

Quantitation of IgE antibody specific for ragweed and grass allergens: Binding of radiolabeled allergens by solid-phase bound IgE

C. Raymond Zeiss, M.D., Doris Levitz, B.S., and Irena M. Suszko, B.S.
Chicago, Ill.

IgE antibody specific for multiple allergens extracted from grass and ragweed pollens was measured by radioimmunoassay. The assay depends on the interaction between IgE antibody bound to a polystyrene solid phase, ^{125}I -labeled grass allergens (GA), and ragweed allergens (RW). The binding of ^{125}I RW by serum IgE antibody from 37 allergic patients ranged from 0.2 ng to 75 ng RW protein (P) bound per ml. This binding of ^{125}I RW by patient's IgE was paralleled by their IgE binding of ^{125}I antigen E (AgE), a purified allergen from ragweed pollen ($r = 0.90$, $p < 0.001$). Inhibition of patient's IgE binding of ^{125}I RW by highly purified AgE ranged from 25% to 85% indicating individual differences in patient's IgE response to inhaled ragweed pollen. The binding of ^{125}I GA by serum IgE antibody from 7 grass-sensitive patients ranged from 0.6 ng GA P bound per ml to 15 ng. This assay should be useful in the study of IgE responses to environmental agents containing multiple allergens and has the advantage that other antibody classes cannot interfere with the interaction between IgE antibody and labeled allergens.

In 1973 we described an assay for specific IgE antibody directed against antigen E (AgE), the major allergen of ragweed pollen. The assay depended upon the interaction between solid-phase bound IgE antibody and radiolabeled AgE. We found that the amount of AgE bound by IgE antibody reached a plateau in antigen excess. This assay offered a method for estimating the ng quantities of specific IgE antibody present in a particular serum.¹ This assay has been used in the study of immunotherapy with both purified AgE and crude ragweed extracts.^{2, 3} It has the advantage that only IgE is bound to a polystyrene solid phase and there is no competition for antigen by antibodies of other classes. This makes this assay especially useful in the study of IgE responses in patients undergoing immunotherapy.

This report describes the extension of this assay to the quantitation of IgE antibody directed against multiple allergens extracted from grass and ragweed pollens. This method depends upon the primary interaction between solid-phase bound IgE antibody and ^{125}I -labeled grass allergens (GA) and ^{125}I -labeled ragweed allergens (RW).

Using this methodology we have been able to compare the IgE binding of AgE with that of RW in serum of patients with ragweed pollenosis. Additionally, we have been able to determine the fraction of total RW binding by a patient's IgE antibody that is attributable to AgE and what fraction of total RW binding that is attributable to other allergens in RW.

MATERIALS AND METHODS

Patients

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

Ragweed AgE

AgE prepared by the method of King, Norman, and Connell⁵ was kindly supplied by Dr. F. C. McIntire. Stock AgE had a concentration of 5.0 mg of P per ml.

From the Department of Medicine, Section of Allergy-Immunology, Northwestern University Medical School.

Supported by United States Public Health Service Grant AI 11403 and the Ernest S. Bazley Grant.

Received for publication Oct. 10, 1977.

Accepted for publication March 21, 1978.

Reprint requests to: C. Raymond Zeiss, M.D., Department of Allergy/Immunology, 303 E. Chicago Ave., Chicago, Ill. 60611.

IgE

IgE myeloma PS was purified as described previously.¹ Purified material had a concentration of 55 mg P per ml and contained 36 mg of IgE per ml as measured by radioimmunoassay.⁶

Antisera

Rabbit antihuman IgE was made in this laboratory as previously described.⁷ The antibody was specific for the Fc portion of human IgE and had no activity against light chains or heavy chains of other immunoglobulin classes. This antibody bound 0.2 mg IgE P per ml. Rabbit antihuman IgE was also purchased from Behring Diagnostics, Somerville, N. J.

