

The cross-reactivity of IgE antibodies with pollen allergens

II. Analyses of various species of ragweed and other fall weed pollens

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Although the antigenic composition of short ragweed pollen has been extensively investigated, very few studies have analyzed the allergenic cross-reactivity among various types of ragweed pollens. Using a serum pool from patients sensitive to short ragweed, we studied the cross-reactivity of IgE antibodies to six ragweeds by the radioallergosorbent test. Extracts were analyzed for their inhibitory activities with solid-phase allergens prepared from all of the ragweed pollens. Also, samples of serum were absorbed with the various solid-phase allergens and the reactivity of the remaining IgE antibodies was determined. Two patterns of reactivity were observed. Short, giant, western, and false ragweeds displayed comparable reactivity in both inhibition and absorption experiments. Slender and southern ragweed were considerably less active, indicating that they lacked allergenic groupings possessed by the other species. These same patterns of cross-reactivity were found using ragweed pollens from four commercial sources. Knowledge of these patterns of cross-allergenicity is of importance for diagnosis and treatment of sensitive patients as well as for in vitro standardization of extracts.

Many wind-pollinated weeds have been identified as causes of fall allergies and among these only the antigenic composition of short ragweed has been extensively characterized.^{1, 2} Very few analyses of the antigenic cross-reactivity among ragweeds and other fall weeds have been performed.³⁻⁶ The radioallergosorbent test (RAST)⁷ may be used to quantitatively measure the reactivity of IgE antibodies with various allergens. In this study we have analyzed the cross-reactivity of IgE antibodies with six types of ragweed pollens and several other fall weeds by applying RAST inhibition and also by testing serum absorbed with different ragweed allergens.

MATERIALS AND METHODS

Source of IgE antibodies

A pool of serums from five individuals highly allergic to short ragweed pollen was used throughout the study as a source of IgE antibodies. These patients are natives of Minnesota

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TABLE I. Classification of the ragweed pollens

Trivial name	Pollen genus and species	Symbols	Present in Rochester area	Solids per ml of extract (mg)
Short ragweed	<i>Ambrosia elatior</i>	Sh	Yes	39.7
Giant ragweed	<i>Ambrosia trifida</i>	G	Yes	19.8
Western ragweed	<i>Ambrosia coronopifolia</i>	W	No	35.5
False ragweed	<i>Franseria acanthicarpa</i>	F	No	29.3
Southern ragweed	<i>Ambrosia bidentata</i>	So	No	26.9
Slender ragweed	<i>Franseria tenuifolia</i>	Sl	No	19.6

and their predominant exposure has been to short and giant ragweed. None of the patients had ever been treated by hyposensitization. This is the same pool used previously for measurement of the potency of short ragweed extracts.⁸

Source of ragweed pollen and other extracts

Pollens from six different types of ragweed were used in the study (Table I). Short (Lot No. 88E3), giant (86ED3), western (91C3), and false (85E3) were purchased from Crystal Labs, Edmond, Okla., and slender (85-50-72) and southern (47-57-72) from Greer Laboratories, Lenoir, N. C. In addition, extracts of these same pollens from several other companies were used in this study. Short (B 468387 D), giant (H 3683240), western (L 368379), false (E 4442202), slender (G 5506001), and southern (K 3950301) ragweeds, burweed marshelder (J 372706), cocklebur (J 4603119), and prairie sage (G 5508001) were purchased from Hollister-Stier, Spokane, Wash. Extracts of giant ragweed (55-35-2x9), western ragweed (58-12-1x3), false ragweed (185-6-1x7), slender ragweed (50-7-1x12), southern ragweed (57-8-1x2), burweed marshelder (44-15-1x12), cocklebur (33-19-2x9), prairie sage (48-7-1x7), annual wormwood (65-11-1x4), giant sage brush (61-9-1x4), and common mugwort (47-10-1x4) were purchased from Greer Laboratories. False ragweed (7314888), slender ragweed (7314887), cocklebur (323801X), prairie sage (7314889), and burweed marshelder (7317418) were also purchased from Center Laboratories, Port Washington, N. Y.

