

Human immunoglobulin E and immunoglobulin G antibody responses to the "minor" ragweed allergen Ra3: Correlation with skin tests and comparison with other allergens

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We report here the use of an antigen-binding assay to measure serum IgE and IgG binding activity (BA) for the "minor" ragweed antigen, Ra3. These studies were carried out in ragweed-allergic individuals, many of whom had been skin tested with Ra3 as well as with the "major" ragweed allergen, antigen E (AgE). IgE BA for Ra3 showed a weak quantitative correlation with skin-test sensitivity to Ra3. More strikingly, there was a threshold of skin sensitivity ($<10^{-3}$ $\mu\text{g/ml}$) above which serum IgE BA was likely to be detectable. Serum IgG BA for Ra3 measured in parallel with IgE BA was found in all sera that showed detectable IgE BA for Ra3. By contrast, 11/13 ragweed-allergic individuals who were skin-test negative for Ra3 showed no detectable IgG BA for Ra3. These results support the view that the known genetic controls over Ra3 sensitivity control IgG antibody as well as IgE antibody responses. Individuals receiving injections of ragweed extract showed increases in IgG BA for Ra3 following therapy. Among nine persons who had no IgG BA for Ra3 before treatment, four out of nine developed a low degree of IgG BA for Ra3. However, the patients who developed IgG BA did not develop detectable IgE BA for Ra3.

Over the last few years purified allergens have been used to investigate specific skin reactivity, lymphocyte response, and IgE antibodies (ab) in patients with allergic rhinitis. Most of this work has been done with major allergens, e.g., antigen E (AgE)¹ and rye grass group I (rye I).² These allergens were originally regarded as "major" because a large proportion of the allergic population was highly sensitive to them. In addition, it has recently become clear that a relatively large proportion of serum IgE may be antibody against pollen allergens, especially the "major" allergens.³⁻⁶ Furthermore, allergic persons who have

IgEab to major allergens also have IgG and IgAab to the same allergens,⁷⁻¹¹ suggesting the possibility of common genetic controls for all classes of antibody to inhalant allergens.

In order to extend our understanding of the controls over humoral antibody responses to minute doses of environmental allergens, we wanted to know more about the antibody responses to a "minor" allergen. In particular, we wanted to investigate whether measurements of serum IgEab and IgGab could replace or complement quantitative skin testing in the assessment of genetic controls. The short ragweed pollen component, Ra3,^{12, 13} molecular weight 12,300 daltons, provides a good model for studies of the antibody response to a minor allergen. Approximately 60% of ragweed-sensitive individuals show positive skin tests with Ra3,^{2, 14} and IgGab, IgEab, and lymphocyte responses to Ra3 have also been demonstrated in humans.^{15, 16} We have recently described highly sensitive antigen-binding assays suitable for measuring IgEab and IgGab.⁵ These assays require very small quantities of purified, radiolabeled anti-

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- 0.1 ml serum or serum dilution
- *0.1 ml carrier (Myeloma PS serum diluted 1:100 for IgE assays)
(Non-allergic serum diluted 1:50 for IgG assays)
- +0.1 ml 125 I labeled Ra3 (or AgE, or Rye I)
wait 4 hours, room temperature
- +0.1 ml Goat anti-IgE (or anti-IgG) to precipitate
overnight 4°C
- Wash 3 times (transferring precipitate before final wash)
count radioactivity in precipitate

FIG. 1. Antigen-binding radioimmunoassay for IgG and IgEab to Ra3.

TABLE I. Quantitative intradermal skin tests and the prevalence of detectable serum IgE binding activity (BA) using purified inhalant allergens

Quantity of allergen necessary to give a 2+ skin test ($\mu\text{g/ml}$)*	Prevalence of detectable IgE BA for:			
	Ra3	AgE	Rye I	Totals
$<10^{-4}$	17/18	10/10	17/19	40/43
10^{-4} to 10^{-3}	9/11	4/7	7/10	20/28
$>10^{-3}$ to 10^{-1}	0/8	1/3	0/4	1/15
Negative†	0/11	0/5	0/7	0/22

*Minimum concentration of antigen ($\mu\text{g/ml}$) that gave a wheal of 8-mm mean diameter when 0.05 ml was injected intradermally.

