

The cross-reactivity of IgE antibodies with pollen allergens

I. Analyses of various species of grass pollens

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Atopic patients with histories of grass pollen allergy often are sensitive to a variety of species of grasses. Using a serum pool from patients sensitive to June grass, we analyzed the reactivity of IgE antibodies to seven grasses by the radioallergosorbent test. Extracts were analyzed for their inhibitory activities with solid-phase allergens prepared from all of the grass pollens. Also samples of serum were exhaustively absorbed with solid-phase allergens and the supernatants tested to determine the reactivity of the remaining IgE antibodies. Three patterns of reactivity were observed: (1) June, orchard, meadow fescue, and perennial rye grasses displayed similar reactivity in both inhibition and absorption studies; (2) sweet vernal and Bermuda grasses were considerably less reactive with the serum pool, indicating that they lacked antigenic determinants possessed by the other grasses; and (3) timothy grass possessed unique antigenic determinants. 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.

Considerable evidence in the literature indicates that there is extensive cross-reactivity among various grass allergens.¹⁻⁴ This information has been derived from in vivo studies utilizing skin testing⁴ and in vitro studies with rabbit antisera to whole extracts or purified antigens.¹⁻³ The introduction of the radioallergosorbent test (RAST) as an in vitro assay for IgE antibodies has made possible quantitative analyses of the cross-reactivity of various allergens.⁵ Such analyses may be performed by determining the direct reactivity of IgE antibody with solid-phase allergens or alternatively by RAST inhibition. In this study we have analyzed the cross-reactivity of IgE antibodies with various species of grass pollen extracts by RAST inhibition and also by testing serum absorbed with the different grass allergens.

MATERIALS AND METHODS

Source of IgE antibodies

A pool of sera from five individuals highly allergic to northern grass pollens was used as the source of IgE antibodies. Eighteen patients responded to an advertisement in an

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TABLE I. Classification of the grass pollens

Trivial name	Pollen genus and species	Tribe	Symbols	Present in Rochester area	Solids per ml of extract (mg)
Timothy grass	<i>Phleum pratense</i>	Agrostideae	Tim	Yes	43.4
Meadow fescue grass	<i>Festuca elatior</i>	Festuceae	MF	Yes	47.1
June grass	<i>Poa pratensis</i>	Festuceae	June	Yes	48.4
Orchard grass	<i>Dactylis glomerata</i>	Festuceae	Orch	Yes	55.7
Perennial rye grass	<i>Lolium perenne</i>	Festuceae*	P-rye	Yes	43.4
Sweet vernal grass	<i>Anthoxanthum odoratum</i>	Phalarideae	SV	No	45.8
Bermuda grass	<i>Cynodon dactylon</i>	Chlorideae	Ber	No	48.6

*Also classified as a member of the Hordeae tribe.⁷

institutional newsletter seeking individuals with symptoms of allergic rhinitis in May-June and the 5 patients' serums having the greatest reactivity with solid-phase June grass allergen were selected for use in the study. All patients had positive skin tests to several northern grass extracts. None of the patients had been treated by hyposensitization.

Grass pollens

Pollens from seven different species of grasses were purchased from Greer Laboratories, Lenoir, N. C. Five of the pollen species were indigenous to southern Minnesota, whereas two species were foreign to this area. These grasses belonged botanically to four tribes,⁶ as indicated in Table I. According to a second classification scheme,⁷ the grasses belonged to five botanical tribes, perennial rye grass being classified as a member of the Hordeae tribe.

Extracts

Five to 10 gm of pollen were defatted overnight with 300 ml diethyl ether in a Soxhlet extractor and crude extracts were prepared by continuous mixing of the dried, defatted pollens in a 1:10 weight per volume (w/v) mixture with distilled water for 48 hr at 4° C using a magnetic stirrer. The extracts were stored at -20° C. Samples of the resulting extracts were dried in an oven at 60° C to determine the weights of dissolved material. These dry weights are shown in Table I.

Solid-phase allergens

Cellulose-grass solid-phase allergens were prepared by reaction of the seven defatted grass extracts with cyanogen bromide (CNBr)-activated microcrystalline cellulose. The details of the coupling procedure have been presented elsewhere.⁸ Briefly, 3.5 gm of microcrystalline cellulose were reacted with 7 gm of CNBr dissolved in 7 ml of N,N-dimethylformamide in a total reaction volume of 90 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 7 aliquots of 0.5 gm and each aliquot was reacted with 3.5-4.7 ml of grass extract containing 200 mg dry weight. These suspensions were tumbled for 30 hr at 4° C and thoroughly washed in 0.2 M H₃BO₃-0.04 M NaOH-0.16 M NaCl pH 8 (borate saline). Each cellulose grass polymer was suspended at a concentration of 1 mg per milliliter in 0.1 M pH 7.4 phosphate buffer containing 0.2% bovine serum albumin, 1% Tween 20, and 0.1% sodium azide (RAST diluent).

