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STUDIES ON THE ALLERGENIC SIGNIFICANCE AND STRUCTURE OF RAT SERUM ALBUMIN

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This study evaluated the capacity of rat serum albumin and of its proteolytic fragments to activate human basophils for IgE-mediated histamine release. The leukocytes from 8 out of 33 patients allergic to rats released histamine with rat serum albumin. Two proteolytic fragments of rat serum albumin, each constituting half of the molecule, were used to study the IgE-reactive antigenic sites. These fragments released histamine with the cells of some of the donors, thus demonstrating the presence of at least 2 antigenic determinants on each fragment for a total minimum of 4 sites on the intact rat serum albumin molecule. Most of the allergenic activity, however, was not recovered in the 2 fragments (total recovery $\bar{x}$ = 6.4%, range between 0.1 and 31%). This loss could be due to cleavage of the rat albumin molecule in the middle of the third domain with loss of antigenic sites and/or due to minor conformational changes in the fragments as compared with the intact molecule. There was up to a 500-fold difference in the percent of activity recovered in the fragments when tested on cells from different patients. Therefore, there is no single immunodominant site on the molecule equally important for all patients. The cells of all 8 patients also reacted with mouse serum albumin but only 2 with bovine serum albumin. At least 1 determinant on mouse and rat serum albumin is cross-reactive with IgE.

Histamine release from washed basophils is an excellent *in vitro* model for IgE-mediated reactions. These studies usually utilize cells from pollen-sensitive patients and trigger the cells with allergen. Although a number of these protein allergens have been purified, the complete amino acid sequence of only a few has been determined, e.g., ragweed Ra5 (1) and cod allergen M (2). There is, however, very little information about the antigenic sites on these molecules capable of reacting with IgE. For example, 2 subunits of ragweed antigen E have been isolated which retained binding activity with human IgE (3). The cod allergen has been split into 2 large peptides, each capable of mediating some of the biologic activity of the native molecule (4). Several smaller tryptic fragments have also been isolated that retain some of the allergenic activity (5, 6).

Serum albumin is one of the most abundant proteins, and the complete amino acid sequence of bovine and human albumin is known (7). Although the complete sequence of rat albumin has not yet been determined, the sequence of several fragments has been analyzed (8, 9). The albumins from different species are structurally similar; out of 300 amino acids identified in rat serum albumin (RSA)[3], 208 were identical with the amino acids at the same positions in human serum albumin and 198 in bovine serum albumin (9). Structural studies indicate that the albumin molecule consists of 9 separate disulfide-bonded loops connected by peptide links of 11 to 26 residues (7). These loops define 3 domains. Limited proteolytic treatment of native albumin produces large fragments probably due to cleavage of unstructured parts of the molecule (10–13). Such fragments appear to retain much of the conformation they assumed in the intact albumin molecule (14). Two fragments have been isolated and characterized from bovine serum albumin. Each fragment consists of one half of the molecule; together they constitute the entire amino acid sequence of bovine albumin (11, 13). The 2 similar half-molecules prepared from RSA, called RSA-$P_{1\text{-}306}$ and RSA-$P_{307\text{-}581}$, were used in the present study.

We utilized RSA as a model for studies of the structure-function relationships of an allergen. Patients who are allergic to animals frequently react to the serum proteins of those same animals (15–18). Thus, serum albumin of various mammalian species (e.g., horses, cats, dogs, and mice) is an important allergen in patients allergic to these animals (18–23). We identified reactivity to RSA in about a quarter of rat allergic individuals; the cells of these individuals also reacted to mouse serum albumin (MSA) but rarely to bovine albumin. Therefore, each of these protein molecules should have at least 2 antigenic sites. Evidence is also presented that 1 antigenic determinant on MSA and RSA is recognized by the same IgE antibodies. Two fragments of the RSA, each constituting half the molecule, retained very little of the allergenic activity of the native albumin. There was wide variation in the reactivity of different individuals to the fragments. The data suggest the presence of at least 4 antigenic sites recognized by IgE on the RSA molecules.

