

Differences in the reactivity of short and giant ragweed with immunoglobulin E antibodies

G. J. Gleich, M.D., A. R. Campbell, M. C. Gleich, and H. A. Swedlund, M.D.
Rochester, Minn.

Currently, the allergens in extracts of SRW and GRW are regarded as equivalent in their ability to react with IgE antibodies. We investigated whether IgE antibodies might recognize differences between these allergens. First, sera from individuals with positive skin tests to both RWs showed higher reactivity with solid-phase SRW in the RAST. Second, immunosorption of 24 sera showed that more than 90% of the IgE antibodies were removed by either SRW or GRW Sepharose. However, all of the sera contained IgE antibodies specific for SRW and six sera contained antibodies specific for both SRW and GRW. Third, the presence of IgE antibodies preferentially reactive with SRW was tested by neutralization of the P-K test. Two individuals were passively sensitized with a serum pool containing IgE antibodies specific for SRW; skin sites were challenged repetitively with an extract of GRW until they no longer reacted with GRW. Challenge of these sites with SRW showed an immediate wheal-and-flare reaction, indicating that antibodies were present in the serum pool which could not be neutralized by GRW and, thus, were specific for SRW. These results indicate that the sera of all RW-sensitive individuals in this study contained IgE antibodies specifically reactive with SRW and many sera contained antibodies specific for GRW. Overall, however, most of the IgE anti-RW reactivity was removed by either GRW or SRW.

Since the P-K skin test neutralization studies of Coca and Grove¹ and Spain and Hopkins,² SRW and GRW pollen have been regarded as allergenically equivalent. Although an occasional study³ questioned whether differences between these pollen extracts might exist, for the most part these early findings that SRW and GRW are allergenically identical have been accepted.

In comparing the reactivity of SRW and GRW by RAST, we frequently noted that sera from RW-sensitive individuals seemed to react more strongly

Abbreviations used

RW:	ragweed
SRW:	short ragweed
GRW:	giant ragweed
RAST:	radioallergosorbent test
P-K test:	Prausnitz-Küstner test
BSA:	bovine serum albumin
FCS:	fetal calf serum

with solid-phase SRW than with solid-phase GRW. We also observed⁴ that in RAST inhibition SRW and GRW were not equivalent in their inhibitory capacities; extracts of SRW appeared more potent than GRW. Because of these observations, we suspected that IgE antibodies could discriminate between SRW and GRW. The results of our study indicate that sera from most RW-sensitive individuals contain IgE antibodies which preferentially react with SRW and sera from many individuals contain antibodies which preferentially react with GRW. Overall, however, the

From the Departments of Medicine and Immunology, the Allergic Diseases Research Laboratory, Mayo Clinic and Foundation.

Supported by Grant AI 11483 and Contract AI 42546 from the National Institute of Allergy and Infectious Diseases, by Contract 223-77-1202, from the Food and Drug Administration, and by the Mayo Foundation.

Received for publication April 20, 1979.

Accepted for publication June 21, 1979.

Reprint requests to: Gerald J. Gleich, M.D., Allergic Diseases Research Laboratory, Mayo Clinic, Rochester, MN 55901.

TABLE I. Analyses in 18 patients of sera for IgE antibodies reactive with SRW and GRW by skin test and by RAST

Patient No.	Skin test*		RAST†	
	SRW	GRW	SRW	GRW
3	4	3	3.82	2.01
5	2	2	6.72	4.75
8	2	2	6.43	3.90
10	3	2	12.97	3.33
16	4	4	1.17	0.61
19	4	3	7.20	4.84
20	4	1	4.47	3.67
22	3	3	1.18	0.25
23	4	3	7.09	0.96
29	4	2	0.79	0.80
36	3	3	3.70	2.69
38	3	2	8.65	5.50
43	3	2	5.51	3.47
45	4	3	8.60	3.76
50	3	2	6.92	1.41
55	4	3	6.92	7.74
60	4	4	10.34	4.10
66	3	3	5.26	4.06
Normal serum No. 1	—	—	0.15	0.20
Normal serum No. 2	—	—	0.31	0.39
Human serum albumin	—	—	0.15	0.19
5-person ragweed allergic serum pool	—	—	15.33	9.80

*Intradermal skin test results are reported as maximum wheal diameter as follows: 4 = >11 mm; 3 = 8 to 11 mm; 2 = 5 to 8 mm; and 1 = 4 to 5 mm. Skin tests were performed with extracts, 100 PNU/ml, purchased from Greer Labs., Lenoir, N. C. (SRW) and Center Labs., Port Washington, N. Y. (GRW). Negative skin tests are less than a 2- to 3-mm wheal diameter, with or without a flare. All positive tests showed a wheal-and-flare greater than the initial wheal due to the injection of the allergen.