Rabbit anti-AgE was made by immunization of rabbits against fraction IV — IVC⁸ which was not as highly purified as the AgE described above which is fraction IVC.⁵

Preparation of allergens

RW and GA were prepared by the aqueous extraction of defatted native pollens followed by ammonium sulfate precipitation.⁹ Pollens were obtained from Hollister-Stier Laboratories, Spokane, Wash. Equal quantities of short and giant ragweed were mixed before extraction. Equal quantities of the following grass pollens were mixed: Bermuda, orchard, timothy, redtop, and June, then extracted. These pollen mixtures were defatted by several additions of ether at room temperature and dried overnight. Defatted pollen was extracted with 0.15 M phosphate-buffered saline (PBS) pH 7.4 at 4° C for 24 hr. This aqueous extract was centrifuged at $17,000 \times g$ for an hour and the supernatant was saved. To this supernatant at 4° C was added ammonium sulfate (60 gm per 100 ml of supernatant) with constant stirring which continued for 3 hr. The resulting precipitate was sedimented at 4° C and the supernatant discarded. The precipitate was dissolved in 50 ml PBS and dialyzed at 4° C against five changes of 5,000 ml PBS, after which time the dialysate was free of ammonium sulfate. The contents of the dialysis bag were centrifuged to remove small amounts of precipitate and the supernatant solution was called GA or RW. RW was further purified by filtration over Sephadex G-25. Protein determination was done by the Biuret technique, and stock solution of these extracts contained approximately 8.0 mg P per ml.

Cutaneous reactivity in allergic subjects

Serial 10-fold dilutions of these allergen preparations were prepared in PBS starting at a concentration of 0.5 mg/ml. These were tested intracutaneously in 4 subjects with the prior diagnosis of grass and/or ragweed pollenosis. The cutaneous reactions were graded on a scale of 0 to 4+ using standard clinical criteria.⁴

Radioiodination

RW, GA, AgE, and IgE were labeled with ¹²⁵I utilizing chloramine-T by the method of Gleich, Averbek, and Svedlund.⁶ The method of labeling the allergens will be described in detail. Solutions of chloramine-T, sodium metabisulfite, and potassium iodide (KI) were made in 0.05

M phosphate buffer (PB). To a 2-mCi vial of ¹²⁵I was added 0.05 ml of 0.5 M PB. In a separate tube, 100 µg of allergen P in PBS was added to 100 µg of chloramine-T for 2 min. The protein was then transferred to the ¹²⁵I vial for 2 min. The reaction was stopped with 0.05 ml (240 µg) sodium metabisulfite. After 30 sec, 0.1 ml of 1% KI, was added. The entire contents of the vial was pipetted onto a small column made from a 10-ml pipet packed with Sephadex G-50 in 0.01 M PB. The vial was then washed with 1 ml of KI and this wash was added to the column. To elute the labeled allergen from the column, 1-ml aliquots of 1% bovine serum albumin (BSA) in 0.01 M PB were added to the column and 16 samples were collected in tubes that contained 1 ml of 1% BSA. These 16 fractions were then counted and an elution profile obtained. The elution profile from the Sephadex column was a repeatable pattern with the first peak containing the labeled protein and the second peak containing free iodine. The tubes containing the highest radioactivity from peak 1 were pooled (pool 1) and the remaining fractions pooled (pool 2).

Estimation of the protein content of the labeled allergen pool

To calculate the protein content of these two pools, 10% trichloroacetic acid (TCA) analysis was done. From this analysis, we estimated the percent of protein in the pool which contained the labeled allergen and the percent of protein in the other pool which contained minor amounts of labeled protein and free iodine. The percent protein in the allergen pool which is used in the assay was calculated in the following manner. Percent TCA precipitable counts per minute (cpm) of allergen pool times total cpm in the pool = protein cpm. Protein cpm in pool divided by total protein cpm (pool 1 + pool 2) equal percent protein in that pool. The percentage of protein in the allergen pool times the micrograms of allergen P labeled gave the micrograms of allergen protein in that fraction. Pool 1 contained between 70% and 80% of the allergen P that was labeled. Since less than 5% of the labeled allergen was retained on the Sephadex column, no correction was made for allergen that may have been entrained on the column.

