

Structure and activity of ragweed antigen E

II. Allergenic crossreactivity of the subunits

Barry R. Paull, M.D.,* Gerald J. Gleich, M.D., and
M. Zouhair Atassi, Ph.D., D.Sc. Rochester, Minn.

Recently we have reported the isolation of the two subunits (α and β) of ragweed antigen E (AgE) in their active form. In the present work we have compared the allergenic activity of the two subunits with native AgE by the radioallergosorbent test (RAST) and by inhibition of the RAST. Also, the amounts of IgE antibodies in 30 ragweed-sensitive sera bound to α and β subunits as well as native AgE were measured by RAST. In all individuals the percentage of RAST binding was greatest to native AgE; however, varying patterns of reactivity to α and β subunits were noted. In RAST inhibition experiments, free β subunit was equally effective in inhibiting the binding of IgE antibody (from a pooled ragweed-sensitive sera) to immobilized α or β subunit. Similarly, fluid-phase α subunit inhibited in a comparable manner the binding of IgE antibody to solid-phase α or β subunits. In further RAST inhibition experiments 7×10^{-13} moles of fluid-phase native AgE was required for 50% inhibition of the binding of IgE antibody to solid-phase native AgE. Larger molar amounts of fluid phase β (3.5×10^{-11}) and α (3×10^{-10}) were needed for 50% inhibition of the binding of IgE antibody to solid-phase native AgE. AgE allergenicity as measured by RAST could not be totally reconstituted by recombination of equimolar quantities of α and β subunits. Less than 1×10^{-12} moles of fluid-phase native AgE inhibited the binding of IgE antibody to solid-phase α or β , greater than 50%. Finally, the isolated chains are biologically active as demonstrated by their ability to provoke wheal-and-flare skin reactions in subjects allergic to ragweed pollen, and the activities of the chains were approximately equivalent on a weight basis to native AgE.

The main allergenic component of ragweed pollen, antigen E (AgE), has received much attention recently because of its importance as an etiologic agent in seasonal allergic rhinitis. It has been demonstrated by skin test and histamine release studies¹⁻³ that AgE is the most active allergen in the crude extract of ragweed pollen. The immunologic activity of the dimeric AgE has been thought to depend on an intact quaternary structure. The two subunits of AgE have been

separated by employing powerful dissociating agents such as 6 M guanidine hydrochloride,¹ 1% sodium dodecyl sulfate, or 6 M urea,⁵ and in each case the separated subunits were biologically inactive. However, it was subsequently demonstrated⁶ that mere pretreatment with 8 M urea caused the complete loss of the allergenic activity of AgE. Therefore it would be expected that AgE which had been inactivated by this treatment would naturally yield inactive subunits.

Determination of the allergenic sites of AgE would obviously require, as a vital first step, the preparation of active subunits from AgE. This obstacle was recently resolved by our introduction of a new approach by which the subunits were obtained in their active form from AgE.⁷ Reversible citraconylation of the amino groups on AgE effected dissociation of the protein into its two (α and β) subunits which were separated by gel filtration. Following unmasking of the amino groups, the α and β subunits thus obtained were found to be quite active by the radioallergosorbent test (RAST). The ability to prepare biologically

From the Departments of Pediatrics, Internal Medicine, and Immunology, Mayo Medical School and Mayo Clinic and Foundation. This work is supported in part by Grants AI 11483 and AI 11974 from the National Institute of Allergy and Infectious Diseases and by contract AI 42546 from the Research Resources Branch of the National Institute of Allergy and Infectious Diseases.

Received for publication March 6, 1979.

Accepted for publication May 30, 1979.

Reprint requests to Professor M. Z. Atassi, Department of Immunology, Mayo Medical School, Rochester, MN 55901.

*Recipient of a training program grant, AI 07047, from the National Institute of Allergy and Infectious Diseases.