†RAST results are reported as percent of total counts bound.

bulk of the IgE antibody to RW is reactive with both SRW and GRW.

MATERIALS AND METHODS

Patients

Two groups of patients were used in this research. In group I, represented in Table I, individuals with a history of hay fever underwent skin testing with both GRW and SRW extracts as a part of their clinical evaluation. Sera from these patients were used in the immunochemical studies described below. In group II patients were selected from respondents to an advertisement in a local newspaper for people with late summer hay fever symptoms. These patients have been described elsewhere.⁵ In this study we used sera from 14 of 50 patients with elevated levels (>100 ng/ml) of IgE antibody to SRW.⁶ The patients from this second group are designated in Tables II and III.

TABLE II. Analysis of reactivity of IgE antibodies following single immunosorption*

Patient No.	IgE antibody levels to SRW and GRW					
	Absorbed with blank Sepharose		Absorbed with GRW Sepharose		Absorbed with SRW Sepharose	
	RAST		RAST		RAST	
	SRW	GRW	SRW	GRW	SRW	GRW
1†	14.14‡	13.75	1.39	0.31	0.33	5.58
4†	9.86	6.69	1.77	0.28	0.38	0.35
6†	14.47	9.22	6.99	0.21	0.34	0.38
10	23.90	18.13	10.67	1.50	1.62	1.33
12†	13.61	8.32	2.11	0.42	0.54	0.26
15†	21.26	19.10	8.34	2.18	2.25	2.58
19	8.83	9.22	1.07	0.55	0.29	0.31
20	9.74	7.79	2.62	0.74	0.71	0.61
21†	3.66	3.66	0.78	0.18	0.21	0.16
30†	19.74	17.23	9.15	0.88	1.28	5.89
34	15.13	8.21	3.02	2.49	0.54	0.87
36	11.21	5.97	2.17	0.83	0.44	0.30
38	18.73	12.43	5.90	0.65	0.52	0.43
43	9.60	6.63	1.24	0.47	0.46	0.45
45	17.65	9.88	4.46	0.32	1.04	2.03
50	10.74	4.37	2.89	0.57	0.43	0.45
52	10.31	9.23	1.00	0.44	0.25	0.61
54†	13.00	11.03	4.04	0.39	3.18	0.57
60	17.46	13.89	5.68	0.93	0.84	0.67
65	5.59	9.39	0.80	0.34	0.45	0.46
66	9.69	11.34	1.14	0.52	0.26	0.43
69	21.74	17.33	13.27	2.64	5.90	4.84
70	10.31	5.71	3.34	0.30	0.31	0.31
71†	16.35	14.61	15.94	0.91	2.21	0.75

*Sera were immunosorbed once using a ratio of 0.50 ml serum to 1.0 ml 10% Sepharose; supernatants were tested by RAST. Negative controls consisted of: human serum albumin, 5%, which gave 0.24% and 0.28% counts bound with SRW and GRW cellulose, respectively; and FCS, which gave 0.19% and 0.16% counts bound with SRW and GRW, respectively. Elevated levels of IgE antibodies were arbitrarily defined as percent counts bound greater than twice that of human serum albumin control.

†Designates those patients in group II; all of the other patients belong to group I.

‡RAST values given as percent of total counts bound.

Ragweed allergic serum pool

A serum pool from five nonhyposensitized individuals who were intensely allergic to SRW was used as a reference standard to establish a dose-response curve in the RAST,⁶ for immunosorption experiments and for P-K neutralization testing (a test for hepatitis B antigen was negative).

Ragweed pollen preparations

SRW pollen, *Ambrosia elatior*, was obtained from Greer Laboratories, Lenoir, North Carolina (Lot No. 18P56-9).

TABLE III. Analysis of the reactivity of IgE antibodies following double immunosorption