Two of the ragweed pollen types, short and giant, are prevalent in the Rochester, Minn., area. The other ragweeds are foreign to the area. The ragweeds belong botanically to two genera as shown in Table I: short, giant, western, and southern ragweeds to the *Ambrosia* genus, the so-called true ragweeds, and false and slender to the *Franseria* genus, the "false ragweeds."

Extracts

The ragweed pollen was defatted with diethyl ether and crude extracts were prepared by continuous mixing of the defatted pollens in a 1:10 weight per volume (w/v) mixture with distilled water. The extracts were stored at -20° C. Samples of the resulting extracts were dried to a constant weight in an oven at 60° C, and the weights of dissolved solids in the various extracts are shown in Table I.

Solid-phase allergens

Six ragweed solid-phase allergens were prepared by reaction of defatted ragweed extracts with cyanogen bromide (CNBr)-activated cellulose.⁹ Briefly, 6.0 gm of microcrystalline cellulose were reacted with 12 gm of CNBr dissolved in 12 ml of N,N-dimethylformamide in a volume of 160 ml at pH 11 and 4° C for 45 min by addition of 4 N sodium hydroxide. After washing, the activated cellulose was divided into six aliquots of 1.0 gm and each aliquot was reacted with 0.7-1.3 ml of ragweed extract containing 25 mg dry weight. These mixtures were reacted overnight at 4° C with rotation and thoroughly washed, up to 17 times, in borate saline.¹⁰ The cellulose ragweed polymers were suspended at a concentration of 1.0 mg per milliliter in RAST diluent. Another solid-phase ragweed polymer was prepared by reacting 500 mg

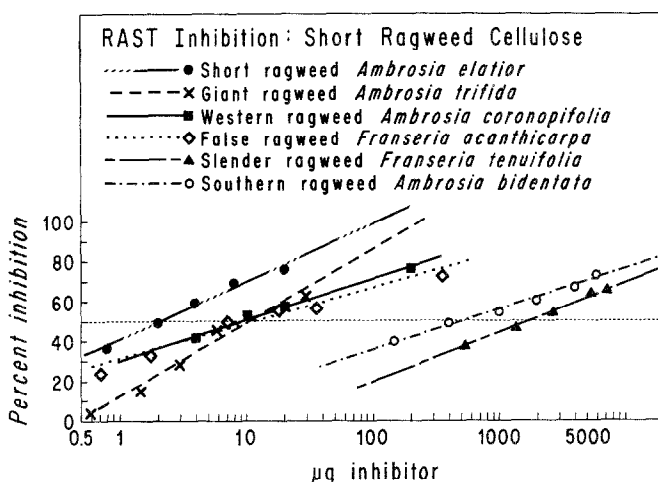


FIG. 1. Comparison of the cross-reactivity of ragweed allergens by RAST inhibition using solid-phase short ragweed allergen. In this and in Figs. 2 and 3, the quantity (μg) of fluid-phase extracts required to produce 50% inhibition in the RAST was used as a measure of cross-reactivity.

TABLE II. Reactivity of IgE antibodies with solid-phase ragweed allergens (counts bound/counts added $\times 100$)

Serum	Solid-phase allergen					
	Short	Giant	Western	False	Southern	Slender
Allergic*	29.29	26.92	28.63	30.00	18.45	11.71
Normal	0.39	0.61	0.45	0.47	0.68	0.67

*0.1 ml of a 1:5 dilution of the allergic serum pool was tested.

activated cellulose with 1.0 mg of lyophilized crude short ragweed extract (Lot 00802FD) obtained from Center Laboratories.