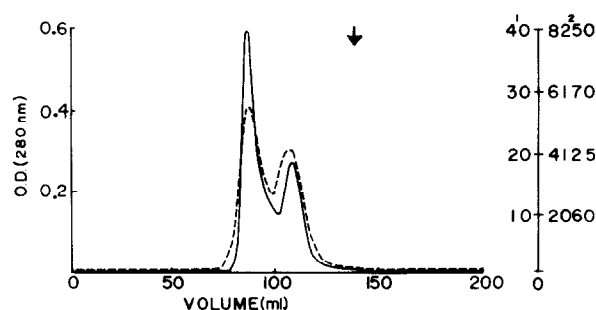


FIG. 1. Gel filtration profile of citraconylated AgE on Sephadex G-100. (—) represents $A_{280\text{ nm}}$, (---) represents IgE binding activity as determined by RAST. On the right ordinate 1 represents activity expressed in percent of total radioactive counts bound, and 2 represents net counts per minute (cpm) bound. The elution peak of native AgE is indicated by the arrow.

active subunits has now made it feasible to undertake immunologic investigations into the molecular basis of the allergenicity of the subunits.

In this communication we have extended our preliminary observations⁷ on the allergenicity of the two active subunits of AgE and have examined their crossreactivity with IgE antibodies. In addition, we have examined the allergenicity and pattern of reactivity of AgE and the α and β subunits with 30 sera from ragweed-sensitive patients and have shown that the subunits of AgE provoke wheal and flare reactions in sensitive patients.

MATERIALS AND METHODS

Sera

Sera used in this study have been described in detail previously.⁷⁻⁹ Briefly, patients were chosen on the basis of the following criteria: past history of seasonal rhinitis, positive immediate intradermal skin test and nasal challenge with crude short ragweed extract, no prior immunotherapy, and a preseasonal level of serum IgE antibodies greater than seven times control binding as measured by the RAST. A ragweed serum pool was obtained from five untreated patients with ragweed hay fever. IgE antibodies to ragweed allergens in the pool exceeded 2,000 ng/ml.⁹

Allergens

Purified AgE and Rye group I were kindly provided by the Research Resources Branch of the National Institute of Allergy and Infectious Diseases. AgE was dialyzed against several changes of 0.05 M NH_4HCO_3 (pH 8.0) buffer to remove glycerol and was lyophilized prior to use.

AgE subunits

The subunits of AgE were obtained essentially as described previously,⁷ except that Sephadex G-100 (Pharmacia Labs. Inc., Piscataway, N. J.) was utilized in the present work and the column length was increased to 200

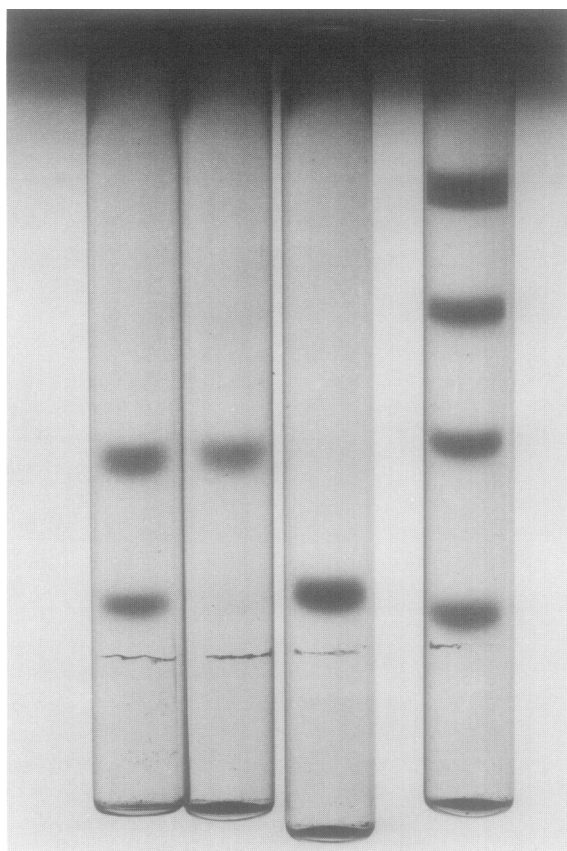


FIG. 2. SDS acrylamide disk gel electrophoresis of AgE subunits. From left to right: AgE, α subunit, β subunit, and marker gel with bovine serum albumin (molecular weight, 69,000 daltons), ovalbumin (49,000 daltons), chymotrypsinogen (25,000 daltons), and cytochrome c (12,500 daltons).