Patient No.	IgE antibody levels to SRW and GRW					
	Unabsorbed sera (0.005 ml)		Sera absorbed with GRW Sepharose (0.05 ml)		Sera absorbed with SRW Sepharose (0.05 ml)	
	RAST		RAST		RAST	
	SRW	GRW	SRW	GRW	SRW	GRW
1*	—†	—	1.14‡(0.28)§	0.21(<0.04)	0.25(<0.03)	3.84(3.51)
6*	5.01(26.3)	0.94(3.80)	4.86(2.51)	0.19(<0.04)	0.16(<0.03)	0.30(0.06)
10	13.6(117.8)	4.65(47.5)	6.74(4.11)	0.55(0.16)	0.58(0.10)	0.21(<0.04)
15*	—	—	3.95(1.84)	0.66(0.22)	0.64(0.12)	0.74(0.26)
30*	—	—	2.41(0.88)	0.22(<0.04)	0.19(<0.03)	1.50(0.80)
34	4.66(23.6)	1.85(11.1)	1.21(0.31)	0.33(0.07)	0.15(<0.03)	0.63(0.20)
38	5.79(32.7)	2.02(12.8)	4.93(2.57)	0.30(0.06)	0.38(0.05)	0.52(0.15)
45	5.02(26.4)	2.14(14.0)	1.93(0.63)	0.18(<0.04)	0.21(<0.03)	0.21(<0.04)
69	6.23(36.5)	3.95(36.7)	4.59(2.31)	0.48(0.13)	0.26(0.03)	0.33(0.07)
71*	2.56(9.6)	1.38(7.0)	6.28(3.69)	0.14(<0.04)	0.30(0.04)	0.32(0.07)
Serum pool						
0.005 ml	9.28(6.64)	6.12(7.33)				
0.05 ml	22.00(>>20)	13.50(>>20)	7.31(4.64)	0.49(0.14)	0.31(0.04)	0.57(0.17)
Normal control						
HSA (5%), 0.05 ml	0.09	0.07				
FCS, 0.05 ml	0.11	0.13				
Normal serum						
0.05 ml	0.18	0.14				

HSA: Human serum albumin.

*Designates patients in group II; all other patients are from group I.

†In some cases insufficient serum was available to include a positive control. Refer to Table II for the reactivity of these sera in the RAST.

‡RAST values given as percent of total counts bound.

§Arbitrary RAST units are given in parentheses. RAST units were determined by interpolating the percent counts bound with each serum on a dose-response curve obtained by utilizing dilutions of a serum pool from 5 ragweed-sensitive patients.⁶ With unabsorbed sera, 0.005 ml of sera were used; however, with the absorbed sera, 0.05 ml of sera were used in the RAST. For purposes of comparison, all RAST units given are per 0.050 ml of serum with the exception of the 0.005 ml of serum pool.

GRW pollen, *Ambrosia trifida*, was obtained from the same source (Lot No. 8P55-9). Following treatment with Calberla's stain, these preparations were homogeneous when examined by light microscopy. Both of these species of RW are indigenous to southern Minnesota.

Preparation of ragweed extracts

Samples of the two RW pollens (45 gm) were defatted by suspending the pollen in diethyl ether, allowing it to settle, and decanting the supernatant. The process was repeated three times with each pollen until the supernatant was almost colorless. The defatted pollens were dried in a pan overnight. Aqueous extracts were prepared by continuous mixing of defatted pollens in a 1:10 w/v mixture with Dulbecco's phosphate-buffered saline (0.0027 M KCl, 0.0015 M KH_2PO_4 , 0.008 M Na_2HPO_4 , 0.14 M NaCl, pH 7.5). The crude extracts were dialyzed against 0.1 M NH_4HCO_3 at 4° C for 48 hr with three changes of buffer. The extracts were lyophilized and stored at 4° C in the presence of calcium

chloride desiccant. We obtained 4.48 gm of SRW extract powder and 2.60 gm of GRW extract powder.

Preparation of solid-phase allergens

The RW extracts were immobilized by reaction with cyanogen bromide-activated microcrystalline cellulose as described previously.⁴ Briefly, the washed, freshly activated microcrystalline cellulose was divided into two 1.0-gm portions and reacted with 400 mg SRW and 400 mg GRW, respectively. The resulting suspensions were tumbled for 60 hr, washed repeatedly and resuspended at a concentration of 1 mg/ml in RAST diluent (0.1 M KH_2PO_4 - K_2HPO_4 , pH 7.4, 0.2% BSA, 1% Tween 20 [ICI United States Inc., Wilmington, Del.] and 0.1% sodium azide).

For the immunosorption experiments, the extracts of SRW and GRW were covalently bound to cyanogen bromide-activated Sepharose 2B (Pharmacia Labs., Piscataway, N. J.). This technique has been described previously for the grass pollens.⁷ Briefly, we used two 30-ml packed-

volume portions of washed, freshly activated Sepharose 2B; one portion was allowed to react with SRW extract, 600 mg, the other with GRW extract, 600 mg. The resulting suspensions were tumbled for 3 days, washed, and resuspended.

Blank Sepharose refers to Sepharose 2B which was not activated nor reacted with protein. Sepharose 2B was washed with and resuspended in borate-saline (0.2 M H_3BO_3 -0.04 M NaOH-0.16 M NaCl, pH 8.0) and served as a control in the immunosorption experiments.

