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Quantitative relationships between IgE antibody and blocking antibodies specific for antigen E in patients given immunotherapy with ragweed antigen E

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SUMMARY

Total antibody binding of ragweed antigen E (AgE) and IgE antibody binding of AgE were quantitatively measured in serum from eleven patients given immunotherapy with purified AgE. From these data the contribution of antibody other than IgE, presumed to be mostly IgG, to total AgE binding could be determined. Binding of AgE by antibody other than IgE antibody was considered to be due to blocking antibody. As immunotherapy progressed, IgG antibody binding of AgE and the IgG/IgE binding ratio were serially determined.

IgG antibody binding of AgE increased from a pretreatment mean of 238 ng AgE bound/ml to 3142 ng AgE bound/ml just prior to the first ragweed season and reached a peak of 4286 ng AgE-bound/ml. Although blocking antibody thus increased progressively with treatment it was not correlated significantly with symptom scores.

The IgG/IgE binding ratio increased from a pretreatment mean of 19–290 just prior to the first ragweed season and reached a peak of 1167. This ratio was related significantly to symptom scores reported by patients during the ragweed season subsequent to immunotherapy.

INTRODUCTION

Since the discovery of blocking antibodies in patients given immunotherapy for ragweed pollenosis (Cooke *et al.*, 1935), there has been considerable interest in blocking antibody and its relationships to clinical improvement. Some immunological methods which have been used to quantitate blocking antibody include Prausnitz-Kustner neutralization (Cooke *et al.*, 1935), passive hemagglutination (Garden, Rose & Sehon, 1958), antigen neutralizing capacity measured by quantitative histamine release (Lichtenstein & Osler, 1966), and immunoglobulin binding of radiolabelled ragweed antigen (Pruzansky & Patterson, 1964; Platts-Mills *et al.*, 1976). The methods and their application to the study of immunotherapy have been reviewed (Irons, Pruzansky & Patterson, 1975; Lieberman & Patterson, 1974). More recently, there has been renewed interest in the relationships between IgE antibody and blocking antibody in patients receiving immunotherapy (Lichtenstein *et al.*, 1973; Evans *et al.*, 1972; Yunginger & Gleich, 1973). In these studies the quantitative relationships between IgE antibody and blocking antibody have been obscure, because of the necessity of expressing IgE antibody in units of activity which cannot be directly related to the activity of blocking antibody.

Recently we developed methods to measure IgE antibody directed against antigen E (AgE) in the serum of allergic patients. This assay measures the interaction between the patient's IgE and radiolabelled AgE. Using this method we determine the ng of AgE bound under conditions of antigen excess by IgE antibody (Zeiss *et al.*, 1973).

This report describes the quantification of total immunoglobulin in the serum directed against AgE by the binding of radiolabelled AgE in antigen excess. It is then possible to assess the contribution of

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IgE antibody to total antigen binding and the combined contribution of all other immunoglobulins to total antigen binding. For simplicity we assume that most of the AgE binding by immunoglobulins other than IgE is by IgG (Platts-Mills *et al.*, 1976; Lichtenstein, Holtzman & Burnett, 1968). For the purposes of this paper the binding of AgE by IgG antibody is defined as blocking antibody.

In this manner we are able to determine the ratio of the IgG to IgE binding of AgE as immunization progresses. This ratio should have biological importance since it represents in a quantitative manner the balance between protective antibody and mast-cell sensitizing antibody in the serum.

We found that there is a marked increase in blocking antibodies during treatment and a marked increase in the ratio of IgG to IgE binding of AgE. It was found that this ratio is related to symptom scores reported by patients during their first ragweed season after immunotherapy with AgE.

MATERIALS AND METHODS

Patients. All patients studied suffered from ragweed pollenosis as judged by standard techniques of history, physical examination, and skin tests. All patients showed a 4+ prick test reactivity to crude ragweed extract.

Immunization and blood samples. AgE or polymerized AgE were given according to a standard protocol (Metzger *et al.*, 1976). The characteristics of polymerized AgE have recently been reported (Patterson, Suszko & McIntire, 1973). The dosage increase was similar to that of standard allergy practice (Melam, 1972) and calculated to be equivalent to the AgE that would be given if the patient was treated with whole ragweed extract. The goal was to reach a maintenance dose which was equivalent to the amount of AgE in 0·5 ml of a 1:50 w/v extract of crude ragweed (20 μg).