cm to affect a better separation. Briefly, 15 mg of AgE was reconstituted in 500 to 1,000 μl of 0.05 M potassium phosphate (pH 8.0) buffer, and a 10^2 - to 10^3 -fold molar excess of citraconic anhydride was added in small aliquots (2 to 5 μl) while pH was kept constant at 8.6. Following citraconylation, the reaction product was subjected to gel filtration on a 1.5×200 cm column of Sephadex G-100 equilibrated and eluted with 0.05 M NH_4HCO_3 (pH 8.0). It gave two peaks of absorbance at 280 nm which coincided with two peaks of IgE activity as measured by RAST (Fig. 1). Fractions belonging to each peak were pooled and lyophilized, and each was subjected to two repeated passages over the Sephadex column. The minor contamination seen in earlier work was thus eliminated. Purity was assessed by disk electrophoresis in 8% polyacrylamide gels containing 1% sodium dodecyl sulfate, and single heavily staining bands were seen for α and β subunits (Fig. 2). Following chromatographic separation the α and β pools were titrated to pH 6.0 with 0.1 M HCl and allowed to remain at 22° C for 48 hr to remove the citraconyl protecting group.^{10, 11} The α and β pools were finally dialyzed against 0.01 M potassium phosphate buffer (pH 7.0) and stored at -20° C prior to use.

Measurement of IgE antibodies

IgE antibodies were measured by the RAST.¹² Solid-phase antigens were prepared by linking 50 μ l of AgE (0.6 to 0.7 μ g) or its α (0.6 to 0.7 μ g) or β (3.25 μ g) subunits to individual cyanogen bromide-activated filter paper disks (6.3 mm diameter) as previously described.¹³ The coupling reaction of proteins to the disks was allowed to continue for 24 hr at 22° C, following which any free reactive sites remaining on the disks were blocked with 500 μ l of 1.0 M ethanolamine (pH 8.5) for an additional 24 hr. Detection of the reaction of allergens with IgE antibody was performed by direct RAST and RAST inhibition as previously described.¹³

Inhibition of the RAST

Inhibition of the binding of IgE antibody from the ragweed serum pool to solid-phase AgE or to the α or the β subunits was tested in the following manner: 50 μ l of inhibitor (α , β , or AgE) was combined with 50 μ l of ragweed pooled sera and allowed to incubate at 22° C for 24 hr, after which a freshly prepared allergen-linked disk (α , β , or AgE) was added and the test was completed as previously described.¹³ Inhibition experiments were also carried out employing a mixture of the chains prepared by combining 500 μ l of equimolar quantities of the α and β subunits and allowing the mixture to stir at 4° C for 48 hr before proceeding with the inhibition as described above.

Initially 30 μ g (50 μ l) of AgE was combined with each cyanogen bromide-activated filter paper disk and allowed to couple for 24 hr. However, larger quantities of inhibitor (AgE, α , or β subunits) were necessary to achieve 50% inhibition, and therefore in later experiments 0.6 to 0.7 μ g (50 μ l) of AgE (as above) was coupled to each disk. In RAST titration experiments with AgE and our ragweed pooled sera, a plateau level of binding was achieved at 0.6 μ g of AgE. Therefore, 0.6 to 0.7 μ g of AgE (or subunit) was coupled to each disk in the inhibition experiments.

Skin tests

Skin tests with AgE, α chain, and β chain were performed in two ragweed-allergic individuals. Concentrations of AgE and α and β chains spanning a range from 68 ng to 8 μ g/ml in sterile phosphate-buffered saline (PBS) (Grand Island Biological Co., Grand Island, N. Y.) were injected intradermally (0.02 ml), and the reactions at 15 min were measured and compared with a PBS control.

RESULTS

Preparation and characterization of the α and β subunits

The α and β subunits of AgE were prepared by gel filtration of citraconylated AgE. As shown in Fig. 1, two peaks of discrete IgE activity as determined by RAST coincided with the two protein peaks on the elution profile of citraconylated AgE, and column fractions from other parts of the chromatograph displayed no RAST activity. In this column, native AgE