Performance of the RAST

Serum IgE antibody to ragweed allergens was measured by RAST essentially as described previously.⁸ Briefly, 0.5 mg of solid-phase ragweed cellulose in 0.5 ml RAST diluent, 0.05 ml test serum or dilution thereof, and 0.2 ml of RAST diluent were added to 10 × 62 mm plastic tubes. The tubes were capped and tumbled overnight; the next day they were centrifuged and the supernatant aspirated. The solid-phase allergen was washed twice with 1 ml of RAST wash (0.1 M KH_2PO_4 - K_2HPO_4 , pH 7.4, containing 1% Tween 20) and 0.65 ml of FCS diluent (same solution as RAST diluent except 20 ml of FCS [Grand Island Biological Co., Grand Island, N. Y.] is substituted for BSA) were added followed by 20 ng of radiolabeled affinity chromatography-purified anti-IgE in 0.10 ml of 0.1 M KH_2PO_4 - K_2HPO_4 , pH 7.4, containing 1% BSA. The tubes were again rotated overnight and washed twice. Radioactive counts associated with the solid-phase allergen were measured in a gamma spectrometer; the results were expressed as counts bound divided by counts added times 100. All serum samples were run in duplicate and the results averaged; as negative controls, normal sera, FCS and human serum albumin (5%; Sigma Chemical Co., St. Louis, Mo.) were tested. We have arbitrarily set a positive test as $\geq$ twice the percent counts bound by either normal sera or negative controls.

In preliminary experiments shown in Table I, a modification of the RAST was employed.⁹ This procedure was identical to that described above except (1) 0.050 mg of the solid-phase allergen, 0.050 ml of serum and 0.150 ml of RAST diluent were added in the first step of the assay, and (2) 0.10 ml of anti-IgE containing 5 ng of antibody plus 0.15 ml of FCS diluent were added in the second step. The tubes were treated in exactly the same way as before except that rotation was parallel to the long axis of the tube.

Absorption of allergic serum with solid-phase ragweed

The protocol employed for this procedure is similar to that described previously.⁷ In preliminary experiments various volumes of a 10% suspension of RW Sepharose 2B in Dulbecco's phosphate-buffered saline were added to a series of tubes. After the tubes were centrifuged and the supernatant aspirated, 0.5 ml of allergic serum was added to each tube. The tubes were tumbled end-over-end overnight, centrifuged, and the supernatants recovered. The supernatants

TABLE IV. Percentages of IgE antibodies specific for GRW or SRW after double immunosorption

Patient No.	Antibodies for SRW not absorbed by GRW	Antibodies for GRW not absorbed by SRW
6	9.5*	1.6†
10	3.5‡	--§
34	1.3‡	1.8
38	7.9‡	1.2‡
45	2.4	---
69	6.3‡	0.2‡
71	38	1.0‡
Allergic serum pool	7.0‡	0.2‡

*Percent calculated by finding the ratio of units (Table III) left after absorption compared to the units in unabsorbed sera, e.g., with SRW for Patient No. 6, $2.51/26.3 \times 100\% = 9.5\%$.

†This patient showed only two types of antibodies in Table II; in Table III a small percentage of antibodies for GRW was detected.

‡These sera showed slightly elevated levels of IgE antibodies to the homologous RW; therefore, the percentage given here is an upper limit.

§Too few counts bound to be determined.

were tested to determine which volume of Sepharose effected the maximum absorption of IgE antibodies as measured by the RAST using both SRW and GRW microcrystalline cellulose. These experiments indicated that a volume of 1.0 ml of the 10% Sepharose 2B RW cellulose was effective in removing the bulk of the IgE antibodies in 0.5 ml of most allergic sera. In Table II a 1.0-ml volume of the 10% suspension was used for the immunosorption experiments. In Table III sera were doubly immunosorbed with the RW Sepharose preparations. In these latter experiments 1.4 ml of the 10% Sepharose were added to each of two tubes; the tubes were centrifuged and the supernatants aspirated. Serum, 0.7 ml, was added to the first tube, tumbled overnight, and centrifuged. The entire supernatant was transferred to the second tube and the process was repeated. Following the second absorption, the supernatants were tested by the RAST as described above.

Neutralization of skin-sensitizing antibodies by the P-K procedure

For the demonstration that IgE antibodies specifically reactive with SRW allergenic determinants were present in serum, we utilized the technique of P-K neutralization.¹ Informed consent was obtained from the recipients. Serum, 0.1 ml, was injected intradermally at sites 1, 2, and 3 into recipients who did not react to GRW and SRW. The skin sites were challenged daily with GRW by intradermal injection until no reaction was produced. The skin sites were then challenged with an extract of SRW.