Symptom scores, reported as an average daily symptom score, were quantitated from data recorded daily by the patients. The grading system used was similar to that described by other investigators (Lichtenstein, Norman & Winkenwerder, 1968). Additionally both patient and physician gave an overall assessment of the treatment i.e. improved or not improved.

Blood was drawn prior to therapy, at 1 μg, 10 μg, 100 μg of AgE given, and just prior to the ragweed season. Serum samples were stored at $-20°$C prior to analysis for IgE and blocking antibody.

Ragweed AgE. AgE prepared by the method of King, *et al.* (King, Norman & Connell, 1964), was kindly supplied by Dr F. C. McIntire. Stock AgE had a concentration of 1·04 mg of protein per ml.

IgE. IgE myeloma PS was purified as previously described (Zeiss *et al.*, 1973). The purified material had a concentration of 55 mg of protein per ml and contained 15 times 10^6 units of IgE per ml as measured by radioimmunoassay (Gleich, Averbeck & Friedlung, 1971).

Radioiodination. AgE and IgE were labelled with ^{125}I according to the method described by Gleich. The ^{125}I-labelled AgE was 82% precipitable with 10% trichloroacetic acid and 70% bound by rabbit anti-AgE in antibody excess. The sp. act. was 5–15 μCi/μg P. The IgE antibody was 95% precipitable with 10% trichloroacetic acid and had a sp. act. of 5–12 μCi/μg protein. Labelled IgE was 78% bound by monospecific rabbit anti-IgE in antibody excess.

IgE antibody. IgE antibody specific for AgE was determined as previously described (Zeiss *et al.*, 1973). Briefly, polystyrene tubes were coated with IgE myeloma, PS. Anti-IgE was added in excess to form an IgE immunoabsorbent. One tenth ml of patients serum was added to the immunoabsorbent tubes. After incubation in the cold for 48 hr the polystyrene immunoabsorbent bound a known fraction of the IgE in the serum. The supernatant was decanted and the tubes washed. ^{125}I-labelled AgE was added in antigen excess and the tubes incubated in the cold for 48 hr. Unbound AgE was then removed by washing and the bound counts were determined. The ng of AgE-millilitre bound by 1 ml of serum in AgE excess was then calculated.

Measurement of total immunoglobulins specific for AgE. For this assay a modification of the ammonium sulfate technique of Lidd and Farr was used (Lidd & Farr, 1962). One-tenth aliquots of serum were incubated with 1, 10, 100, and 1,000 ng of ^{125}I-labelled AgE. The total reaction volume was 0·6 ml. The antigen tubes were incubated for 2 hr at 37° C and 16 hr at 4°C. Saturated ammonium sulphate (pH 7·0), four-tenths ml was added in the cold, dropwise, with constant strirring. The precipitates formed for 2 hr at 4°C and were then centrifuged at 3000 rev/min for 15 min. The supernatant and precipitate counts were determined and true percent Age bound was calculated. The true percent AgE bound was plotted on the y axis of semilog paper and the ng of AgE added were plotted on the log x axis. The ng AgE bound at the 20% binding point was determined and multiplied by the dilution factor. This gave the ng of AgE bound by 1 ml of serum in antigen excess.

Several correction factors were used to determine the true per cent AgE bound. It was found by experimentation that over the range of labelled antigen added the precipitate would entrain 10% of the supernatant counts. Additionally, ammonium sulphate at 40% saturation will precipitate 10% of the total counts added. Before each assay the per cent immune reactivity of the ^{125}I-labelled AgE was assessed by the ability of rabbit anti-AgE to precipitate the radiolabelled antigen in antibody excess. True per cent AgE bound is calculated by the following formula:

$$\text{true per cent AgE bound} = \frac{(\text{PPT ct/min} - 10\%\ \text{SUP ct/min}) - (10\%\ \text{total ct/min added})}{(\text{per cent total immune reactivity} \times \text{total ct/min added}) - (10\%\ \text{total ct/min added})}.$$

Calculation of blocking antibody and the IgG to IgE binding ratio. Subtraction of IgE from total immunoglobulin binding of AgE was defined as blocking antibody. As stated earlier this was taken to be IgG antibody to AgE. The IgG to IgE-binding ratio was obtained by dividing this value by the corresponding IgE antibody.