†Wheal less than 8-mm diameter or no wheal when 0.05 ml of a solution containing 0.1 $\mu\text{g/ml}$ was injected intradermally.

gen, and assays for each class can be run under directly comparable conditions in the same experiment with the same antigen preparation. In the present paper we report the use of this assay to measure antibodies to Ra3 and compare these measurements to quantitative skin tests with Ra3.

MATERIALS AND METHODS

Patients

Our subjects were 87 white individuals (54 male, 33 female, aged 15 to 59 yr, mean 31 yr) who volunteered for various clinical and immunologic studies of hay fever to ragweed and grass pollens at the Good Samaritan Hospital. Of these, 80 were highly sensitive to ragweed pollen (with or without grass sensitivity) and seven were sensitive only to grass pollen, as determined by clinical history and skin testing with crude ragweed and grass extracts. Sera were available from 26 subjects before, during, and after therapy with injections of ragweed extract (desensitization). Sera from 10 nonallergic English individuals were used as controls and to establish the background for the assays. Three individuals were studied only after desensitization while the remaining subjects had either never received injections of ragweed extract or had received such therapy at least 4 yr previously (eight patients). Serum IgE BA (see below) was

studied in 76 of the above 87 patients. The remaining 11 patients were used to provide additional data in the study of variation of IgG BA following desensitization.

Antigens and skin testing

The sources and purification of Ra3, AgE, and rye I have been described previously.¹⁷ All preparations were physicochemically pure and gave single precipitin lines by immunoelectrophoresis against potent antisera to whole ragweed extract. Intradermal skin-test titrations were performed as described previously^{14, 17} with a series of tenfold dilutions of the allergen in the range 10^{-7} to 0.1 $\mu\text{g/ml}$. The allergen concentration producing a 2+ end point (8-mm mean wheal diameter) was taken as the sensitivity of the patient to the allergen. End points between the log dilutions were interpolated to give results to the nearest 0.5 log.

Antigen-binding radioimmunoassay

The assay for IgE binding activity (BA) has been described in detail using rye I or AgE,⁵ and is a modification of the antigen-binding assays used previously for IgG.^{9, 15, 18} The labeling efficiencies and specific activities of ^{125}I -rye I and ^{125}I -AgE were similar to those described previously. Ra3 was radiolabeled by the chloramine-T technique giving 50% efficiency and a specific activity of 4,500 cpm/ng. In an attempt to improve the radiolabeling, Ra3 was labeled with the Bolton-Hunter reagent.¹⁹ However, the efficiency of labeling was only 34% and the technique appeared to offer no advantages over the chloramine-T technique. The assays (Fig. 1) were carried out exactly as described previously. IgE BA and IgG BA were assayed in the same experiment and values were obtained from IgG BA control curves. The background binding of Ra3 by nonallergic sera was $0.2 \pm 0.03\%$ of the total counts per minute added and results were regarded as positive if they were more than two standard deviations above the background values. The volumes and hence antigen concentrations were kept constant, maintaining at least a fourfold excess of antigen over all classes of ab. Under these conditions, dilution curves for IgG and IgEab are parallel. Because of the interference in IgEab measurements at high levels of IgG, sera from treated patients and sera that were found to have more than 20-fold more IgG BA than IgE BA were fractionated with saturated ammonium sulfate (SAS).⁵ IgE BA for Ra3 was assayed in the IgE-rich fraction precipitating between 33% to 50% SAS. The results were adjusted to compensate for the proportion of total IgE precipitating in this fraction.⁵ Total IgE was measured by double-antibody inhibition radioimmunoassay.^{20, 5}

RESULTS

IgE binding activity for Ra3 and quantitative skin tests

IgE BA for Ra3 was detected in 31/69 sera from patients with ragweed hay fever. No IgE BA for Ra3 was detected in sera from 10 nonallergic controls, or in sera from seven patients with grass pollen hay fever alone. Forty-nine of the patients whose sera were as-