Performance of the RAST

The RAST was performed as described in the accompanying paper.¹⁰

RAST inhibition

The RAST inhibition procedure was performed as described in the accompanying paper.¹⁰

Absorption of allergic serum with solid-phase ragweeds

Samples of serum were incubated with each of the ragweed cellulose polymers to remove IgE antibodies specific for each allergen. The protocol for this procedure is illustrated in the accompanying paper.¹⁰ A portion of the cellulose allergen suspension, 1.5 mg, in 1.5 ml RAST diluent was first centrifuged and the buffer aspirated. Samples, 1.5 ml, of a 1:5 dilution of allergic serum pool in RAST were added and rotated for at least 24 hr at room temperature. The suspensions were centrifuged and the supernatants were absorbed 12 times more in the same manner. We found that this absorption procedure removed most of the IgE antibody reactivity with each specific immunosorbent so that the final supernatant in the RAST with the homologous solid-phase ragweed had markedly reduced reactivity. Following absorption the serums were reacted in the RAST with the various solid-phase ragweeds to determine the reactivity of the remaining IgE antibodies to these allergens. Serum absorbed in the same

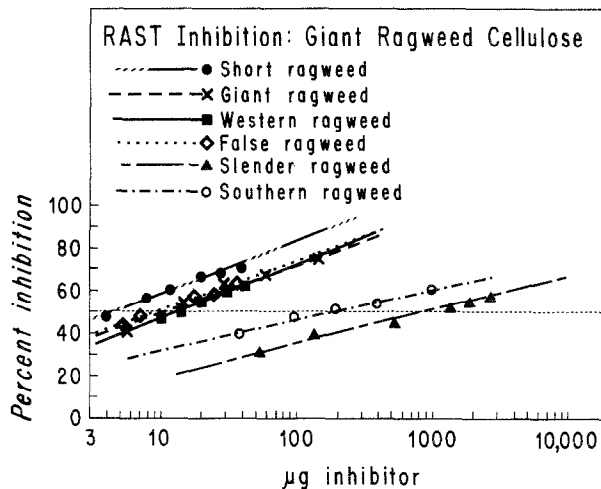


FIG. 2. Comparison of the cross-reactivity of ragweed allergens by RAST inhibition using solid-phase giant ragweed allergen.

manner with bovine serum albumin-cellulose did not lose reactivity with solid-phase ragweed allergens.

RESULTS

In initial RAST experiments we tested the serum pool from ragweed-sensitive subjects with the six solid-phase ragweed allergens. The results are shown in Table II. The allergic serum pool yielded significantly positive reactions with all of the solid-phase allergens. Normal serum yielded very slight binding of radiolabeled anti-IgE, a maximum of 0.7%.

The antigenic relationships among the various ragweeds were tested further by RAST inhibition and representative results from three experiments are shown in Figs. 1 through 3. In Fig. 1 the inhibition patterns of the ragweed extracts competing with the IgE antibodies for solid-phase short ragweed are shown. Four of the ragweeds, short, giant, western, and false, were similar in their inhibitory activities, yielding 50% inhibition end points within sixfold of one another. Short ragweed was the most potent inhibitor in its own system. In contrast, southern and slender ragweed were markedly less efficient as fluid-phase inhibitors. It is evident from the graphs that the regression lines were not parallel. In Fig. 2 the reactivity of the fluid-phase allergens competing with giant ragweed cellulose is shown. Essentially the same pattern of reactivity as shown in Fig. 1 was seen, and the four similarly reacting ragweeds had 50% inhibition end points within a factor of three of each other. Slender and southern were again markedly less active. In Fig. 3 the reactivity of the fluid-phase allergens competing with slender ragweed cellulose for IgE antibodies is shown. Five of the ragweeds, including slender ragweed, were quite comparable in their inhibitory activities, but southern ragweed was markedly less active.

The results of each inhibition experiment are summarized in Fig. 4. Short, giant, western, and false ragweed extracts were the most active inhibitors in the

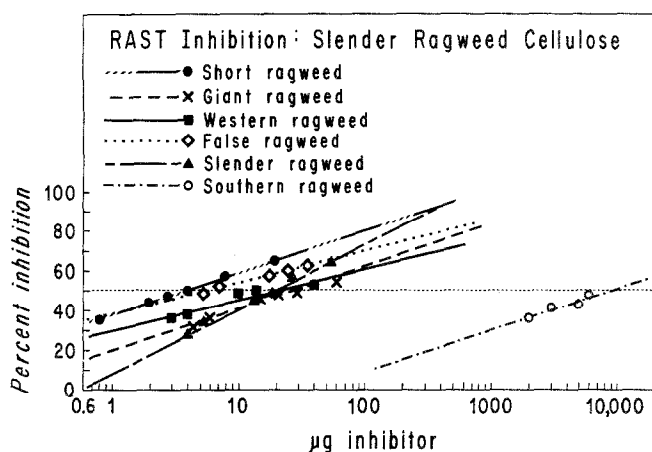


FIG. 3. Comparison of the cross-reactivity of ragweed allergens by RAST inhibition using solid-phase slender ragweed allergen.