Sephacrose-grass solid-phase allergens were also prepared by reaction of the seven defatted grass extracts with CNBr-activated Sepharose 2B (Pharmacia Laboratories, Piscataway, N. J.).⁸ Briefly, 105 ml of packed Sepharose 2B (2,100 mg) were reacted with 11 gm CNBr dissolved in 11 ml N,N-dimethylformamide in a total reaction volume of 210 ml at pH 11 and 4° C for 50 min by addition of 4 N sodium hydroxide. After washing, the activated Sepharose was divided into 7 aliquots of 300 mg and each aliquot was reacted with 5.5 to 7.0 ml of grass extract containing 300 mg dry weight. The capacity of activated Sepharose 2B to bind proteins is greater than activated microcrystalline cellulose.⁸ Therefore, the activated Sepharose

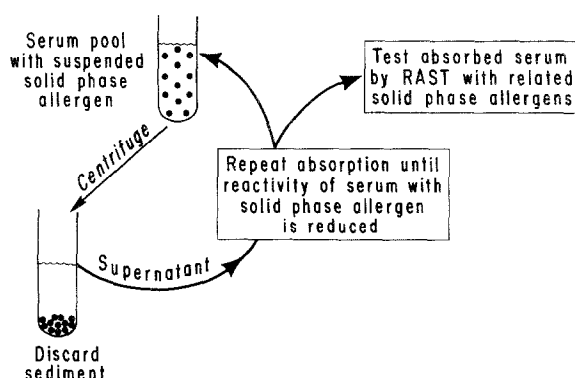


FIG. 1. Procedure for absorption of allergic serum with solid-phase grasses.

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

Serum	Solid-phase allergen						
	Timothy	Meadow fescue	June	Orchard	Perennial rye	Sweet vernal	Bermuda
Allergic*	10.2	16.1	11.8	14.6	11.9	5.5	4.3
Normal	0.3	0.4	0.2	0.2	0.2	0.2	0.1

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

polymers were reacted with larger amounts of extract than the cellulose polymers. These suspensions were tumbled for 48 hr at 4° C and thoroughly washed in borate saline. Each grass Sepharose polymer was suspended in RAST diluent at a concentration of 10 mg per milliliter.

Performance of the RAST

Serum IgE antibody to grass pollen allergens was measured by RAST⁹ essentially as described previously.¹⁰ Briefly, 0.5 mg solid-phase grass cellulose in 0.5 ml RAST diluent, 0.10 ml test serum or dilution thereof, and 0.90 ml RAST diluent were added to 10 $\times$ 62 mm plastic tubes. The tubes were capped and were slowly rotated overnight at room temperature, after which they were centrifuged and the supernatant aspirated. The solid-phase allergen was washed twice with 1 ml of 0.1 M pH 7.4 K_2HPO_4 - KH_2PO_4 containing 1% Tween 20; 1.4 ml of diluent consisting of 0.1 M pH 7.5 KH_2PO_4 - K_2HPO_4 , 470 ml, fetal calf serum (Grand Island Biologicals, Grand Island, N. Y.), 20 ml, 10% NaN_3 , 5 ml, and Tween 20, 5 ml (FCS diluent) were added followed by 20 ng of radiolabeled affinity chromatography purified anti-IgE in 0.1 ml of pH 7.5 phosphate albumin. The tubes were rotated overnight and washed again. Radioactive counts associated with the final washed polymer were measured in a gamma spectrometer and the results were expressed as counts bound divided by counts added $\times 100$. All serum samples were run in duplicate and the results averaged.

RAST inhibition

The RAST inhibition procedure was carried out essentially as described previously.¹¹ Briefly, in rapid succession, inhibitor in RAST diluent or a neat extract was added in a volume up to 0.5 ml. Then 0.1 ml of a 1:2 dilution of pooled allergic serum in RAST diluent was added. The volume was adjusted to 1.0 ml with RAST diluent, and 0.5 ml of the solid-phase cellulose-grass allergen at a concentration of 1 mg per ml was added. The tubes were capped and rotated overnight at room temperature. Following washing, 1.4 ml of FCS diluent and 20 ng radiolabeled anti-IgE in 0.1 ml of pH 7.5 phosphate albumin were added, followed by

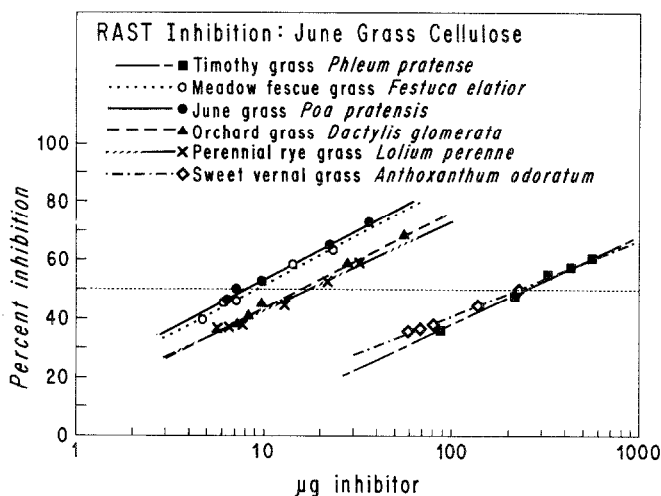


FIG. 2. Comparison of the cross-reactivity of grass allergens by RAST inhibition using solid-phase June grass allergen. In this and in Figs. 3 to 5, the quantity (μg) of fluid-phase extracts required to produce 50% inhibition in the RAST was determined as a measure of cross-reactivity.