MATERIALS AND METHODS

Materials. Rat allergen extract was obtained as an aqueous

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[3] Abbreviations used in this paper: D_2O, deuterium oxide; MSA, mouse serum albumin; RSA, rat serum albumin; RSA-$P_{1\text{-}306}$, peptic fragment of rat serum albumin composed of approximately the first 306 residues; RSA-$P_{307\text{-}581}$, peptic fragment of rat serum albumin composed of approximately residues 307 to 581; Pipes ACM, Pipes buffered medium containing Ca^{++} and Mg^{++}; EDTA, ethylenediaminetetraacetic acid.

extract in 50% glycerin from Hollister Stier Laboratories, Spokane, WA. This allergen is prepared from the whole pelt of rats. For histamine release experiments, the extract was used after dialysis against phosphate-buffered saline (NaCl 0.15 M, PO_4 0.02 M, pH 7.4). The deuterium oxide (D_2O) was purchased from Bio-Rad Laboratories, Richmond, CA.

Rat albumin (RSA). RSA was purified by Dr. Richard C. Feldhoff from serum of adult male Sprague-Dawley rats. The albumin was extracted from a trichloroacetic acid precipitate of the serum proteins with ethanol, dialyzed, and further purified by chromatography with DEAE-cellulose (13). Half-cystine was coupled to the thiol group by a previously described procedure (24) to prevent dimerization.

Bovine serum albumin. Bovine albumin was crystalline bovine plasma albumin from Miles Laboratories, Elkhart, IN.

Mouse albumin (MSA). MSA (Fraction V) was obtained from Sigma, St. Louis, MO. Fatty acids were removed by charcoal treatment as described by Chen (25). The albumin was then dialyzed against phosphate-buffered saline and purified by gel chromatography on a BioGel P100 column.

Solutions. A Pipes-buffered (pH 7.4) medium was used containing NaCl 110 mM, KCl 5 mM, NaOH 40 mM, Pipes 25 mM, and 0.03% human serum albumin (Pipes A). To this medium, 2 mM Ca^{++} and 0.5 mM Mg^{++} were added to prepare Pipes ACM used for diluting antigens and cells for the histamine release reaction. Stock EDTA[3] solution was 0.1 M, pH 7.2. Media containing D_2O were prepared by substituting D_2O for water in Pipes ACM.

Peptide fragments. The 2 fragments of RSA were prepared by Dr. Richard C. Feldhoff by limited peptic digestion as described for bovine albumin (13). Purity of all fragments was judged to be greater than 98% by electrophoresis on polyacrylamide gel or on cellulose. Their sizes and locations in the albumin molecule were identified by residue numbers and by the number of disulfide loops in each fragment. Fragment RSA-$P_{1\text{-}306}$ consists of amino acid residues approximately 1 to 306, with the disulfide loops 1 to 5, and has a m.w. of about 35,000; fragment RSA-$P_{307\text{-}581}$ consists of amino acid residues approximately 307 to 581, disulfide loops 6 to 9, and has a m.w. of about 31,500.

Clinical evaluation. The patients in this study were seen in the Employee Allergy Clinic at the N.I.H. They developed allergic symptoms upon exposure to rats. Most of the patients also developed symptoms on exposure to several other laboratory animals. Rat allergy was confirmed by history, intracutaneous skin test, and leukocyte histamine release. For skin tests, 0.02 ml of allergen was injected on the upper arm and the reactions were read at 15 min. Skin test grading was based on measurement of the wheal with the use of the following criteria: neg, site no different from a diluent control; 1+, ≤5 mm wheal; 2+, 5 to 10 mm wheal; 3+, 10 to 15 mm wheal without pseudopods; 4+, >15 mm wheal with or without pseudopods or >10 mm wheal with pseudopods. The highest concentration used for skin tests was 100 PNU/ml, and where appropriate, the skin tests were initiated with lower allergen concentrations.

Leukocytes. Blood was collected in plastic syringes and anticoagulated with 0.01 M EDTA, pH 7.2. It was sedimented at room temperature for 60 to 75 min with 2.5 ml of dextran (clinical dextran 6%, in 0.85% NaCl, to which 3% glucose was added) per 10 ml of blood. The upper layer, containing leukocytes, platelets, and plasma, was transferred by pipetting to another plastic tube and then centrifuged at 300 × G for 8 min at 4°C. The pelleted cells were suspended in Pipes A buffer containing 4 mM EDTA and centrifuged at 300 × G for 8 min at 4°C. The cells were then washed in Pipes A. After centrifugation, the cells were suspended in Pipes ACM buffer.