TABLE V. IgE antibody specific for SRW: Demonstration by P-K neutralization

	Recipient 1				
	Site				
	1	2	3	4*	5*
Test†/vol (μl)	GRW/100	GRW/100		GRW/100	
Initial result (mm)	10 × 4	8 × 8		4 × 3	
15-min result, W (mm)	15 × 12	20 × 22		4 × 3	
15-min result, E (mm)	40 × 50	55 × 65		None	
24 hr later, test/vol (μl)	GRW/100	GRW/100			
15-min result, W (mm)	16 × 11	12 × 10			
15-min result, E (mm)	40 × 35	30 × 30			
24 hr later, test/vol (μl)	GRW/100	GRW/100			
15-min result, W (mm)	14 × 10	14 × 12			
15-min result, E (mm)	16 × 20	26 × 28			
24 hr later, test/vol (μl)	GRW/100	GRW/100			
15-min result, W (mm)	8 × 8	10 × 10			
15-min result, E (mm)	None	None			
24 hr later, test/vol (μl)	SRW/50	SRW/50	SRW/50		SRW/50
Initial result (mm)	7 × 4	5 × 6	3 × 3		6 × 6
15-min result, W (mm)	12 × 7	11 × 12	12 × 9§		7 × 6
15-min result, E (mm)	15 × 17	32 × 20	50 × 45		None
24 hr later, test/vol (μl)					
15-min result, W (mm)					
15-min result, E (mm)					
24 hr later, test/vol (μl)					
Initial result (mm)					
15-min result, W (mm)					
15-min result, E (mm)					

*Unsensitized site.

†GRW and SRW, 100 μg/ml, were contained in sterile, pyrogen-free 0.135 M NaCl, 0.00667 M NaH₂PO₄-Na₂HPO₄, 0.5% human serum albumin, pH 7.4. The initial result shows the size of the injection site; 15 min later the wheal (W) and erythema (E) sizes were measured.

‡This recipient showed reactions at 15 min which were erythematous and slightly larger than the initial wheal. This may be due to irritation from the large volume of solution injected, as injection of a smaller volume of GRW later did not provoke a reaction (see site 2).

§Pseudopods.

RESULTS

Typical results of analyses of sera by the RAST are shown in Table I; these sera display the full spectrum of reactivity. In all cases save one, Patient No. 55, solid-phase SRW showed more reactivity with IgE antibodies in the RAST than did solid-phase GRW. These experiments suggested that IgE antibodies are more reactive with SRW allergens than they are with GRW allergens. Alternatively or in addition, the relative quantities of allergens present on the solid-phase microcrystalline cellulose may be different for SRW and GRW.

To determine whether IgE antibodies reactive with one RW could be removed and leave IgE antibodies reactive with the other RW, we performed immunosorption experiments. In Table II we show the results from 24 sera following a single immunosorption. First, exposure of serum to either GRW or SRW

Sephacrose removes the bulk of the IgE antibody activity. Thus, these results confirm prior studies indicating a marked cross-reactivity between GRW and SRW. Second, only two sera, No. 1 and 52, clearly showed residual amounts of IgE antibody which were preferentially reactive with GRW. For the other sera, exposure to SRW allergenic determinants removed all or almost all reactivity with GRW. Third, following exposure to solid-phase GRW, all or virtually all of the reactivity with GRW was removed while significant reactivity remained with SRW.

In many sera, such as No. 30, 45, and 69, the sera were not completely absorbed by the homologous immunosorbent; that is, the percent of counts bound was not ≤ twice the negative control. Therefore, we doubly immunosorbed certain sera as described in the Materials and Methods section. Table III shows the double immunosorption results. For serum No. 1

Recipient 2					
Site					
1	2	3	4*	5*	6*
GRW/100	GRW/100		GRW $\pm$ /100		
7 $\times$ 7	6 $\times$ 7		9 $\times$ 7		
24 $\times$ 17	25 $\times$ 29		10 $\times$ 9		
45 $\times$ 38	55 $\times$ 55		15 $\times$ 15		
GRW/100	GRW/100		GRW/100		
14 $\times$ 12	17 $\times$ 12		9 $\times$ 9		
35 $\times$ 35	35 $\times$ 35		13 $\times$ 13		
GRW/100	GRW/100				
14 $\times$ 8	14 $\times$ 8				
14 $\times$ 10	22 $\times$ 15				
GRW/100	GRW/100				
15 $\times$ 11	20 $\times$ 20				
17 $\times$ 10	20 $\times$ 12				
GRW/100	GRW/100				
8 $\times$ 8	11 $\times$ 8				
14 $\times$ 15	10 $\times$ 12				
GRW/50	GRW/50				
6 $\times$ 6	6 $\times$ 6				
10 $\times$ 10	None				
SRW/30	SRW/30	SRW/30		SRW/30	SRW/10
5 $\times$ 5	7 $\times$ 5	7 $\times$ 7		7 $\times$ 6	6 $\times$ 6
20 $\times$ 20§	17 $\times$ 10§	30 $\times$ 20§		6 $\times$ 6	7 $\times$ 6
33 $\times$ 43	25 $\times$ 25	45 $\times$ 45		None	None