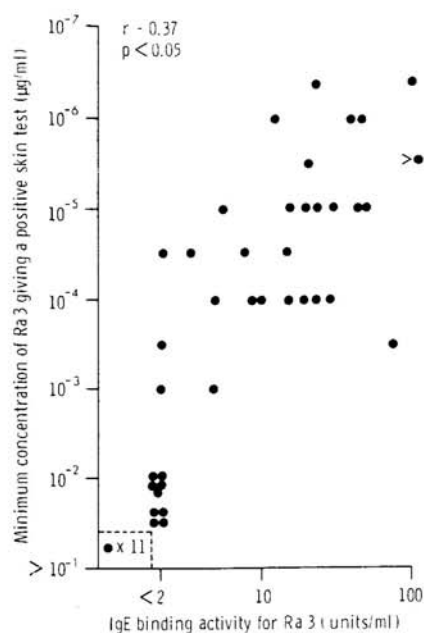


FIG. 2. Skin-test reactivity to Ra3 and serum IgE BA for Ra3 in 49 ragweed-allergic patients. Eleven patients had negative skin tests and negative IgE BA.

sayed for IgE BA against Ra3 had previously been skin tested with Ra3 by the intradermal end-point titration method. Of these, 26 had detectable IgE BA and positive skin tests at $<10^{-3}$ $\mu\text{g/ml}$, 11 had negative skin tests (no reaction up to 10^{-1} $\mu\text{g/ml}$) and no detectable IgE BA, and 12 had positive skin tests, but no detectable IgE BA (Fig. 2). There appeared to be a threshold for skin sensitivity ($2+$ reaction at about 10^{-3} $\mu\text{g/ml}$) above which serum IgE BA was likely to be detectable (Fig. 2). A similar threshold was apparent for other allergens studied in the same group of patients (Table I). Comparing the log concentration of Ra3 necessary to give a skin test and the log (IgE BA) in the 38 skin test-positive subjects, there was a weak inverse correlation ($r = 0.37$; $p < 0.05$) (Fig. 2). This relationship between skin-test sensitivity and IgE BA shows considerable scatter, presumably because of the inherent inaccuracy of skin testing and because skin responses depend not only on IgEab but also on a large number of variables from patient to patient.

Serum IgG BA for Ra3

In the present study, IgG BA for Ra3 was measured in 65 sera in parallel with IgE BA (Fig. 3). In keeping with previous results all sera with detectable IgE BA for Ra3 contained detectable IgG BA for Ra3. For the

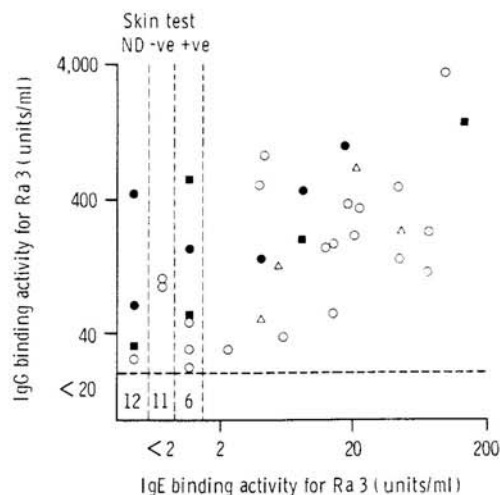


FIG. 3. IgG and IgE BA for Ra3 in sera from ragweed-allergic patients. Patients who had received injections of ragweed extract within the last 2 yr (\blacksquare) or at some time in the past (\bullet) are indicated. Patients with no detectable IgE BA (<2) are divided according to their skin response to Ra3 (ND = not done). Patients with IgE BA who had not been skin tested are shown (Δ). Values below the horizontal dashed line indicate the number of patients in each group with no detectable BA of either class.