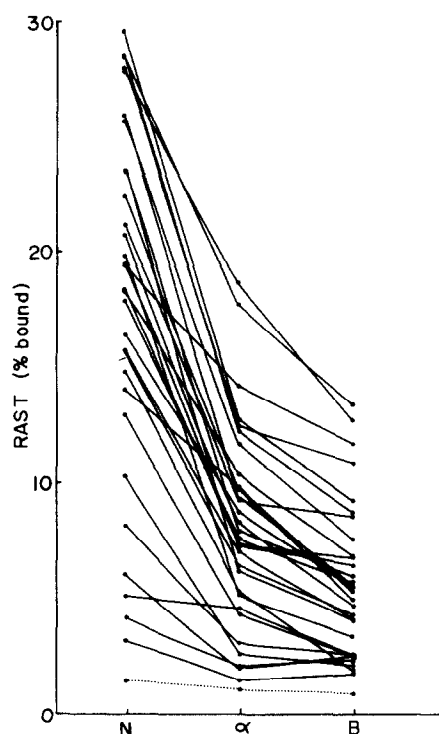


FIG. 3. RAST reactivity of sera from 30 ragweed-sensitive individuals tested with the α and β subunits and native (N) AgE. Lines are drawn between points to facilitate identification of individual patient's patterns of reactivity. RAST binding by a control serum from an individual without a history of ragweed hay fever is indicated by the dotted line. Percent binding represents percentage of 270,000 total ¹²⁵I counts of anti-IgE added which bound to AgE or α and β subunits.

eluted at 140 ml, and neither an absorbance peak nor an allergen peak, as detected by the RAST, was seen at that position. The result suggests that all of the AgE was dissociated into subunits. Following deblocking, the α and β subunits were tested for purity in polyacrylamide gels containing 1% sodium dodecyl sulfate (Fig. 2). Two bands were noted with native AgE corresponding to molecular weights of 25,000 and 13,900 daltons. The α and β subunits were homogeneous in heavily loaded gels and had molecular weights of 25,000 and 13,900 daltons, respectively. The amino acid composition of each subunit agreed well with the reported values.⁵

Binding of IgE antibodies to AgE and its subunits by RAST

The reactivity of IgE antibodies in 30 ragweed-sensitive sera with solid-phase AgE and α and β subunits was measured by RAST. The results are shown in Fig. 3. All patients displayed the highest percentage of RAST binding to the immobilized native AgE. In general, a higher reactivity with AgE was accom-

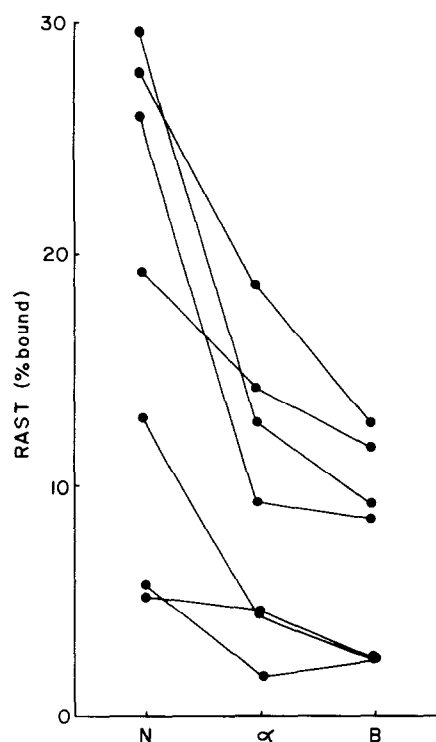


FIG. 4. RAST reactivity of seven patients to native (*N*) AgE and the α and β subunits (from Fig. 3). Three high-binding patients (greater than 25% of total radioactive counts bound), two patients with medium levels of binding (10% to 20%), and two patients with a low level of binding (5% to 10%) are shown.

panied by a higher reactivity to the subunits. However, there were numerous exceptions. The individual patterns of reactivity are better demonstrated for seven patients in Fig. 4. Three patients with high percentages of binding to native AgE (greater than 25% of counts bound) each showed different levels of binding to β subunit. Two of these patients had similar binding to α subunit, while the other had noticeably higher binding to the α . Two patients with an intermediate level of binding to AgE (10% to 20% of counts bound) bound different amounts of IgE antibody to α and β subunits. In one of the intermediate-binding sera, the pattern of reactivity to α and β was similar to the pattern seen in two patients with low binding to AgE (<10% of total counts bound). One patient with a low percentage of IgE antibody to AgE showed a slightly higher percent of binding to β than to α subunit.