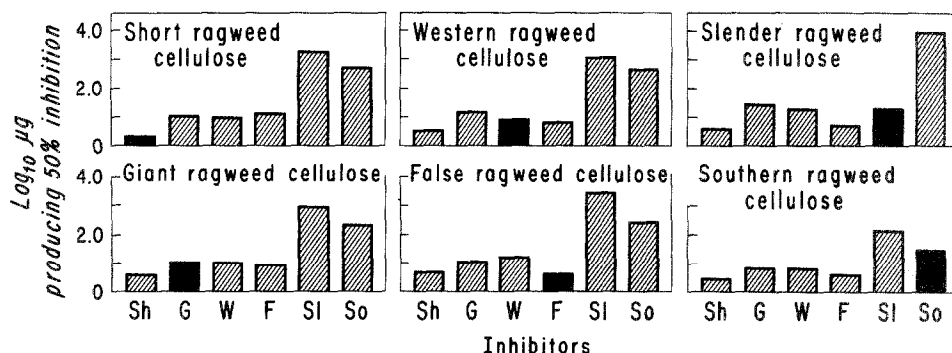


FIG. 4. Inhibitory activities of the various fluid-phase ragweed extracts competing for IgE antibodies with solid-phase short, giant, western, false, slender, and southern ragweed allergens (inhibitory activity is designated as the log of the micrograms of soluble antigens required to produce 50% inhibition in the RAST).

short ragweed system (*top left*); slender and southern were considerably less active. Essentially the same pattern of reactivity with IgE antibodies was found with the fluid-phase antigens and solid-phase giant, western, and false ragweeds. With solid-phase slender ragweed, soluble slender ragweed was considerably more efficient as an inhibitor than in the previous comparisons, while southern ragweed remained a poor inhibitor. Finally, with solid-phase southern ragweed, short, giant, western, false, and southern ragweed extracts were quite similar in inhibitory activity, while slender ragweed was the least potent inhibitor.

To determine whether a given solid-phase ragweed allergen could remove not only the IgE antibodies reactive with the homologous allergen but also related allergens, we absorbed samples of serum with solid-phase ragweed allergens and tested the reactivity of the supernatants by the RAST. As shown in Fig. 5 (*top left*), when solid-phase short ragweed cellulose was reacted with samples of serum