skin test-positive patients who reported no desensitizing injections and had detectable IgG BA there was a direct correlation between log (IgG BA) and log (IgE BA) for Ra3 ($r = 0.52$; $p = 0.01$). The patients with no detectable IgE BA shown in Fig. 3 are divided according to their skin-test results. There were 13 ragweed-allergic patients who had negative skin tests for Ra3 and, of these, only two had detectable IgG BA for Ra3. This prevalence of IgG BA for Ra3 in skin test-negative patients was significantly lower than the prevalence of IgG BA in skin test-positive patients (by chi-square test, $p < 0.002$). Thus, ragweed-allergic patients generally do not develop IgG BA for Ra3 unless they develop skin sensitivity to Ra3. However, as with other allergens, there are a few interesting exceptions, patients who develop IgG BA without IgEab.^{5, 15, 20}

Injections of aqueous crude ragweed extract containing 8 to 10 μg Ra3/10,000 PNU might be expected to increase IgG BA for Ra3. For 26 patients IgG BA was determined before and after a course of injections of ragweed extract, giving a cumulative dose of approximately 8,000 PNU (Fig. 4). The results show that, in all patients with detectable IgG BA for Ra3, the level of IgG antibody increased as a result of treatment. In addition, four out of nine pa-

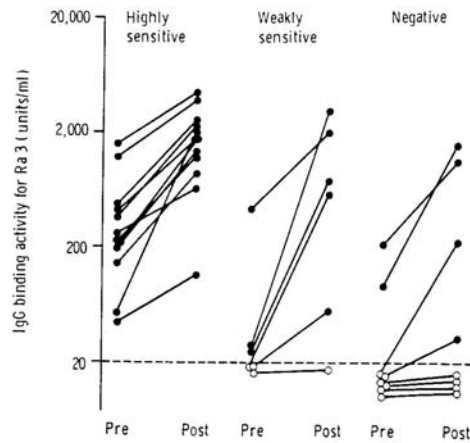


FIG. 4. IgG binding activity for Ra3 in patients before (*pre*) and after (*post*) desensitization with ragweed extract. Patients were divided into those with 2+ skin test to $<10^{-2}$ μ g Ra3 (highly sensitive); those with 2+ skin test to $\geq 10^{-2}$ μ g Ra3 (weakly sensitive); and those with negative skin tests to Ra3. Sera with no detectable IgG BA for Ra3 are indicated by open symbols.

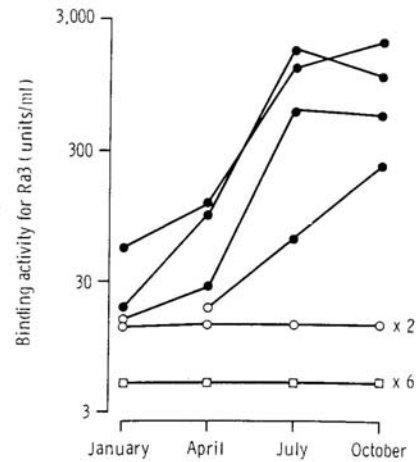


FIG. 5. IgG and IgE binding activity for Ra3 in patients treated with ragweed extract. Four patients showed IgG BA (\bullet — \bullet) responses, two patients had no detectable IgG BA after treatment (\circ — \circ), and none of the patients showed detectable IgE BA (\square — \square). Sera with no detectable IgG BA for Ra3 are indicated by open circles.

tients who had no detectable IgG BA for Ra3 before treatment developed such antibody as a result of treatment. The data suggested that the patients who gave negative skin tests with Ra3 were less likely to produce IgG BA for Ra3 in response to desensitization (Fig. 4). Nine additional ragweed-allergic patients who were not receiving desensitizing injections were followed over the same period and none of these showed a greater than twofold increase in IgG BA for Ra3 (data not shown).

Previous studies have shown that desensitizing injections with allergens can produce an initial rise followed by a modest fall in serum IgEab.^{9, 18} In the present study, a few patients who had very little or no detectable IgG BA before therapy developed IgG BA for Ra3 and it seemed possible that they might also have developed IgE BA for Ra3. In order to assay IgE BA in sera with high levels of IgG BA, the sera were fractionated with ammonium sulfate and IgE BA was assayed in the "IgE-rich" fraction (precipitated between 33% and 50% SAS).⁵ The results in Fig. 5 show that, in four patients, a sharp rise in IgG BA for Ra3 (closed circles) was not associated with detectable IgE BA for Ra3 (open squares). Three of these patients were skin-test positive and one was skin-test negative. Two further skin test-negative patients who did not develop IgG BA for Ra3 also showed no detectable IgE BA (Fig. 5).