RAST inhibition studies with AgE and its subunits

By the RAST, significant percentages of total radioactive counts were bound to the α , β , or AgE disks (10 to 30 times control binding) when tested with the pool of ragweed-sensitive sera. To determine if α

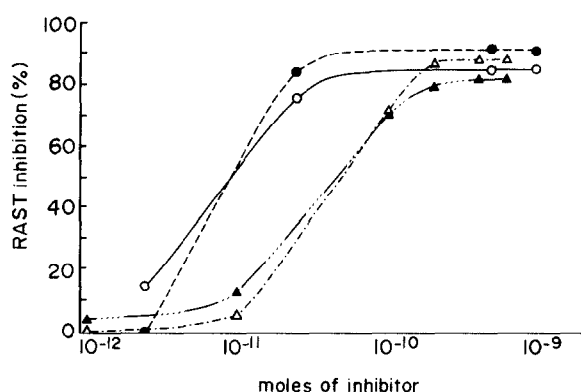


FIG. 5. Inhibition of the binding of antiragweed IgE antibody (from a ragweed pooled serum) to disk-immobilized α and β chains by fluid-phase α or β subunit. (○), Solid-phase β inhibited with fluid-phase β . (●), Solid-phase α inhibited with fluid-phase β . (▲), Solid-phase β inhibited with fluid-phase α . (Δ), Solid-phase α inhibited with fluid-phase α .

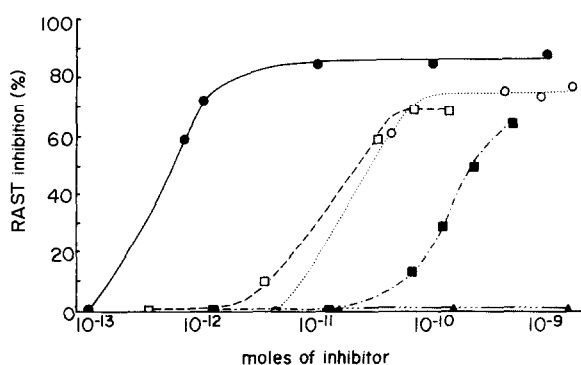


FIG. 6. Inhibition of the binding of antiragweed IgE antibody (from a ragweed pooled serum) to solid-phase AgE by (○), AgE; (□), β subunit; (■), α subunit; and (□), equimolar mixture of α and β subunits. (▲), An unrelated allergen rye group 1.

and β subunits of AgE possessed significant crossreactivity with native AgE and with each other, they were tested by RAST inhibition using the ragweed serum pool. In the RAST inhibition experiments, α and β subunits were coupled to activated filter paper disks, and the ability of fluid-phase α and β subunits to inhibit the binding of IgE antibody to the disk adsorbents was tested (Fig. 5). Essentially identical patterns of inhibition were generated when fluid-phase β subunit inhibited the binding of IgE antibody to either immobilized α or β subunit, with 50% inhibition occurring at 1×10^{-11} moles of fluid-phase β added. Similarly, when fluid-phase α was the inhibitor, it showed virtually identical inhibitory activities toward the binding of IgE antibody to solid-phase α and β subunits, and 50% inhibition occurred at 5×10^{-10} moles of fluid-phase α added.

TABLE I. Skin tests with AgE and the α and β subunits in ragweed allergic individuals

		Concentration			
		68 ng/ml	340 ng/ml	1.7 μ g/ml	8 μ g/ml
Patient No. 1					
AgE					
Wheal		5 $\times$ 4	6 $\times$ 7	9 $\times$ 8	14 $\times$ 28
Flare		9 $\times$ 20	9 $\times$ 16	25 $\times$ 25	50 $\times$ 30
α					
Wheal		4 $\times$ 4	5 $\times$ 5	9 $\times$ 10	17 $\times$ 11
Flare		—	15 $\times$ 10	30 $\times$ 30	40 $\times$ 40
β					
Wheal		3 $\times$ 3	4 $\times$ 4	8 $\times$ 7	11 $\times$ 9
Flare		—	10 $\times$ 10	35 $\times$ 30	35 $\times$ 35
Patient No. 2					
AgE					
Wheal		3 $\times$ 3	6 $\times$ 3	8 $\times$ 10	12 $\times$ 10
Flare		6 $\times$ 6	7 $\times$ 4	12 $\times$ 18	45 $\times$ 25
α					
Wheal		—	6 $\times$ 3	5 $\times$ 5	7 $\times$ 8
Flare		—	5 $\times$ 7	—	10 $\times$ 7
β					
Wheal		—	5 $\times$ 5	5 $\times$ 5	12 $\times$ 13
Flare		—	7 $\times$ 6	9 $\times$ 8	25 $\times$ 30

Four concentrations of AgE, α or β subunits, were used to elicit skin reactivity, and wheal-and-flare measurements (in millimeters) were recorded at 15 min. PBS controls showed no skin reactivity at 15 min. The volume injected intradermally in each case was 0.02 ml.