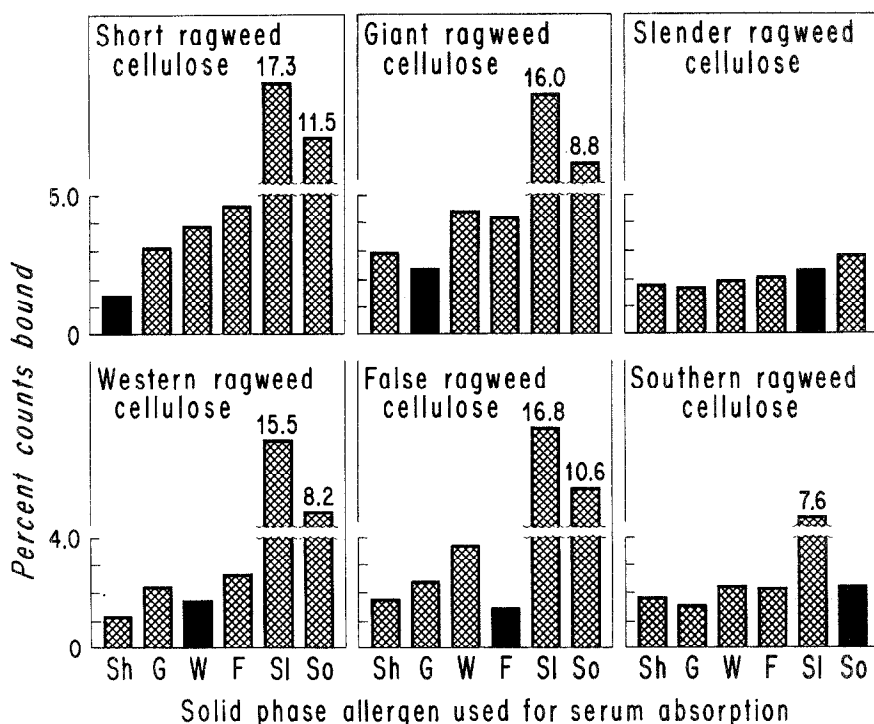


FIG. 5. Reactivity of absorbed serum samples with short, giant, western, false, slender, and southern solid-phase ragweed allergens. Samples of serum were individually absorbed with each solid-phase allergen indicated on the abscissa and subsequently tested by RAST with the solid-phase allergens indicated in the panels.

absorbed with each of the individual ragweed solid-phase allergens, the least reactivity was seen with the homologous allergen, short ragweed. For example, before absorption the serum pool bound 25.8% of total counts added, and following absorption this reactivity was reduced to 1.4%. Also, much of the reactivity with short ragweed allergen was removed from the serum pool by absorption with solid-phase giant, western, and false ragweeds. In contrast, slender and southern were poorer immunosorbents and left considerable reactivity with solid-phase short ragweed. Essentially the same pattern of reactivity of the absorbed serums was seen with the solid-phase western, giant, and false ragweeds. In contrast, when the absorbed serum samples were reacted with southern ragweed cellulose (Fig. 5, lower right), five of the solid-phase ragweeds removed the bulk of the reactivity while the sample of serum absorbed with slender ragweed cellulose retained significant reactivity. Finally, with slender ragweed cellulose all of the ragweeds were equally efficient in removing IgE antibodies reactive with slender ragweed allergens.

The possibility that the cross-reactivity observed with the short, giant, western, and false ragweed extracts was due to contamination of a given lot of ragweed with short ragweed was tested by comparing different lots of ragweed pollen from three additional companies in separate RAST inhibition experiments. In

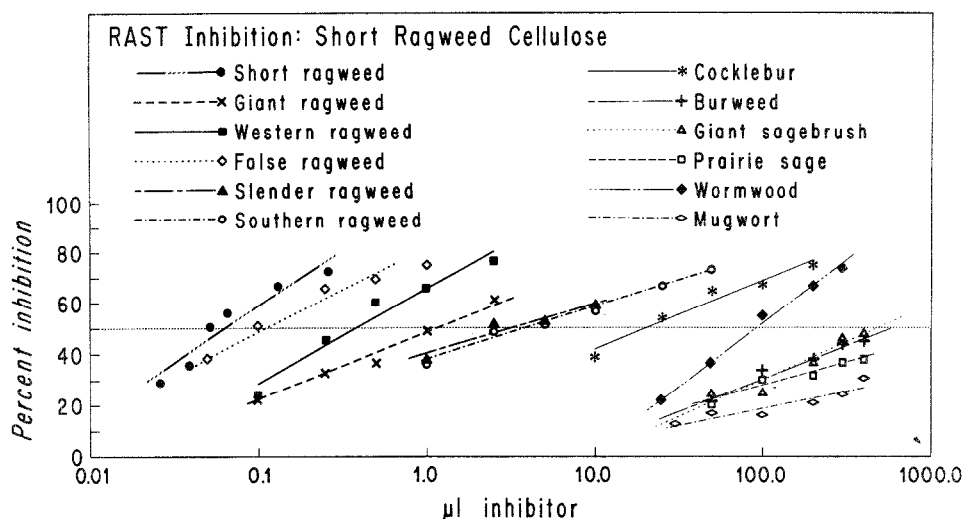


FIG. 6. Comparison of the cross-reactivity of ragweed allergens and other fall weed allergens by RAST inhibition using solid-phase short ragweed allergen. As a measure of cross-reactivity, the volume (μ l) of extract required to produce 50% inhibition was determined. The inhibitor extracts were: a partially purified preparation of short ragweed⁸ and selected extracts of giant ragweed, western ragweed, false ragweed, southern ragweed, slender ragweed, cocklebur (*Xanthium commune*), burweed marshelder (*Iva xanthifolia*), giant sagebrush (*Artemisia tridentata*), prairie sage (*Artemisia graphalodes*), annual wormwood (*Artemisia annua*), and common mugwort (*Artemisia vulgaris*).