Histamine release. Histamine release reactions were carried out in 12 x 75 mm plastic tubes; 0.25 ml of Pipes ACM or antigen was added to the tubes in an ice bath, 0.25 ml of the washed leukocytes was then added to all the tubes, and racks were transferred to a 37°C bath. The racks were shaken every 10 min to resuspend the cells. In each experiment, perchloric acid at a final concentration of 3% was added to some samples to obtain the total histamine content of the cells. At the end of 60 min, the tubes were transferred to an ice bath and centrifuged at 1500 rpm (650 × G) for 15 min at 4°C. The supernatants were decanted and assayed for histamine. Peptide fragments were also used as haptens for inhibition of histamine release; washed leukocytes (0.2 ml) in Pipes ACM were incubated with the fragment (0.2 ml) at 37°C for 10 min, and then 0.2 ml of rabbit serum albumin was added and the reaction was allowed to proceed for 60 min. All experiments were repeated at least twice, and in all tests experimental tubes were in duplicate.

Histamine assay. Histamine was assayed by an automated fluorometric technique (26, 27). Calculations were as described previously (28).

Total serum IgE. Determination of total serum IgE was kindly performed by Dr. T. Waldmann, N.I.H. The values are expressed as nanograms per milliliter.

Statistical methods. The means, standard error, and *t*-statistic for 2 means were calculated using a Hewlett-Packard 65 calculator.

RESULTS

RSA as an allergen. Thirty-three patients were identified who developed allergic symptoms on exposure to rats, had positive skin tests with rat pelt extract, and whose leukocytes released histamine on challenge with rat pelt extract. The leukocytes from these patients were tested for histamine release with RSA at concentrations of 0.01 to 10 μg/ml (1.5×10^{-10} M to 1.5×10^{-7} M). The cells from only 8 out of 33 patients (24%, of rat allergic individuals) released at least >10% and up to 100%, of their histamine with RSA. The summary of the clinical and laboratory information on these patients is shown in Table I. On exposure to rats, 4 of the patients developed hay fever and asthma, 3 developed only hay fever type of symptoms, and 1 patient developed only asthma. Most of the patients had elevated total serum IgE levels.

The 8 patients allergic to RSA were significantly more sensitive to rat pelt extract than the 25 RSA negative subjects on the basis of both skin test and leukocyte histamine release (Table II). The concentration of rat pelt allergen required for 50% histamine release was 15 PNU/ml for the leukocytes from RSA-negative patients *vs* 1.6 PNU/ml for the RSA-positive patients.

Histamine release with RSA and peptic fragments of the RSA. The leukocytes of all RSA allergic patients were challenged with the RSA and 2 peptic fragments of the molecule, RSA-$P_{1\text{-}306}$ and RSA-$P_{307\text{-}581}$. The dose-response curves of the leukocytes of two patients are shown in Figure 1. The cells from E.S. respond to both peptic fragments of RSA with histamine release (Fig. 1*A*). Therefore, there are at least 2 separate determinants on each RSA-$P_{1\text{-}306}$ and RSA-$P_{307\text{-}581}$ fragment. Compared with the whole RSA molecule, RSA-$P_{1\text{-}306}$ had retained 26% activity and RSA-$P_{307\text{-}581}$ 5%. The results with the leukocytes of patient L.W. demonstrate that here even more of the original allergenic activity is lost (Fig. 1*B*). Fragment RSA-$P_{307\text{-}581}$ was inactive at concentrations as high as 3×10^{-7} M,

TABLE I

Clinical data and histamine release results of 8 patients reactive to rat serum albumin

Clinical Data						Total IgE (ng/ml)	Leukocyte Histamine Release (%)					
							Rat Pelt Extract (PNU/ml)			Rat Albumin (μg/ml)		
NR	Pat.	Age/Sex	Hay-fever*	Asthma*	Skin test Rat Pelt**		1	10	100	0.01	0.1	1
1	C.L.	41/♂	+	−	4+	1857	55	74	74	65	71	68
2	T.O.	24/♂	+	+	3+	558	64	87	50	74	75	55
3	L.R.	27/♀	+	−	4+	698	N.D.	100	100	94	100	70
4	T.S.	39/♂	+	+	4+	595	62	67	82	36	54	45
5	L.W.	31/♂	+	+	3+	221	22	75	75	13	52	66
6	E.S.	48/♂	+	+	4+	387	17	57	59	65	49	30
7	S.Y.	32/♂	−	+	3+	1250	N.D.	54	44	56	67	70
8	S.W.	35/♀	+	−	4+	519	34	72	31	46	71	71

