decline very slowly so that even after nine months (October to July) they are about one-half of the October values. This slow decline is difficult to explain in view of the half-life of serum IgE of 5.1 days.³⁰ Clearly IgE antibodies to ragweed continue to be produced over long periods of time, a phenomenon warranting further investigation.

As expected, administration of pollen extract resulted in the production of IgG blocking antibody^{31, 32} to AgE, and the levels of these IgG antibodies were strikingly elevated in the sera of our treated patients. However, in contrast to the results with IgE antibodies there were no clear-cut seasonal changes in the levels of IgG antibodies. Comparison of the levels of IgG and IgE antibodies in October, 1974, revealed a positive correlation ($r_s = 0.67$) ($p < 0.001$) in keeping with our earlier finding.¹¹ This result is at variance with that of Lichtenstein and co-workers⁹ and Evans and co-workers,¹⁰ who found, respectively, no relationship and a significant inverse relationship between the amount of IgG and IgE antibodies. The reasons for the discrepancies among these reports are not clear, but the result of Evans and co-workers¹⁰ is precisely what one would expect if IgG antibodies were interfering by competing with IgE antibodies for the solid-phase ragweed allergens. Although we used a partially purified ragweed extract rather than AgE in the preparation of our solid-phase allergen, our earlier results¹⁹ and more recent studies¹ indicate that the specificity of the RAST using solid-phase ragweed is primarily for AgE. Thus AgE is an excellent inhibitor of the reaction between IgE antibodies with solid-phase ragweed while AgK, Ra3, Ra4, and Ra5 are poor inhibitors. Moreover, the very low background associated with the particular solid-phase ragweed used in this study, less than 0.2% of counts bound, permitted measurements of IgE antibodies and dose-response curves in all of the 63 treated patients.

Previously we had suggested that prolonged immunotherapy might result in a form of partial immunologic tolerance. This suggestion was based on the findings that patients with low levels of IgE antibody tended to have low levels of IgG antibody¹¹ and on the report of Sherman, Stull, and Cooke³³ in 1940 that sera from patients treated for 10 or more years had low levels of blocking (IgG) antibody and transferred skin-sensitizing antibody (IgE) poorly. We believe the results of Evans and associates showing a decrease in lymphocyte responsiveness to ragweed AgE following immunotherapy¹⁰ lend support to this suggestion even though these authors rejected it because of the increased levels of IgG antibody. However,

levels of IgG antibody are distributed over a 50-fold range, and, as shown in Fig. 9, treated patients whose levels of IgG antibody fell into the lower portions of this range also had lower levels of IgE antibody. If immunotherapy causes a progressive reduction in the levels of IgE antibody (because of suppression of seasonal rises), then perhaps IgG antibodies also diminish with continued antigen exposure. Clearly prospective studies of immunotherapy are needed to answer this question.

We thank Dr. Frank Adkinson for suggesting that AgE be labeled after gel filtration to remove the glycerol. We are also grateful to the physicians of the Allergy Division of the Department of Medicine and to the nurses in the Allergy Treatment Center for their cooperation and to Ms. Kristin Leiferman for assistance in obtaining the serum samples from the patients. Finally, we thank Mr. Ken Offord for aid in the statistical analyses of the data.

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