The patterns of inhibition of binding of IgE antibody to immobilized AgE by fluid-phase AgE or α or β subunit and by an equimolar mixture of the two subunits are shown in Fig. 6. Small molar quantities of fluid-phase native AgE (7×10^{-13} moles) were required for 50% inhibition of the binding of IgE antibody in the ragweed serum pool. Larger quantities of fluid-phase β (3.5×10^{-11} moles) and fluid-phase α (3×10^{-10} moles) were needed for 50% inhibition of the binding of IgE antibody to the solid-phase AgE. An attempt was made to reconstitute native AgE by combining equimolar quantities of α and β subunits as described in the Methods section. In several experiments, one of which is shown in Fig. 6, some reconstitution was seen as evidenced by a shift of the activity curve to the left from the activity expected on the basis of simple mixing. However, in no experiment was the inhibitory activity of the native protein fully regenerated.

In inhibition studies using AgE as the inhibitor, greater than 70% inhibition of the binding of IgE antibody to α or β disks was accomplished with less than 1×10^{-12} moles of fluid-phase native AgE. Control experiments demonstrated that an unrelated allergen, rye group I, showed essentially no inhibition when added as an inhibitor to prevent IgE antibody from binding to immobilized AgE or α or β subunit. Fur-

thermore, solid-phase rye group I did not bind IgE antibody from the ragweed-sensitive pooled serum.

Skin tests

Skin tests with AgE and α and β subunits were performed in two ragweed-allergic (hay fever) individuals, and positive skin reactivity was elicited in both individuals to AgE and the α and β subunits. Table I shows the results of skin tests in these volunteers. An increase in the size of wheal and flare occurred with increasing concentrations of AgE, α , or β ; appropriate PBS controls showed no skin reactivity.

DISCUSSION

It is pertinent to discuss at the outset the purity and activity of the AgE subunits. Citraconylation of AgE has the effect of reversing the positive charges on the side chains of lysine residues and the α -amino groups. As a result of the abundant distribution of net negative charges on the surface of the protein, the latter is expected to undergo considerable unfolding.^{10, 11, 12} In oligomeric proteins, in which the subunits are not covalently linked, the unfolding attending the citraconylation will very frequently cause dissociation of the protein into its constituent subunits.¹¹ Indeed, gel filtration of citraconylated AgE afforded two discrete

peaks as expected. Perhaps it should be pointed out here that in gel filtration a citraconylated protein will elute earlier than expected from its molecular weight. This is due to the fact that the unfolding of the protein causes an increase in the Stoke's radius, and in gel filtration the elution position correlates more precisely with the Stoke's radius than with molecular weight.¹⁵⁻¹⁷ This explains why the citraconylated subunits elute faster than native AgE (Fig. 1). It is highly significant that the two ultraviolet-absorbing peaks coincided completely with discrete peaks of allergenic activity (Fig. 1). The two protein peaks, each after rechromatography twice on the same column, were homogeneous (Fig. 2) by disk electrophoresis in heavily loaded gels. The molecular weights and the amino acid compositions obtained were in excellent agreement with the values reported⁵ for the α and β subunits of AgE. It is noteworthy that native AgE was completely absent in the gel filtration profile of citraconylated AgE, the latter giving only the subunits. The fact that two discrete activity peaks were obtained which coincided completely with the elution positions of the two subunits and the fact that no activity was detected elsewhere in the elution profile (especially where AgE emerges) is very critical. It argues very strongly in favor of the conclusion that the activity is inherent in the subunits and is not the byproduct of contamination. And it is to emphasize visually this critical point that Figs. 1 and 2 are shown, even though similar (but not identical) diagrams were reported previously in a preliminary communication.⁷

Other evidence that the activity is inherent in the chains and not due to contamination can be seen in the patterns of reactivity of the individual patient sera with immobilized allergens in the RAST (Figs. 3 and 4). When tested with IgE antibody from sera of 30 ragweed-sensitive allergic individuals, the subunits show varying patterns of reactivity. Although comparison of the results of measurement of IgE antibody by RAST among patients is difficult because the efficiency of coupling various allergens may differ, in these experiments cellulose disks were sensitized with the same preparations of subunits, so patient results should be comparable. All patients tested bound IgE antibody (greater than control binding) to α and β subunits, and among patients the amounts of IgE antibody bound to α and β subunits varied markedly. In any event, these patterns are such that the results are difficult to explain by contamination of the isolated chains with AgE.