three populations of IgE antibodies are present. One population is reactive with both GRW and SRW because: absorption with either solid-phase RW lowers binding (cf Table II); a second population of IgE antibody is specific for GRW, in that it is not removed by SRW Sepharose; and a third population is specific for SRW in that it is not absorbed by GRW Sepharose. Sera No. 30 and 34 behave similarly to serum No. 1. In addition, although absorption was incomplete for the homologous RW with sera No. 15 and 38, it appears likely that residual amounts of antibody remain to the nonhomologous RW. Therefore, we have classified sera No. 1, 15, 30, 34, 38, and 52 (cf Table II) as having three types of IgE antibodies to RW. The remaining sera contain two populations of IgE antibodies, one reactive with both GRW and SRW and a second specific for SRW, in that the IgE antibody activity is not completely removed by immunosorption with GRW. The latter reaction pattern is shown best by sera from Patients No. 4, 6, 19, 21, 43, 65, and 70 (Table II) and by 10, 45, 69, and 71 (Table III). Even after double immunosorption, the serum pool showed residual amounts of IgE antibodies for SRW and GRW; however, the quantity specific for

GRW was very small compared with the quantity specific for SRW. In the experiments shown in Table III, we analyzed the supernatants in a quantitative RAST in which a linear relationship between the log of the IgE antibody and the log of the percent counts bound exists⁶; thus, the percentages of IgE antibody reactive with SRW and GRW can be calculated. The results shown in Table IV indicate that the bulk of the antibody, greater than 90%, is reactive with either SRW or GRW. Only one serum, No. 71, shows greater than 10% of its reactivity for one of the RW species.

The above results indicate that certain sera contain significant quantities of IgE antibodies which are specific for SRW. To test this concept in another manner, we performed P-K neutralization experiments. The results, shown in Table V, indicate that following neutralization of the antibody reactive with GRW by injections of GRW extract into the skin site, reactivity with SRW allergens remains.

DISCUSSION

The two main sources of RW pollen in the United States east of the Rocky Mountains are GRW (*A.*

trifida) and SRW (*A. elatior*). Many earlier studies have compared the reactivity of these pollen extracts and have questioned whether or not there are antigenic and allergenic differences between them. Of the 12 early studies (1916 to 1938) reviewed below, five concluded that SRW and GRW were identical as allergens, but the other seven concluded that there were either allergenic or antigenic differences. In 1916 Cooke and Vander Veer showed, by direct skin testing in 26 patients, that individuals who reacted to one pollen extract also reacted to the other and their reactions were roughly equal in intensity.¹⁰ In 1924 Armstrong and Harrison confirmed earlier studies that pollen extracts were antigenic in rabbits and showed by complement fixation that GRW and SRW extracts could be distinguished.¹¹ Also in 1924, Bernton, commenting on the occurrence of hay fever in the Washington, D. C., area, observed that the date of pollination for GRW occurred with remarkable regularity on or about August 10, whereas pollination of the SRW began roughly a week to 10 days later.¹² By correlating the results of clinical treatment with either GRW or SRW and the chronologic onset of the patient's symptoms, Bernton concluded that the allergens derived from the pollens of SRW and GRW differed and he advocated the utilization of both extracts in the treatment of individuals with autumnal hay fever. In 1925 Coca and Grove utilized the P-K passive transfer procedure to test the identity of SRW and GRW allergens by local passive desensitization; they concluded that the allergens derived from GRW and SRW did not differ.¹ In 1927 Brown investigated whether individuals injected with extracts of one RW extract would have a constitutional reaction following injection of the same dose of the other RW extract.¹³ In 31 patients Brown found that a change could be made from one RW extract to the other without a reaction. He concluded that the allergens present in GRW and SRW were identical. In 1929 Rappaport and Johnson prepared fractions of GRW and SRW pollen extracts and were able to divide 40 patients with fall hay fever into two distinct groups of 20 each on the basis of skin testing with partially purified allergen extracts.¹⁴ The individuals in one group were sensitive only to SRW while those of the other were sensitive to both GRW and SRW. In 1930 Spain and Hopkins tested whether or not the allergens from GRW and SRW were identical by neutralization of the P-K passive transfer test.² In this report they referred to prior experiences in the early 1920s with 800 cases of fall hay fever tested by intracutaneous and ophthalmic procedures in which no patient was found who reacted to one pollen extract without reacting to the other. They confirmed this observation in 106 patients with fall hay fever by demonstrating that each