Specific activity of the labeled allergens

The specific activity of the proteins in the allergen pool was determined by dividing the total cpm in the fraction by the µg of allergen protein in that fraction. This gave cpm/ng allergen P. In a typical labeling experiment, ¹²⁵I RW had a specific activity of 10,000 cpm per ng RW P and ¹²⁵I GA had a specific activity of 6,000 cpm/ng P. This procedure gives the average specific activity of the labeled proteins but no information as to the specific activity of any individual allergen such as antigen E contained in the labeled material.

¹²⁵I AgE

Highly purified AgE was labeled with ¹²⁵I as described above. The AgE was 89% precipitable with 10% TCA and was 78% bound by rabbit anti-AgE in antibody excess. The ¹²⁵I AgE had a specific activity of 10 to 15,000 cpm/ng P.

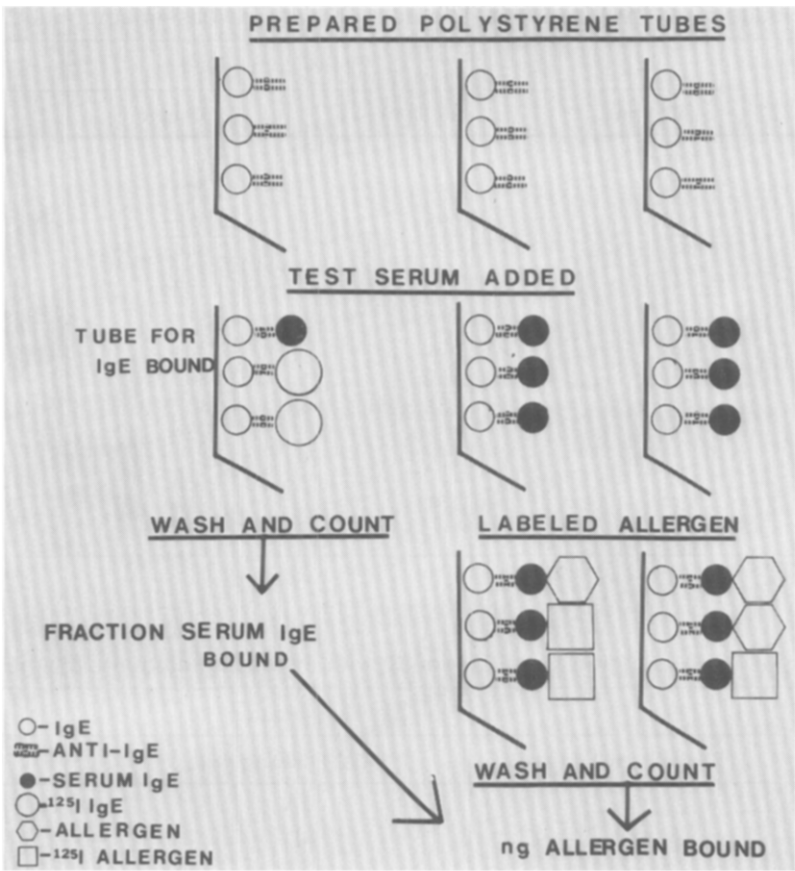


FIG. 1. The methodology of the polystyrene tube radioimmunoassay for IgE antibody directed against multiple allergens.

Solid-phase assay for IgE against ^{125}I AgE, ^{125}I RW, and ^{125}I GA (Fig. 1)

Binding of IgE in patient's serum to the solid phase was done according to the method described previously.¹ One-ml aliquots of a solution containing 430 μg IgE myeloma PS per ml in 0.1 M bicarbonate buffer, pH 8.6, were added to a series of polystyrene tubes (Lab-Tek Products, Division Miles Laboratories, Inc., Westmont, Ill.). Adsorption of IgE to the polystyrene surface was carried out at room temperature for one hour, after which the IgE was removed and saved for reuse. The tubes were washed three times with PBS buffer, and 2 ml of 1% BSA in PBS were added for one hour at room temperature. The BSA was added to fill all free solid-phase adsorption sites. The tubes were then washed three times with PBS and 1 ml of rabbit anti-IgE was added to each tube. This reaction proceeded for 48 hr at 4° C. The rabbit anti-IgE was removed, saved for reuse, and the tubes rinsed three times in PBS. These tubes then acted as a solid-phase immunoabsorbent for the patient's serum IgE. The prepared tubes can be used immediately or stored at 4° C for several weeks without losing binding efficiency. The tubes are absolutely specific for IgE and will not bind ^{125}I -labeled immunoglobulins of the IgG, IgM, or IgA class.