* Symptoms on exposure to rats

** Intradermal skin test with 100 PNU/ml

TABLE II

Skin test and leukocyte histamine release with rat pelt extract in 33 rat allergic patients

Reactivity to Rat Albumin	N	Rat Pelt Extract						
		Intradermal skin test (100 PNU/ml)				Histamine release with[a]		
		<1+	2+	3+	4+	1 PNU/ml	10 PNU/ml	100 PNU/ml
		No. of subjects				%		
Negative[b]	25	0	9	10	6	16 ± 3	47 ± 4	64 ± 4
Positive	8	0	0	3	5	42 ± 9[c]	76 ± 6[c]	68 ± 8

[a] Histamine release $\bar{x}$ ± SEM.

[b] Less than 10% histamine release with 10 μg/ml of RSA (histamine release $\bar{x}$ = 2.1% ± 0.6).

[c] These values are significantly different than those in the first line (paired *t*-test, p = 0.005).

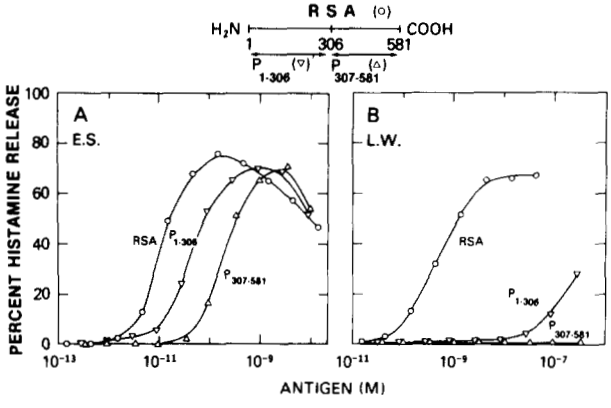

Figure 1. Histamine release by rat serum albumin and fragments RSA-$P_{307\text{-}581}$ and RSA-$P_{1\text{-}306}$ from the leukocytes of two individuals allergic to rats. *A*, cells from donor E.S.; fragment RSA-$P_{1\text{-}306}$ had 26%, and fragment RSA-$P_{307\text{-}581}$ 5% of the activity of intact rat albumin. *B*, cells from donor L.W.; both fragments RSA-$P_{1\text{-}306}$ and RSA-$P_{307\text{-}581}$ retained essentially no activity (less then 0.1%).

whereas RSA-$P_{1\text{-}306}$ has some activity at very high concentrations (about 0.1%, of the activity of RSA).

A summary of the data from the 8 patients sensitive to RSA is shown in Table III. The antigen dose required for 30% histamine release was calculated from the ascending portion of the antigen dose-response curve. The cells from 4 patients released well with RSA but less than 10% with the peptic fragments. The cells from these patients were retested in buffer containing D_2O to enhance the release. It was found that D_2O shifts the antigen dose-response curve so that RSA was active at lower concentrations and the peptic fragments now released some histamine. The effect of this was to increase the activity estimates of the peptic fragments (data not shown). Therefore, the activities estimated in the peptic fragments are greater than those that would have been determined in the absence of D_2O.

The antigen dose required for 30% histamine release varied more than 100-fold among the different patients. In general, there was more activity recovered in RSA-$P_{1\text{-}306}$ than in fragment RSA-$P_{307\text{-}581}$ (RSA-$P_{1\text{-}306}$ $\bar{x}$ = 5.0%, ± 8.7%; RSA-$P_{307\text{-}581}$ $\bar{x}$ = 1.4% ± 2.1%). The sum of the total antigenic activity recovered in both fragments was low ($\bar{x}$ = 6.4%, range 0.09% to 31%). This indicates that 70 to 99% of the ability of the antigen to trigger basophils was lost by the cleavage of the molecule in the middle of the second domain. The variability in the response of different patients to the fragments is apparent; e.g., there is a 200- to 500-fold difference in the percent of activity recovered in the fragments as tested with the cells of different patients (Table III).

The variation in reactivity of the different patients with the fragments allows estimates to be made of the possible contamination of these fragments with intact RSA. With the RSA-$P_{1\text{-}306}$ fragment, the least reactivity was 0.09% of RSA; therefore, this is the maximum possible limit of contamination with rat albumin in the RSA-$P_{1\text{-}306}$ preparation. The RSA-$P_{307\text{-}581}$ preparation could contain even less intact RSA; clearly it is less than 0.01%.