patient showed positive cutaneous reactions of equal intensity to extracts of GRW and SRW. Next they studied sera from nine patients with fall hay fever by P-K neutralization. In all patients the cutaneous reactivity was abolished by either injection of SRW or GRW extracts, indicating that there was no difference between the pollen extracts. Stull et al. reached the same conclusions in 1932 in tests of five sera from ragweed-sensitive subjects by P-K neutralization.¹⁵ In the same report 12 patients with RW hay fever with similar skin reactivity were treated with an extract of SRW only (six patients) and GRW only (six patients). At the end of the treatment period, the patients receiving the SRW were switched to an equivalent dose of the GRW and the patients receiving GRW were switched to a similar dose of the SRW extract. No constitutional reactions resulted; the authors interpreted this as further evidence of the equivalence of the allergens in these extracts. In 1933 Cromwell and Moore demonstrated differences between GRW and SRW extracts utilizing skin tests in rabbits and precipitin reactions with rabbit sera.¹⁶ In their discussion they drew attention to an earlier report utilizing passive transfer experiments in humans in which sites desensitized with GRW still were able to react to mixed ragweed pollen extract, suggesting that antibodies specific for the SRW were present.¹⁷ They also cite a patient who had marked cutaneous sensitivity to SRW extract, but no reaction to GRW extract.¹⁶ In 1935 Cooke et al. reexamined the then-accepted belief that the extracts of the two ragweed pollens were identical.¹⁸ They had found that when patients were changed from treatment by injection of GRW to injection of SRW, many of them suffered constitutional reactions. In this study the authors first compared extracts of the same pollen collected in different years to determine whether marked differences existed in allergenic composition. In the case of two GRW extracts they were not able to detect a difference. They then tested whether individuals treated with SRW extract could be switched to GRW extract and whether individuals treated with GRW extract could be switched to SRW extract without reaction. They found that SRW extract had a greater capacity to produce constitutional reactions than GRW extract. They also observed that the serum-neutralizing capacities of SRW extracts were three to ten times stronger than similar extracts of GRW. The authors concluded that differences between the activities of the allergens present in extracts of GRW and SRW pollens could be demonstrated. In 1938 Grubb and Vaughan, commenting on evidence of sensitization to specific pollens, noted that they had occasionally seen positive reactions to SRW with completely negative reactions to GRW and these had been confirmed on

retesting.³ They also noted that the reverse situation occurs.

Using the RAST, we found earlier that solid-phase SRW was more reactive with allergic serum than was solid-phase GRW. Also, we noted that SRW appeared more potent than GRW in RAST inhibition.⁴ In more recent studies, Bernstein et al. demonstrated common allergenic determinants in various species of RW.¹⁹

The present study confirms our earlier observation that solid-phase SRW tends to be more reactive with sera from individuals with fall hay fever than does solid-phase GRW; examples are shown in Table I. It is of interest that the serum of one individual with fall hay fever, No. 22, though reactive with SRW, did not react with solid-phase GRW using the criterion of at least twice the percent of total counts bound with normal serum. However, the experiments in Tables II and III are more crucial in judging the equivalence of allergens present in SRW and GRW. In these experiments we exposed sera from individuals with fall hay fever to both GRW and SRW immunosorbents; we then tested the supernatants for residual IgE antibodies to both GRW and SRW determinants. In all cases these experiments indicated that the bulk of the IgE antibodies were removed by exposure to either solid-phase SRW or solid-phase GRW. Thus, a marked degree of cross-reactivity between the allergens derived from these pollens exists. In addition, IgE antibodies specific for SRW and GRW also exist. For example, all of the sera tested in Tables II and III by immunosorption with solid-phase GRW retained reactivity with SRW in the RAST. In contrast, only six of these 24 sera showed IgE antibodies specific for GRW. These six sera contain three populations of IgE antibodies: antibodies reactive with both GRW and SRW, antibodies reactive only with SRW, and antibodies reactive only with GRW. Finally, to show that these reactions have an in vivo parallel, we tested a pool of sera which demonstrated the preferential reactivity with SRW particularly well. The results in Table V show that, following repeated injection of GRW into skin sites passively sensitized with the serum pool, reactivity with this allergen was lost. However, these sites still reacted with a wheal-and-flare reaction following the injection of SRW extract.