Nine-tenths milliliter of 1% BSA and 0.1 ml of test

serum were added to the prepared polystyrene tubes. A control serum was treated in the same manner to determine nonspecific binding. The fraction of the patient's serum IgE which was bound to the tube was determined by adding a trace quantity ^{125}I IgE to one solid-phase tube containing 0.9 ml of 1% BSA and 0.1 ml of the patient's serum. All tubes are then incubated at 4° C for 48 hr. After incubation, the tubes which had been trace-labeled with ^{125}I IgE were counted in a gamma counter, washed 10 times in tap water, and recounted. The fraction of the patient's serum IgE which had bound to the solid phase was calculated from the pre- and post-wash counts after a small correction had been made for the immune reactivity of the ^{125}I IgE.¹

The fractions of patient's IgE in 0.1 ml of serum bound to the polystyrene immunoabsorbent ranged from 20% to 80%. Usually 50% to 80% of a patient's IgE will be bound. Patients with high levels of total IgE, e.g., 24,000 ng/ml, will still have 20% of their IgE bound to the solid phase.

Tubes with serum only were washed three times in PBS and labeled allergens were added in 1 ml of 1% BSA. For ^{125}I RW, 1×10^6 cpm were added which contained approximately 100 ng of RW P. For ^{125}I GA 1×10^6 cpm were added. For ^{125}I AgE, approximately 100,000 cpm were added, which was equivalent to 10 ng of AgE. The tubes

TABLE I. Reproducibility of ^{125}I RW binding by IgE antibody with increasing amounts of ^{125}I RW added

Patient	^{125}I RW cpm added			
	10^4 cpm	10^5 cpm	10^6 cpm	2×10^6 cpm
A	352*	2,919	12,195	15,469
	316	2,853	13,079	16,825
	516	2,695	12,658	15,402
Mean $\pm$ SD	394 ± 87	$2,822 \pm 93$	$12,644 \pm 361$	$15,898 \pm 655$
CV	22.1%	3.3%	2.8%	4.1%
B	137	1,015	4,657	5,737
	228	891	4,238	7,287
	204	969	3,886	6,129
Mean $\pm$ SD	189 ± 38	958 ± 51	$4,260 \pm 315$	$6,384 \pm 658$
CV	20.1%	5.3%	7.4%	10.3%
Average Control Binding	34	151	842	1518

CV: coefficient of variation.

*All values in table cpm ^{125}I RW bound (–) control values. Binding of ^{125}I RW by IgE antibody in 0.1 ml serum.**TABLE II.** Inhibition of ^{125}I RW binding by IgE antibody with increasing amounts of AgE

Patient	% inhibition		
	^{125}I RW + 100 ng AgE	^{125}I RW + 500 ng AgE	^{125}I RW + 1,000 ng AgE
1	25	ND	25
2	21	ND	41
3	25	50	42
4	34	34	46
5	36	ND	48
6	30	46	49
7	46	58	54
8	29	ND	55
9	36	ND	61
10	32	45	61
11	49	ND	69
12	44	69	69
13	72	ND	72
14	41	69	72
15	53	67	73
16	43	59	77
17	62	ND	79
18	63	72	81
19	74	ND	82
20	78	ND	85
Mean $\pm$ SD	44.7 ± 16.9	56.9 ± 12.1	62.1 ± 16.1

containing the labeled allergens were incubated at 4°C for 48 hr. After incubation, the tubes were washed 10 times in tap water and counted. To determine the ng of RW, GA, or purified AgE which had been bound by specific IgE antibody, the cpm bound were divided by the specific activity of the labeled allergen (cpm per ng), which gave ng of allergen bound or ng of AgE bound. Control binding was then subtracted and the ng of allergen bound or AgE bound by the IgE antibody in 1 ml of patient serum was calculated by the following formula: ng allergen or ng AgE bound times dilu-

tion divided by the fraction of patient's IgE which is bound to the polystyrene tube.