The abilities of the α and β subunits to bind IgE antibodies were comparable when each was tested in

its immobilized form. In the fluid state each subunit had lesser reactivity than native antigen E. Thus in RAST inhibition studies AgE was considerably more successful in competing for IgE antibodies than the β or the α chain, while the β chain had a considerably higher affinity (10-fold) than the α chain. This is not entirely unprecedented and presumably is primarily due to conformational factors. For example, in recent studies we have synthesized antigenic sites of human adult hemoglobin¹⁸ and bovine serum albumin¹⁹ which, when immobilized on Sepharose, quite efficiently bound radiolabeled IgG antibodies against the respective native protein but were incapable of inhibiting the binding of homologous protein with its antibodies. Also, we have carried out detailed optical rotatory dispersion and circular dichroism measurements on the isolated subunits and have found that the free subunits are considerably unfolded relative to the parent native protein.*

The results of the RAST inhibition studies with the antiserum pool indicate marked crossreactivity between the isolated subunits, with the fluid-phase β subunit being able to compete 10 times more successfully than fluid-phase α subunit for IgE antibodies. It is remarkable that the ability of free β subunit to inhibit binding of IgE antibody in the antiserum pool to immobilized β chain was almost superimposable with its ability to inhibit the binding to the immobilized α chain. Similarly, when α was the inhibitor, the inhibition curves toward immobilized α or immobilized β subunits were virtually identical. This magnitude of crossreactivity between the chains was totally unexpected. On the other hand, the results with the individual sera (Figs. 3 and 4) indicate that the activities of the subunits are not always equal. This suggests either that the extent of the allergenic recognition of the subunits as being functionally equivalent may depend on the serum, or more likely that the subunits bind to the same IgE antibody population but not always with equal affinity. In the latter case the functional equivalence of the allergenic sites does not require complete identity in all the binding residues of the sites. Conservative substitutions may occur, and the extent of their influence on the affinity constant will vary with the antiserum. A similar situation has recently been observed with the functionally equivalent (but not completely identical) antigenic sites of serum albumin.¹⁹ At any rate, the present findings clearly show that the major allergenic sites on the α subunit are equivalent to those on the β subunit, at least in functional terms.

By mixing the α and β subunits, the allergenicity of

*Paull BR, Atassi MZ: In preparation.

the native AgE could not be regenerated appreciably. Although a shift in the inhibition curve was observed which was greater than what would be expected by mere additivity of the independent activities of the subunits, the results clearly indicate that reassociation of the subunits to produce the native dimeric AgE had been quite limited. Recent circular dichroism measurements by us have indeed confirmed the inability of the subunits to reassociate upon mixing to regenerate the native dimeric protein.

The results of skin testing with AgE and the α and the β subunits, shown in Table I, indicate that the chains are potent allergens whose abilities to provoke wheal-and-flare reactions are roughly comparable on a weight basis to native AgE. Furthermore, the quantities of AgE required to elicit skin reactions in the two patients shown in Table I are in the range reported by King et al.^{1, 20} for their preparations of AgE. Although the quantities of α and β chains required to elicit skin reactions are roughly five times more than for AgE, the activity of the chains is difficult to explain on the basis of AgE contamination. For example, if the activity of the chains is due to AgE contamination, one must assume 20% contamination, and this degree of contamination is not supported by any of our other results. Finally, the potent skin reactivity of the chains supports the utilization of the RAST for measurement of the allergenic activity of the chains.