Thus, our results demonstrate two major patterns of immunologic reactivity in cases of RW sensitivity. In the first and more frequent of these, sera from individuals with fall hay fever display a preferential reactivity to SRW allergens. In the second, sensitive sera possess antibodies specific for both SRW and GRW allergens. As shown in Table IV, it should be emphasized that in both of these cases the vast bulk of the antibody is cross-reactive.

We thank Ms. Cheryl R. Adolphson and Ms. Linda H. Callister for assistance in the preparation of this manuscript.

REFERENCES

1. Coca AF, Grove EF: Studies in hypersensitiveness: XIII. A study of the atopic reagins. *J Immunol* **10**:445, 1925.
2. Spain WC, Hopkins M: On the identity of the atopens of high ragweed and low ragweed pollen. *J ALLERGY* **1**:209, 1930.
3. Grubb GD, Vaughan WT: Evidence of group specific and species specific sensitization to pollen. *J ALLERGY* **9**:211, 1938.
4. Leiferman KM, Gleich GJ, Jones RT: The cross-reactivity of IgE antibodies with pollen allergens. II. Analyses of various species of ragweed and other fall weed pollens. *J ALLERGY CLIN IMMUNOL* **58**:140, 1976.
5. Welsh PW, Yunginger JW, Tani DG, Toussaint NF, Jr, Larson LA, Bourne WM, Gleich GJ: Topical ocular administration of cromolyn sodium for treatment of seasonal ragweed conjunctivitis. *J ALLERGY CLIN IMMUNOL* **64**:209, 1979.
6. Gleich GJ, Jacob GL, Yunginger JW, Henderson LL: Measurement of the absolute levels of IgE antibodies in patients with ragweed hay fever. *J ALLERGY CLIN IMMUNOL* **60**:188, 1977.
7. Leiferman KM, Gleich GJ: The cross-reactivity of IgE antibodies with pollen allergens. I. Analyses of various species of grass pollens. *J ALLERGY CLIN IMMUNOL* **58**:129, 1976.
8. Gleich GJ, Yunginger JW: Standardization of allergens, in Rose NR, Freidman H, editors: *Manual of clinical immunology*. Washington, D. C., 1976. American Society for Microbiology, chap. 77, pp. 575-584.
9. Gleich GJ, Adolphson CR, Yunginger JW: The mini-RAST: Comparison with other varieties of the radioallergosorbent test (RAST) for the measurement of IgE antibodies. *J ALLERGY CLIN IMMUNOL* **65**:20, 1980.
10. Cooke RA, Vander Veer A Jr: Human sensitization. *J Immunol* **1**:201, 1916.
11. Armstrong C, Harrison WT: A study of ragweed pollen extracts for use in the treatment of ragweed pollen hypersensitiveness. *U.S. Public Health Report* **39**:2422, 1924.
12. Bemton HS: Immunologic observations in autumnal hay-fever. *JAMA* **82**:1435, 1924.
13. Brown A: Studies in hypersensitiveness. XXIV. On the question of the identity of the atopens of the pollens of high ragweed and low ragweed. *J Immunol* **13**:73, 1927.
14. Rappaport BZ, Johnson CA: Fractionation of pollen proteins and their skin reactions on sensitive individuals. *Proc Soc Exp Biol Med* **26**:771, 1929.
15. Stull A, Cooke RA, Chobot R: The identity of the allergically active substance in the giant and low ragweed pollen. *J ALLERGY* **3**:120, 1932.
16. Cromwell HW, Moore MB: Studies on pollen and pollen extracts. X. Antigenic differences between short and giant ragweed pollens. *J ALLERGY* **4**:347, 1933.
17. Moore MB, Cromwell HW, Moore EE: Studies on pollen and pollen extracts. V. Skin reactions to pollen fractions. *J ALLERGY* **2**:85, 1931.
18. Cooke RA, Stull A, Hebal S, Barnard JH: Clinical and serologic study of the relationship of giant (*Ambrosia trifida*) and low (*Ambrosia artemisiifolia*) ragweed pollen. *J ALLERGY* **6**:311, 1935.
19. Bernstein IL, Perera M, Gallagher J, Michael JG, Johansson SGO: In vitro cross-allergenicity of major aeroallergenic pollens by the radioallergosorbent technique. *J ALLERGY CLIN IMMUNOL* **57**:141, 1976.