Cell desensitization is an operational term that refers to the unresponsiveness of cells to the repetitive addition of an agent. Desensitization occurs when the cells are exposed to an antigen under nonoptimal conditions (e.g., addition of antigen in the absence of Ca^{++}). In nonspecific desensitization, cells desensitized with 1 antigen are unresponsive to other IgE-mediated reactions. With human basophils, both antigen-specific and nonspecific desensitization can occur (29). In order to test whether there are unique or homologous antigenic sites along the RSA molecule, desensitization experiments were done with the cells from each of these 8 patients. Cells were preincubated for 60 min at 37°C in the absence of Ca^{++} with the desensitizing antigen (RSA, RSA-$P_{1\text{-}306}$ or RSA-$P_{307\text{-}581}$) at concentrations previously shown to be capable of releasing histamine. The cells were then washed twice and challenged with the nondesensitizing antigens and anti-IgE (data not shown). RSA caused nonspecific desensitization of the cells of all these patients; fragments that released histamine also caused nonspecific desensitization. However, fragment RSA-$P_{307\text{-}581}$ did not release histamine or desensitize the cells of 3 of these patients. Therefore,

TABLE III

Histamine release with rat serum albumin, peptic fragments RSA-P_{1-306} and RSA-$P_{307-581}$ from the leukocytes of 8 RSA-sensitive patients

Patients		Testing in Presence of D_2O	RSA Histamine Release Ag_{30}[a]	Fragment RSA-$P_{(1-306)}$		Fragment RSA-$P_{(307-581)}$		Total Activity in Fragments (% of RSA)
No.	Initials			Histamine release Ag_{30}[a]	% Activity of RSA[b]	Histamine release Ag_{30}[a]	% Activity of RSA[b]	
		%	*M*	*M*		*M*		%
1	(E.S.)		8.5×10^{-12}	3.3×10^{-11}	25.8	1.6×10^{-10}	5.3	31.1
2	(C.L.)		1.5×10^{-11}	2.8×10^{-10}	5.4	4.8×10^{-10}	3.1	8.5
3	(T.O.)		8.5×10^{-12}	3.0×10^{-10}	2.8	3.0×10^{-10}	2.8	5.6
4	(S.Y.)[c]	20	3.5×10^{-11}	1.2×10^{-9}	2.9	1.0×10^{-7}	0.04	2.9
5	(T.S.)	25	3.7×10^{-11}	3.0×10^{-9}	1.2	6.5×10^{-7}	<0.01	1.2
6	(S.W.)	10	3.1×10^{-10}	3.0×10^{-8}	1.0	$<10^{-6}$	<0.01	1.0
7	(L.R.)		1.0×10^{-12}	2.5×10^{-10}	0.4	1.0×10^{-9}	0.1	0.5
8	(L.W.)	20	3.7×10^{-10}	4.3×10^{-7}	0.09	$<10^{-6}$	<0.01	0.09

[a] Ag_{30} = Antigen dose required for 30% histamine release.

[b] Percent activity of RSA $= \frac{Ag_{30} \text{ for RSA}}{Ag_{30} \text{ for fragment}} \times 100$.

[c] On immunotherapy with rat pelt extract.

the desensitization experiments were not useful for studies of the antigenic sites on RSA.

Previous studies have demonstrated that the 2 halves of the bovine serum albumin, which are similar to RSA-P_{1-306} and RSA-$P_{307-581}$, can associate in solution (24, 30). The complex formed has some of the biologic and immunologic activities of intact bovine albumin molecules. Equimolar concentrations of both peptide fragments were mixed at 10^{-7} to 10^{-9} M and then added to leukocytes (data not shown). All the histamine release induced by mixture of the 2 fragments could be accounted for by the activity of each fragment added separately. Therefore, there was no evidence for the aggregation on the cell surface of the 2 halves of the RSA molecule.