DISCUSSION

In previous studies using quantitative intradermal skin testing it was found that patients with ragweed hay fever varied in their skin response to the minor ragweed allergen Ra3.¹²⁻¹⁵ The present results suggest that measurements of serum IgE BA for Ra3 can be used to complement skin testing. Measurements of IgE BA for Ra3 showed a quantitative correlation with skin-test results but were less sensitive. A similar correlation between skin-test and RAST results has been reported for crude ragweed extract.²¹ On the other hand, we found that the level of intradermal skin sensitivity above which serum IgEab was likely to be detected was surprisingly consistent for Ra3, AgE, and rye I. Similarly, serum IgE BA was detectable only in patients who were sensitive to $<10^{-3}$ μ g/ml of the house dust mite allergen F₄P₁.²⁰ It is clear that measurements of serum IgE BA for Ra3 would correctly identify patients who were highly skin sensitive to Ra3, but that most weakly sensitive patients would not be detected. Patients whose skin reacts to 10^{-2} μ g allergen are regarded as weakly positive. However, these results must be interpreted with caution because they could be explained by a $<1\%$ impurity of the allergen extract (with an allergen to which the individual was highly sensitive). This problem in defining negative skin tests has been discussed elsewhere.²² Antigen-binding assays have sensitivity similar to

RAST assays.^{5, 20, 23} In previous studies using RAST for IgEab to Ra3, results obtained were broadly similar to these reported here.¹⁶ Those studies used 1 mg of purified Ra3 to bind to RAST disks and ~1 µg Ra3/assay. One of the advantages of antigen-binding techniques is that they require only very small quantities of purified allergen. It is possible to radiolabel 5 µg of protein and assays can be carried out with ~5 ng/assay.^{5, 20, 24}

The results of IgG BA and IgE BA measurements for Ra3 appear to be similar to previous results obtained with major allergens.^{9, 18, 25} First, all the sera with detectable IgE BA had detectable IgG BA for Ra3. Second, in untreated patients there was a quantitative correlation between IgG BA and IgE BA. Third, during desensitization serum IgG BA increased. Most previous studies on desensitization have studied only antigens to which the patients were allergic. The present results show that some patients developed IgGAb to Ra3 although they were not allergic to Ra3. On the other hand, half the patients who were skin-test negative had no IgGAb response to Ra3 (Fig. 4). More recent results suggest that it is rare for patients to develop IgG antibody against allergens to which they are skin-test negative. The present results may reflect the fact that at least one of the skin-test-negative patients who produced IgG antibody had received allergen in a depot preparation in the past. It has always seemed possible that desensitizing injections could induce IgEab responses to new antigens. Certainly injections of grass pollen extract adsorbed to alumina gel can induce IgE-mediated sensitivity in nonallergic subjects.²⁶ The present results show that, at least in the short term, injections of aqueous ragweed extract did not induce IgE BA for Ra3 in Ra3 skin test-negative individuals. Thus, as with other allergens, desensitizing injections appear to produce a selective rise in IgGAb.^{9, 18, 25, 27} A possible explanation of this selective IgGAb response comes from studies on nasal secretions. These studies suggest that the IgGAb response to desensitizing injections occurs "centrally" in contrast to the predominantly local response to inhaled allergens.²⁵ These results are in keeping with the view that IgG and IgE antibodies to Ra3, as to other allergens, are produced in parallel as part of a local response.²⁵ This implies that the genetic controls known to influence skin sensitivity to inhaled allergens, including Ra3, act on IgGAb as well as IgEab responses.²⁸⁻³⁰ For studying IgEab responses to purified allergens, quantitative skin testing remains the most sensitive technique. On the other hand, antigen-binding assays can give parallel information about IgG and IgEab. These assays are safer than skin

testing, can be applied repeatedly to stored sera and, in principle, can be extended to a wide variety of highly purified allergens that may be available only in minute amounts.

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