RESULTS

Cutaneous reactivity of the allergen extracts

Serial 10-fold dilutions of RW and GA were made from an initial solution containing 0.5 mg P per ml and were tested intradermally to determine the highest dilution giving a 4+ reaction. In the 4 patients stud-

ied, 4+ skin reactivity was elicited at dilutions between 10^{-6} and 10^{-8} .

Reproducibility and quantitative aspects of the binding of ^{125}I RW by IgE antibody

The IgE antibody in 0.1 ml of serum from 2 patients was bound to a series of polystyrene tubes through the IgE anti-IgE immunoabsorbent. ^{125}I RW was added in increasing concentration in triplicate from 10,000 cpm to 2×10^6 cpm (Table I). As can be seen from Table I, as the concentration of ^{125}I RW is increased the cpm bound by IgE antibody steadily increases. After 1×10^6 cpm is added, there is little additional increase in cpm bound by adding another million counts. This point is in moderate antigen excess. We therefore have done most of our subsequent assays using 1×10^6 cpm ^{125}I RW, which is equivalent to adding 100 ng of ragweed allergen P. Triplication was good with a coefficient of variation of 2.8% for Patient A and 7.4% for Patient B at 1×10^6 cpm added.

Comparison of patient's IgE binding of ^{125}I AgE and ^{125}I RW

The ability of patient's IgE antibody to bind ^{125}I AgE and ^{125}I RW was compared. To a series of polystyrene tubes to which the patient's IgE from 0.1 ml serum had been bound was added in duplicate either ^{125}I AgE (100,000 cpm, 10 ng AgE) or 1×10^6 cpm ^{125}I RW (100 ng RW P). After appropriate incubation and washing, the cpm of ^{125}I RW and ^{125}I AgE bound were determined and the ng of AgE or RW bound by IgE antibody in 1 ml of serum was calculated. The results of this experiment are shown in Fig. 2. As can be seen, the binding of AgE by a patient's IgE was paralleled by the binding of RW. The ability of the patient's IgE to bind AgE covered a wide range from 0.2 ng AgE bound per ml of 94 ng AgE bound per ml. The ability of patient's IgE to bind RW likewise covered a wide range from 0.2 ng of RW P bound to 75 ng RW P bound. The correlation coefficient, r , was 0.90, $p < 0.001$. These results suggested that each of the 37 patients had some of their serum IgE antibody directed against AgE but gave no information as to the other allergens that may have been bound along with AgE.

Inhibition of patient's binding of ^{125}I RW by AgE

To establish that other allergens in ^{125}I RW in addition to AgE were being bound by patient's solid-phase bound IgE antibody, inhibition analysis was done. Using the serum from 20 patients with high binding of ^{125}I RW, 1×10^6 cpm ^{125}I RW was added in the presence of 0, 100, 500, and 1,000 ng of highly puri-

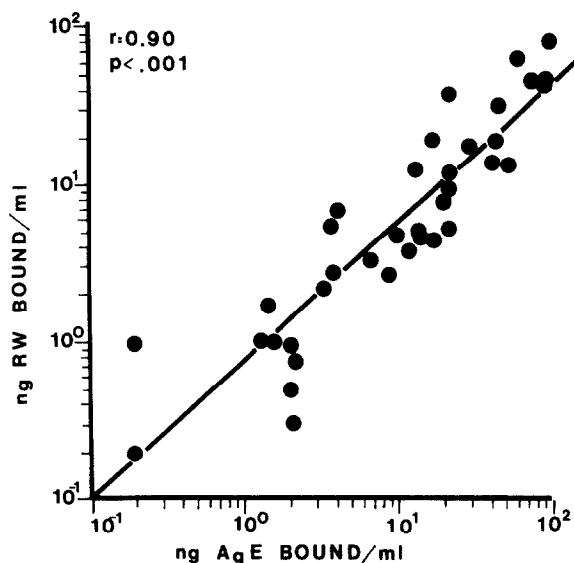


FIG. 2. The relationship between patient's IgE binding of AgE and multiple ragweed allergens, RW.