each case short, giant, western, and false ragweeds were quite comparable in their inhibitory activities, whereas slender and southern were markedly less active. Finally, several other fall weed extracts were tested in inhibition experiments with solid-phase short ragweed. Fig. 6 shows the inhibition results from one of these experiments. With the exception of cocklebur and wormwood, the other fall weeds were exceedingly poor inhibitors and 50% inhibition was not reached with quantities of extract up to 400 μ l.

DISCUSSION

Our results with the various species of ragweeds show that considerable cross-reactivity exists. In these experiments we utilized a serum pool from individuals sensitive to ragweeds indigenous to the southern Minnesota area. Principally, short and giant ragweed grow in this area, but the results indicate that western and false ragweed display considerable cross-reactivity. In all of the studies these four ragweeds were quite similar in their activities. In contrast, slender and southern ragweed extracts were considerably less active, indicating that they were antigenically deficient compared to the other ragweeds. Because western, false, slender, and southern ragweeds are not predominant in our area, the serum pool may have been deficient in specific reactivity with these allergens. Therefore, we cannot exclude the possibility that unique allergenic determinants may be present on these pollens.

In general the results of the absorption experiments showed the same patterns of allergenic cross-reactivity with IgE antibodies as did the RAST inhibition experiments with one exception. In the inhibition experiments (Fig. 4) it appeared that slender ragweed was distinct from southern ragweed. Southern was a very poor inhibitor in the slender solid-phase system and slender was a rather poor inhibitor in the southern solid-phase system. In the absorption experiments (Fig. 5) serum absorbed with solid-phase slender ragweed retained considerable reactivity with southern ragweed cellulose, whereas southern ragweed cellulose was as efficient in removing IgE reactivity with slender ragweed cellulose as slender was itself. A second absorption experiment using ragweeds coupled to Sepharose 2B (Pharmacia Laboratories, Piscataway, N. J.) as the solid-phase immunosorbent showed the same results. We have no explanation for the differences found in these two test systems.

We tested ragweed and fall weed pollen extracts from three other suppliers in an additional inhibition experiment using a different solid-phase short ragweed. Essentially the same patterns of reactivity were seen among the ragweed extracts. The other fall weed extracts showed very little inhibitory activity, and in most cases 50% inhibition was not achieved with additions of relatively large volumes of extract. The two extracts which showed some inhibitory activity, though markedly less than any of the ragweed extracts, could have been contaminated with trace amounts of ragweed pollen. This is consistent with the finding of contamination in other extracts from weeds pollinating during the ragweed season.⁶ However, we do not believe that the patterns of allergenicity seen among the ragweeds were due to cross-contamination of the various ragweed extracts. Our reasons for this conclusion are: (1) the results with ragweed extracts from four different sources yielded the same patterns of reactivity and (2) in the case of one of the suppliers,¹¹ the false and western ragweed pollens are collected in California while the short ragweed pollen is collected in Ohio and Illinois. Because short and giant ragweed do not grow in California, it is extremely unlikely that the false and western extracts would contain short or giant ragweed contaminants. Similarly, the short ragweed should not contain false or western allergenic components.

Knowledge of these patterns of cross-allergenicity has application for diagnostic testing as well as in vitro standardization of allergy extracts and treatment of sensitive patients. A standardized solid-phase allergen prepared with any of the similarly reacting ragweeds, short, giant, western, or false, or a mixture of the four ragweeds reacting with a pool of IgE antibodies, could be utilized in standardizing allergy extracts by RAST inhibition or in diagnosing ragweed sensitivity in patients by the RAST. It seems likely that, because of the antigenic differences of slender and southern ragweed, additional systems would be necessary for standardization of extracts and detection of sensitivity to these pollens.

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