In conclusion, with a serum pool from ragweed-sensitive patients, the α and β polypeptide chains of ragweed AgE appear to have a high degree of allergenic crossreactivity, the β chain being more effective than the α chain in competing for antiragweed IgE antibodies. Reassociation of the α and β subunits to regenerate the native dimeric molecule is impaired by some unknown factors. Neither subunit is allergenically as active as native AgE, but they are more effective as allergens in direct RAST than in RAST inhibition. Also, by direct RAST in sera from 30 ragweed-sensitive individuals, AgE is generally more active than either of the subunits. Finally, skin tests show that the subunits are potent allergens able to provoke strong wheal-and-flare reactions.

REFERENCES

- King TP, Norman PS, Connell JT: Isolation and characterization of allergens from ragweed pollen. II. *Biochemistry* **3**:458, 1964.
- Lichtenstein LM, King TP, Osler AG: In vitro assay of allergenic properties of ragweed pollen antigens. *J ALLERGY CLIN IMMUNOL* **38**:174, 1966.
- King TP, Norman PS, Lichtenstein LM: Studies on ragweed pollen allergens. V. *Ann Allergy* **25**:541, 1967.
- Griffiths BW: The subunits of antigens of ragweed pollen. *J Chromatogr* **69**:391, 1972.
- King TP, Norman PS, Tao N: Chemical modifications of the major allergen of ragweed pollen, antigen E. *Immunochemistry* **11**:83, 1974.
- Takatsu K, Ishizaka K, King TP: Immunogenic properties of modified antigen E. III. Effect of repeated injections of modified antigen on immunocompetent cells specific for native antigen. *J Immunol* **115**:1469, 1975.
- Paull BR, Gleich GJ, Atassi MZ: Separation of active subunits of ragweed antigen E. *Immunochemistry* **15**:199, 1978.
- Welsh PW, Yunginger JW, Kern EB, Gleich GJ: Preseasonal IgE ragweed antibody levels as a predictor of response to therapy of ragweed hayfever with intranasal cromylin sodium solution. *J ALLERGY CLIN IMMUNOL* **60**:104, 1977.
- Adolphson CR, Goodfriend L, Gleich GJ: Reactivity of ragweed allergens with IgE antibodies: Analysis by leukocyte histamine release and the radioallergosorbent test and determination of cross-reactivity. *J ALLERGY CLIN IMMUNOL* **62**:197, 1978.
- Habeeb AFSA, Atassi MZ: Enzymic and immunochemical properties of lysozyme. III. Evaluation of several amino group reversible blocking reagents. *Biochemistry* **9**:4939, 1970.
- Atassi MZ, Habeeb AFSA: Reaction of proteins with citraconic anhydride. *Methods Enzymol* **25B**:546, 1972.
- Wide L, Bennich H, Johansson SGO: Diagnosis of allergy by an in vitro test for allergen antibodies. *Lancet* **2**:1105, 1967.
- Gleich GJ, Yunginger JW, in Rose NR, Friedman H, editors: *Manual of clinical immunology, standardization of allergens*, chap 77. Washington, D.C., 1976, American Society for Microbiology, p. 575.
- Singhal RP, Atassi MZ: Immunochemistry of sperm whale myoglobin. IX. Interaction of peptides obtained by cleavage at arginine peptide bonds. *Biochemistry* **10**:1756, 1971.
- Ackers GK: Molecular exclusion and restricted diffusion processes in molecular-sieve chromatography. *Biochemistry* **3**:723, 1964.
- Siegel LM, Monty KJ: Determination of molecular weights and frictional ratios of proteins in impure systems by use of gel filtration and density gradient centrifugation. Application to crude preparations of sulfite and hydroxylamine reductases. *Biochim Biophys Acta* **112**:346, 1966.
- Atassi MZ, Caruso DR: Immunochemistry of sperm whale myoglobin. II. Modification of the two tryptophan residues and their role in the conformation and antigen-antibody reaction. *Biochemistry* **7**:699, 1968.
- Kazim AL, Atassi MZ: Prediction and confirmation by synthesis of two antigenic sites in human haemoglobin by extrapolation from the known antigenic structure of sperm-whale myoglobin. *Biochem J* **167**:275, 1977.
- Atassi MZ, Sakata S, Kazim AL: Immunochemistry of serum albumin. 9. Localization and verification by synthesis of five antigenic sites of bovine serum albumin. *Biochem J* **179**:327, 1979.
- King TP, Norman PS, Lichtenstein LM: Isolation and characterization of allergens from ragweed pollen. IV. *Biochemistry* **6**:1992, 1967.