fied AgE to polystyrene tubes to which their IgE had been absorbed. Each level of inhibition was done in duplicate and the average percent inhibition was determined. As can be seen from Table II with increasing amounts of AgE added, percent inhibition either reached plateau or approached plateau levels. In 4 patients who showed less than 50% inhibition at 1,000 ng AgE added, inhibition analysis was extended to 2,000 ng AgE added utilizing another ^{125}I RW label on a different day. Inhibitions at 2,000 ng AgE added were 38%, 40%, 41%, and 47%. For these 20 patients, inhibition of ^{125}I RW binding by AgE ranged from 25% to 85% with a mean of 62.1% at 1,000 ng AgE added. This indicated that IgE antibody binding of ^{125}I RW in these patients was directed not only against AgE in RW but also directed against other allergens in RW.

Inhibition of ^{125}I RW binding by AgE and RW

To further confirm that other allergens were contained in RW, we designed an experiment which compared the inhibition of binding of ^{125}I RW by IgE antibody in the presence of 1,000 ng of AgE and 10,000 ng of RW in 2 patients (Table III). Maximum inhibition was obtained in these two patients at 1,000 ng AgE added and the percent inhibition was 58% and 64%, respectively. The addition of 10,000 ng RW resulted in 97% and 99% inhibition. The RW used for inhibition was identical to that which had been labeled with ^{125}I . This experiment again indicated that there were other allergens besides AgE in the RW which were recognized by IgE antibody, and that the binding of ^{125}I RW by IgE antibody could be completely inhibited in the presence of excess RW.

TABLE III. Inhibition of ^{125}I RW binding by IgE antibody with excess AgE and RW

Patient	^{125}I RW	^{125}I RW + 1,000 ng AgE		^{125}I RW + 10,000 ng RW	
	cpm bound	cpm bound	Average % inhibition*	cpm bound	Average % inhibition*
1	20,231	9,449	58	1,549	97
	24,807	10,250		1,643	
2	12,993	5,342	64	991	99
	13,675	5,462		1,304	
Control	1,026	1,002		865	
	1,438	1,395		1,111	

*% inhibition = $\frac{\text{Uninhibited cpm} - \text{Inhibited cpm}}{\text{Uninhibited cpm}}$. (All values calculated minus control.)

TABLE IV. Binding of ^{125}I GA by IgE antibody with increasing amounts of ^{125}I GA added

Patient	^{125}I GA cpm added			
	10^4 cpm	2×10^5	1×10^6	2×10^6
C	118*	908	3,502	5,078
	120	804	3,526	4,969

*All values in table cpm ^{125}I GA bound (—) control values. Binding of ^{125}I GA by IgE antibody in 0.1 ml serum.

Studies utilizing ^{125}I GA

Increasing concentrations of ^{125}I GA were added to a fixed amount of a patient's IgE from 0.1 ml serum that had been bound to the polystyrene immunoabsorbent. As can be seen from Table IV, IgE binding of ^{125}I GA increased dramatically with increasing concentrations of ^{125}I GA added. The binding of ^{125}I GA approached plateau levels at 1×10^6 cpm ^{125}I GA added (135 ng added). Increasing the concentration of ^{125}I GA added from 1×10^6 cpm to 2×10^6 cpm resulted in only a small increase in ^{125}I GA bound; therefore subsequent assays were done utilizing ^{125}I GA at 1×10^6 cpm added.

The sera of 7 clinically grass-sensitive patients were analyzed for the ability of IgE antibody to bind the ^{125}I GA. There was a 25-fold range in binding from 0.6 to 15 ng of GA bound by IgE antibody per ml of serum (Table V).

DISCUSSION

The polystyrene tube assay for specific IgE antibody directed against purified and complex allergen mixtures measured the primary interaction between ^{125}I -labeled allergen and IgE antibody. This assay has the advantage that the labeled allergens are in solution and are free to interact with IgE antibody. IgE bound allergen is easily separated from free allergen by washing the polystyrene tubes. Additionally, this assay eliminates the possibility that other antibody

TABLE V. Binding of ^{125}I GA by IgE antibody of grass-sensitive patients

Patient	Ng ^{125}I GA bound/ml serum (135 ng added)
1	15.0
2	6.0
3	5.3
4	3.5
5	2.5
6	2.0
7	0.6

will interfere with the interaction between IgE and relevant allergens.