RESULTS

Initial studies

Typical antibody binding curves obtained with the ammonium sulfate technique are shown in Fig. 1. During immunization there was a shift to the right of the binding curves indicating increased antibody binding of AgE in post-treatment serum. These curves are representative of low, mid and high antibody content.

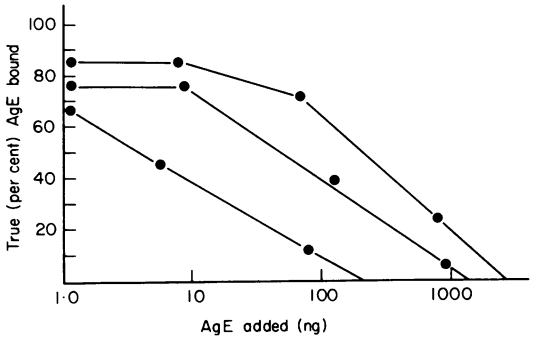

FIG. 1. Antibody binding of radiolabelled AgE at different levels of AgE added. The rightward shift in these binding curves illustrate the increase in total serum antibody against AgE as immunization progresses.

TABLE 1. Antigen-E binding by total serum antibody illustrating saturation of antibody in antigen excess

Low antibody		Mid antibody	High antibody
Per cent bound	AgE bound (ng)	AgE bound (ng)	Age bound (ng)
60	4·2	14·0	72·0
30	6·0	48·0	144·0
20	9·0	66·0	188·0
10	10·0	64·0	160·0

These curves show that as the 20% binding point is reached the antibody binding sites are saturated and antigen bound reaches a plateau. This is outlined in Table 1 using the antibody binding curves in in Fig. 1.

Immune response

The antibody response to immunization is shown in Table 2. The table shows means and ranges of IgG- and IgE-antibody binding of AgE and the IgG to IgE binding ratio for eleven patients. Prior to therapy there was a low level of IgG antibody which increased over tenfold just prior to the ragweed season. There was a statistically significant increase in IgG antibody from pretreatment levels after a 10 μg of AgE were given. There was no significant increase in IgG antibody after 1 μg of AgE given. The mean IgE binding of AgE did not change during immunotherapy. Minor individual fluctuations in IgE binding did occur.

TABLE 2. Immune response and IgG/IgE ratio during immunotherapy

	Pretreatment	Amount of AgE given (μg) 10 μg	100 μg	370 μg
				Ragweed season
IgG $\left(\frac{\text{ng AgE bound}}{\text{ml}}\right)$	238 (1–779)	738* (60–1985)	2137† (103–7171)	3142† (409–7171)
IgG/IgE	19 (0–64)	54* (5–137)	161† (15–619)	290† (33–690)
IgE $\left(\frac{\text{ng AgE bound}}{\text{ml}}\right)$	24 (2–119)	26 (3–119)	25 (1–114)	23 (1–96)

Student's *t*-test * $P<0{\cdot}01$; † $P<0{\cdot}005$.

IgG to IgE ratios and symptom scores

The IgG to IgE ratios increased dramatically during therapy. There was a fifteen-fold increase in the IgG to IgE ratio from pretreatment to just prior to the ragweed season. There was a statistically significant increase in IgG to IgE ratio after 10 μg of AgE were given.

Seven of eleven patients returned complete symptom scores during the ragweed season *prior* to therapy. All of the patients studied returned completed symptom scores during the ragweed pollen season after therapy. All of the patients received at least 100 μg of AgE protein prior to the second ragweed season. The symptom scores and their relationships to the IgG to IgE-binding ratio, IgE and

TABLE 3. Symptom scores: the IgG/IgE ratio and IgG, IgE anti-AgE. (A) Good clinical response; (B) poor clinical response

Symptom Scores				
Pretreatment	Post-treatment	IgG/IgE	IgG	IgE
A				
9·7	1·9	570	3194	5·6
n.d.	3·4	124	5357	43·3
16·5	3·8	264	2590	9·8
36·6	5·9	690	3794	5·5
n.d.	11·1	372	409	1·1
27·0	11·1	245	7171	29·3
23·0	11·2	619	4393	7·1
	6·9±3·8	412±200	3844±1980	14·5±14·5
B				
n.d.	14·2	83	1423	17·1
19·2	18·0	77	2172	28·1
n.d.	18·2	33	3104	95·6
31·8	34·8	112	951	8·5
	21·3±7·9	76±28	1912±814	37·3±34·3

Mean ±s.d.