Peptide fragments as haptens. The leukocytes of 3 of the donors did not release histamine with RSA-$P_{307-581}$ at any of the concentrations tested. This peptide therefore was used in inhibiting the release induced by RSA. Leukocytes were preincubated for 10 min with this peptide, and then RSA at an optimal histamine releasing concentration was added to all the tubes. The cells of 2 of the donors demonstrated no haptenic inhibition of RSA-induced release with RSA-$P_{307-581}$. However, there was significant inhibition by the fragment RSA-$P_{307-581}$ from the cells of the third donor (Fig. 2). The molar ratio for 50% inhibition of RSA-albumin induced release is 60:1. The cells of this donor also released with MSA. When the cells were preincubated with RSA-$P_{307-581}$ and then challenged with MSA, there was inhibition of release (Fig. 2). Although a higher molar ratio of the fragment was required to inhibit MSA than RSA release, this antigenic site on RSA and MSA is clearly similar and reacts with the same IgE molecule.

In inhibition studies with the cells of the only patient with low reactivity to RSA-P_{1-306}, no monovalent haptenic activity could be demonstrated (patient L.W.). These cells, however, release with RSA-P_{1-306} at higher concentrations.

Cross-reactivity between rat, mouse, and bovine serum albumins. The leukocytes from all 8 RSA-sensitive patients released histamine with MSA. The dose-response curves with both albumins were similar: 50% histamine release was induced by 8×10^{-11} M RSA and 1.5×10^{-10} M MSA (Fig. 3). The leukocytes from 27 out of the 33 patients allergic to rats released histamine with mouse pelt extract. However, the leukocytes from only 2 out of the 8 RSA-allergic patients released histamine with bovine serum albumin. In both cases the concentration of bovine serum albumin for 50% histamine release was 100- to 1000-fold higher then the concentration of the RSA (data not shown).

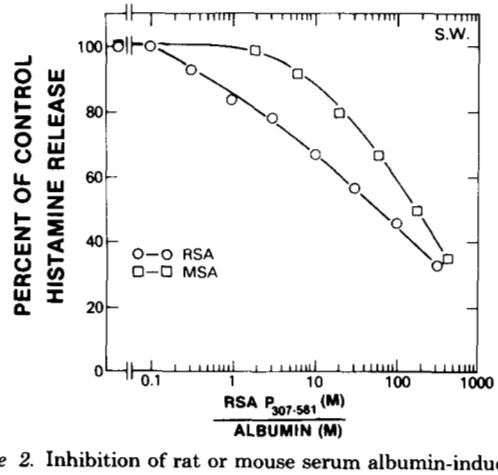

Figure 2. Inhibition of rat or mouse serum albumin-induced histamine release by the RSA-$P_{307-581}$ fragment. Cells were preincubated with different concentrations of RSA-$P_{307-581}$ fragment for 10 min at 37°C before the addition of either 3.0×10^{-10} M rat serum albumin (○) or 1.5×10^{-10} M mouse serum albumin (□). Histamine release in the absence of RSA-$P_{307-581}$ was 32% with rat serum albumin and 33% with mouse serum albumin.

DISCUSSION

The results indicate that RSA is an allergen for about 25% of rat-allergic individuals. In previous experiments it has been

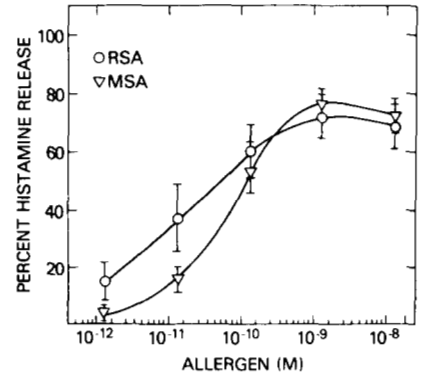

Figure 3. Comparison of the ability of rat or mouse serum albumin to release histamine from the cells of rat serum albumin sensitive patients. ○, Rat serum albumin; ▽, mouse serum albumin. The values represent mean ± SEM (N = 8).

shown that MSA is 1 of the 2 major allergens in about 30% of mouse-sensitive patients (23). In the present study no fractionation of the rat pelt allergen was attempted, and therefore we cannot speculate on the relative importance of the albumin as an allergen compared with other proteins present in the pelt extracts.