The assay does depend on the availability of IgE myeloma protein. It is very sparing of IgE protein in that 20 mg of IgE P will serve to prepare 5,000 polystyrene tubes.

The aqueous allergens used in this study were partially purified by ammonium sulfate precipitation and in the case of RW further purified by passing the ammonium sulfate precipitated material through a Sephadex G-25 column. The starting material is very similar to fraction A described by King, Norman, and Connell⁵ which is the starting material for purification of all the major ragweed allergens. These partially purified allergen extracts were skin-reactive in allergic patients at high dilutions.

The allergen extracts obtained in this manner were easily labeled with ^{125}I utilizing the chloramine-T method to high specific activity. Unfractionated RW extract would not label well for unknown reasons. TCA analysis of the labeled RW and GA enable us to determine the percent of protein and therefore the micrograms of labeled allergen P in the label. Knowing the total ^{125}I cpm and the μg of allergen P present, the average specific activity, e.g., cpm/ng of allergen P, can be determined. In this manner, cpm of allergen bound by IgE antibody can be converted to ng of

allergen P bound. The optimum level of added ^{125}I RW was 1×10^6 cpm representing approximately 100 ng of RW protein. At this level of allergen added triplication was excellent with a coefficient of variation ranging from 3% to 7% (Table I).

The individual patient's value is expressed as the ng of allergen protein bound by their IgE antibody in 1 ml of serum. This value gives no information as to the nature of the particular allergens being bound by an individual's IgE antibody but is an estimate of their total binding of a complex allergen such as RW.

As an approach to the problem of what allergens are being bound by an individual's IgE antibody, we compared patient's binding of RW with that of purified AgE. The binding of RW by IgE antibody in patient's serum ranged from 0.2 to 74 ng/ml and paralleled the binding of purified AgE (Fig. 2). Patients with low levels of IgE antibody directed against AgE tended to have low levels of IgE antibody against RW, and conversely patients with high levels of IgE antibody against AgE tended to have high levels of antibody activity against RW. This indicated that AgE was one of the primary allergens in RW bound by the IgE antibody of these patients but gave little information as to individual differences in IgE binding of the other allergens present in RW.

In this regard, the interaction between solid-phase bound IgE antibody and ^{125}I RW was used to determine the percentage of total ^{125}I RW binding by IgE antibody of a patient's serum which was attributable to AgE and the percentage of total ^{125}I RW binding which was attributable to other allergens. In this analysis, increasing concentrations of purified AgE added to ^{125}I RW competitively inhibited the binding of labeled AgE in ^{125}I RW by IgE antibody. By adding large excesses of AgE to a point where inhibition reached a plateau, the fraction of the binding of ^{125}I RW by a patient's IgE which was AgE-specific could be determined. There was a continuous range of inhibition from 25% to 85%, indicating that these patients had individual variation in the fraction of their ragweed-specific antibody which was directed against AgE and other allergens in RW. In a recent review, King¹⁰ has underscored the importance of AgE, but other antigens such as antigen K, Ra3, Ra5, Ra4, and as yet to be defined rapidly extracted components are of importance in some allergic patients. This assay would be of value in determining individual patient differences in the ability of their ragweed-specific IgE to bind these various other allergens of ragweed pollen.

Our studies utilizing ^{125}I GA indicate that the binding is reproducible and reached a useful working range at 1×10^6 cpm ^{125}I GA added (135 ng). The

quantity of ^{125}I GA bound by IgE antibody in 1 ml of serum ranged from 0.6 to 15 ng in the 7 patients studied (Table V). These observations indicate that IgE antibody activity against other major allergen (GA) is readily quantifiable.