IgG levels are shown in Table 3. Seven patients' overall impression was that of a good response to therapy. Their symptom scores were less than 12. Of these patients, five showed a substantial reduction in symptom score. Two of the four patients who reported no overall improvement showed either no change in symptom score or a slight increase. There was a statistically significant difference between the mean symptom score of the patients reporting a good response and the patients reporting no benefit. The mean of the symptom score for those with a good response was 6·9 and for those with a poor response 21·3 ($P<0·01$).

As can be seen in Table 3, the patients reporting improvement had a significantly higher IgG to IgE ratio with a mean of 412 for the improved group and a mean of 76 for the other group ($P<0·001$). Although there was a trend to higher IgG levels in the improved group, this difference was not statistically significant ($P = 0·10$). The mean IgE level was higher in the patients with no improvement but this difference was not statistically significant ($P>0·01$).

Of those patients reporting symptom scores in the ragweed season prior to treatment and then again after treatment the symptom score was 23·4 prior to treatment and 12·4 after treatment. The difference was significant at the $P = 0·05$ level.

Treatment was continued through the ragweed season and the peak level of IgG and the peak IgG to IgE ratio determined. The mean IgG level rose to 4286 ng AgE protein bound per ml and the IgG to IgE ratio rose to 1167. At this time, the mean cumulative dose of AgE given was 600 μg of AgE protein.

DISCUSSION

During the course of studies of a polymerized form of AgE we were able to do extensive serial immunologic studies of patients receiving either the polymerized antigen or the monomolecular form of AgE (Metzger *et al.*, 1976). Both patient groups showed a significant immune response and there was no difference between the groups. This study was primarily an investigation of the immune response to purified AgE and not one contrasting the differences between polymerized and monomer AgE. In both groups, patients were treated to a definite end point and blood was drawn at predetermined levels of antigen given.

The ammonium sulphate antigen binding technique and the polystyrene tube radioimmunoassay for IgE against AgE enabled us to follow the binding of AgE by IgE and the binding of AgE by other immunoglobulin classes throughout immunotherapy. These techniques gave us the opportunity to quantitatively follow the IgG-blocking antibody activity as related to IgE-antibody activity. The antibody activity of these two biologically important antibodies were expressed in the same units i.e. the ability to bind AgE.

Prior to immunotherapy there was a low level of IgG antibody activity, mean 238 ng AgE bound/ml which contrasted with a mean IgE antibody activity of 24 ng AgE bound|ml. The mean of each individual IgG to IgE-binding ratio prior to immunotherapy was 19. A fairly wide range in initial immunologic values was seen (Table 2). This probably represents differences in individual immune responsiveness and allergen exposure.

As immunization with AgE progressed there was a substantial increase in IgG antibody activity while IgE antibody activity remained at pretreatment levels. This resulted in a marked increase in the IgG to IgE-binding ratio which reached a mean of 290 just prior to the next ragweed season.

This ratio was related to the clinical results of immunotherapy. The patients reporting an overall good clinical response had lower symptom scores ($P<0·01$) and significantly higher IgG to IgE-binding ratios ($P<0·001$) than patients reporting a poor clinical response. There was a statistically significant linear inverse correlation between this important ratio and symptom scores reported by the eleven patients (Spearman's $r = -0·636$, $P<0·05$).

All patients had a substantial increase in IgG-antibody activity from pretreatment levels. The patients with a poor clinical response attained a level of IgG antibody activity which was not statistically different from that attained by patients with a good clinical response. However, as can be seen in Table 3 their

IgG response was not high enough in relation to their IgE level to result in a high IgG to IgE-binding ratio. There was no correlation between the IgG antibody and symptom scores reported by the patients. This lack of correlation between blocking antibody and symptom scores has been reported by others (Irons *et al.*, 1975).

Using newer immunological techniques we have been able to better understand the quantitative relationship between IgG and IgE antibody specific for AgE in patients receiving immunotherapy for ragweed pollenosis. Not only is blocking antibody level important but its relationship to IgE antibody with which it competes for antigen at the mast-cell surface may also be an important determinant in the overall success of immunotherapy.

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