The recognition of RSA as an allergen allowed the testing of fragments of this molecule for their capacity to release histamine. The leukocytes from some patients release histamine with either of 2 proteolytic fragments, each of which constitutes half of the molecule. Release of histamine requires at least the bridging of 2 adjacent IgE molecules (31). Therefore, each of these 2 fragments (RSA-P_{1-306} and RSA-$P_{307-581}$) must contain at least 2 antigenic sites. The total number of antigenic sites recognized by human IgE on the molecule appears to be at least 4. Studies with rabbit antibodies to bovine serum albumin indicate at least 6 antigenic sites distributed throughout the whole molecule (32, 33). Some studies have suggested that rabbit sera late after immunization contain antibodies predominantly to the third domain (34). In contrast to the results with rabbit antibodies to bovine albumin, the leukocytes of our patients appear to react more to the amino-terminal portion of the RSA.

The large fragments obtained by limited proteolysis of albumin retain intact disulfide bonds and unaltered amino acid side chains. Therefore, they resemble the native molecule more than fragments prepared by chemical cleavage techniques (7, 14, 24, 30). Proteolytic cleavage occurs at unstructured parts of the molecule and has the effect of breaking the middle domain in half. The low total recovery of allergenic activity in the 2 fragments could be due to several possibilities. The second (middle) domain might have some important antigenic sites that are lost after cleavage. In general, antigenic sites are on the exposed surface of proteins and constitute amino acids either in continuous peptide linkage or spatially adjacent surface residues (35–37). The sites in the second domain would probably be of spatially adjacent amino acids. It is also possible that cleavage of the albumin molecule into 2 halves results in some minor conformational changes in the fragments. Studies by circular dichroic spectroscopy and magnetic osmometry suggest that there is retention of some of the secondary structure but the fragments are not in their native configuration (14, 30). Immunochemical studies with rabbit antibodies to bovine serum albumin indicate that the 2 fragments are capable of binding precipitating anti-native albumin antibodies (32). The complete loss of the tertiary structure of the albumin molecule results in a loss of its reactivity with antibody to native albumin and the appearance of new-antigenic determinants (33, 38). Important functional interaction between the 2 parts of the second domain of bovine albumin has been observed previously (11, 39).

The importance of conformational determinants has been suspected in a number of allergens (40, 41). The treatment of ragweed antigen E with 8 M urea or 1%, sodium dodecyl sulfate to dissociate the noncovalently bound subunits results in a loss of its biologic reactivity (42, 43). In contrast, the allergenic activity of cod allergen M is stable on heating and on treatment with dissociating agents, suggesting that conformational determinants are not important (44). Our results would suggest that conformational determinants are important for the IgE reaction with RSA.

The 2 peptide halves of the albumin molecule associate when mixed at high concentrations (24, 30). The association constant is low (at 22°C, pH 7.4, it is $7.4 \times 10^3\ M^{-1}$ and $\sim 1.8\ \mu M^{-1}$ at pH 8.6). The fragments mixed at low concentrations did not trigger the basophils although at the cell surface the IgE-molecules could trap the RSA fragments and result in higher localized concentrations than in solution.

There was significant heterogeneity in the response of the 8 RSA-allergic donors to the proteolytic fragments; the amount of activity recovered in fragment RSA-P_{1-306} varied from 0.09%, to 26% (over 250-fold difference). The variation in activity recovered in RSA-$P_{307-581}$ was similar. Out of the 8 donors, 2 did not recognize any determinant in RSA-$P_{307-581}$, 1 recognized 1 (haptenic) determinant, and 5 reacted to at least 2 antigenic sites. On repeated testing, the maximum variation with the cells of the same individual was found to be 6-fold. Therefore, these differences cannot be due to experimental error. Clearly, there is no single immunodominant site in the molecule that is important for all these patients. Whether this is true for other allergens is a crucial question for studies of the genetics of the IgE-response.

The leukocytes from all 8 RSA-sensitive patients released histamine with MSA. The dose-response curves were similar. The simplest explanation would be cross-reactivity due to the IgE reacting with similar antigenic sites on both molecules. Evidence for this cross-reactivity was found with the cells of 1 donor by the ability of RSA-$P_{307-581}$ to block MSA-induced histamine release. Surprisingly, despite similarities in the amino acid sequence of rat and bovine albumin, the leukocytes of only 2 out of 8 subjects reacted with bovine serum albumin. However, haptenic fragments of bovine albumin did not inhibit RSA induced release.

Our results indicate the importance of cross-reactivity in the reaction to allergens, the heterogeneity of the IgE immune response to a well-defined protein, and the substantial loss of the allergenic activity of the RSA by cleavage into 2 fragments.

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