When purified allergens such as AgE are used in this assay system, it is possible to estimate the absolute quantity of IgE antibody present which is directed against AgE because the molecular weight of AgE is known and large AgE excess insures complete saturation of IgE binding sites.¹ Since the molecular weights, the exact proportion of multiple allergens in RW, and only moderate antigen excess is reached, this assay can determine only the relative binding activity of IgE antibody in a serum directed against these complex allergen mixtures. The results are therefore expressed as ng of allergen bound by IgE antibody per ml of serum.

There are presently two other published methods for determining the absolute quantity of IgE antibody directed against purified¹¹ and multiple allergens¹² using RAST technology. In these systems, IgE antibody reacts with solid-phase bound allergen, while in our system solid-phase bound IgE antibody reacts with labeled allergen in solution. The major advantage of our assay system is that *only* IgE antibody reacts with labeled allergen. In a radioallergosorbent test (RAST) system, there is the possibility that antibody other than IgE directed against the solid-phase bound allergen will interfere by competing with IgE antibody for allergenic determinants.

This assay offers the potential of quantitating specific IgE antibody responses directed against multiple allergens in patients who have IgE-mediated hypersensitivity to a variety of complex environmental agents, where the major allergens have not as yet been characterized. The assay lends itself to inhibition analysis by which a patient's IgE response to multiple allergens e.g., in ragweed pollen, can be dissected when purified allergens such as AgE are available.

REFERENCES

1. Zeiss, C. R., Pruzansky, J. J., Patterson, R., and Roberts, M.: A solid phase radioimmunoassay for the quantitation of human reaginic antibody against ragweed antigen E, *J. Immunol.* **110**:414, 1973.
2. Metzger, W. J., Patterson, R., Zeiss, C. R., Irons, J., Pruzansky, J. J., Suszko, I., and Levitz, D.: Polymerized ragweed antigen E. Clinical and immunologic studies, *N. Engl. J. Med.* **295**:1160, 1976.
3. Irons, J., Pruzansky, J. J., Patterson, R., and Zeiss, C. R.: Studies of perennial ragweed immunotherapy. Associated changes in cellular responsiveness, total serum antigen binding capacity, and specific IgE antibody concentrations, *J. ALLERGY CLIN. IMMUNOL.* **59**:190, 1977.
4. Booth, B. H.: Diagnosis of immediate hypersensitivity, *In* Pat-

- terson, R., editor: Allergic diseases diagnosis and management, Philadelphia-Toronto, 1972, J. B. Lippincott Co., p. 63.
5. King, T. P., Norman, P. S., and Connell, J. L.: Isolation and characterization of allergens from ragweed pollen. II, *Biochemistry* **3**:458, 1964.
 6. Gleich, G. J., Averbek, A. K., and Svedlund, H. A.: Measurement of IgE in normal and allergic serum by radioimmunoassay, *J. Lab. Clin. Med.* **77**:690, 1971.
 7. Patterson, R., Talbot, C. H., and Roberts, M.: Reverse passive respiratory reactions due to anti IgE in rhesus monkeys, *Clin. Exp. Immunol.* **110**:1402, 1972.
 8. Patterson, R., Suszko, I. M., and McIntire, F. C.: Polymerized ragweed antigen E. I. Preparation and immunologic studies, *J. Immunol.* **110**:1402, 1973.
 9. Patterson, R., Suszko, I. M., Pruzansky, J. J., Zeiss, C. R., Metzger, W. J., and Roberts, M.: Polymerization of mixtures of grass allergens, *J. ALLERGY CLIN. IMMUNOL.* **59**:314, 1977.
 10. King, T. P.: Clinical and biological properties of some atopic allergens, *Adv. Immunol.* **23**:77, 1976.
 11. Schellenberg, R. R., and Adkinson, N. F., Jr.: Measurement of absolute amounts of antigen-specific human IgE by a radioallergosorbent test (RAST) elution technique, *J. Immunol.* **115**:1577, 1975.
 12. Gleich, G. J., Jacob, G. L., Yunginger, J. W., and Henderson, L. L.: Measurement of the absolute levels of IgE antibodies in patients with ragweed hayfever, *J. ALLERGY CLIN. IMMUNOL.* **60**:188, 1977.