rotation overnight and washing. Duplicate samples were analyzed and the associated radioactivity averaged. In the performance of RAST inhibition, varying quantities of extracts were added to determine the amount necessary to produce 50% inhibition of the binding of IgE antibody to the solid-phase allergens. In all cases the negative control with normal human serum was subtracted from the experimental results. The results were plotted on semilog paper and all of the resulting least squares regression lines had correlation coefficients of +0.98 or greater. Finally, differences in the slopes of regression lines given by individual extracts were tested by analysis of covariance¹² using a programmable Hewlett-Packard 9810A calculator.

Absorption of allergic serum with solid-phase grasses

The protocol employed for this procedure is illustrated in Fig. 1. Samples of the serum pool were incubated with each of the grass-Sepharose polymers to remove IgE antibodies specific for each allergen. Thirty-five mg of the grass-Sepharose in 3.5 ml RAST diluent were first centrifuged and the buffer aspirated. Samples, 2.5 ml, of a 1:5 dilution of pooled allergic serum in RAST diluent were added and rotated for at least 8 hr at room temperature. The suspensions were centrifuged and the supernatants were absorbed twice more in the same manner. We found that this absorption procedure removed virtually all of the IgE antibodies reactive with specific immunosorbent, so that the reactivity of the final supernatant in the RAST with the homologous solid-phase grass was essentially the same as normal serum. Following absorption the serums were tested in the usual RAST with the various solid-phase (cellulose) allergens to determine the reactivity of the remaining IgE antibodies.

RESULTS

In initial RAST experiments we tested the serum pool from grass-sensitive subjects with the seven solid-phase grass allergens. The results are shown in Table II. The allergic serum pool yielded significantly positive reactions with all of the solid-phase allergens, including those foreign as well as those indigenous to the Minnesota area. Normal serum yielded very slight binding of radiolabeled anti-IgE, a maximum of 0.4% of counts being associated with the meadow fescue

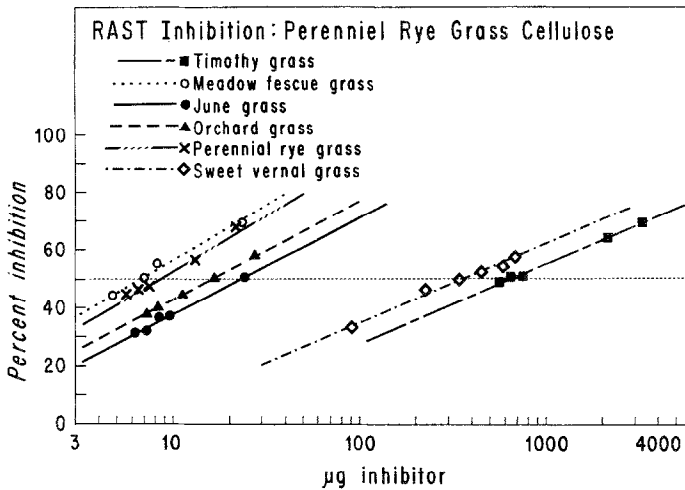


FIG. 3. Comparison of the cross-reactivity of grass allergens by RAST inhibition using solid-phase perennial rye grass allergen.

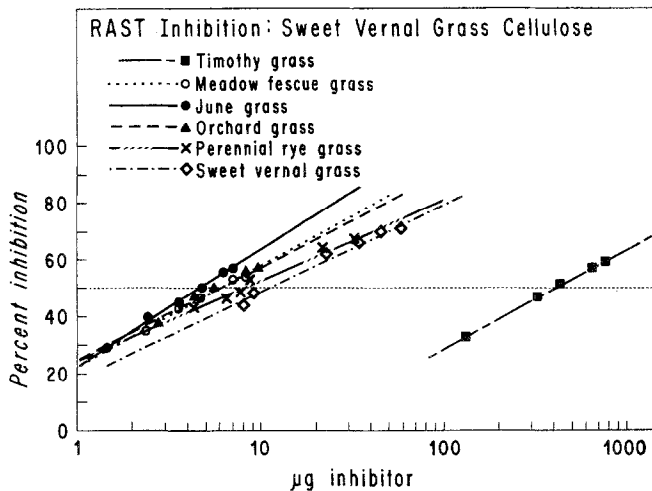


FIG. 4. Comparison of the cross-reactivity of grass allergens by RAST inhibition using solid-phase sweet vernal grass allergen.

solid-phase allergen. These results indicated that the IgE antibodies in the serum pool reacted well with all of the indigenous grasses and less strongly with those foreign to the area, namely, sweet vernal and Bermuda.

The allergenic relationships among the various grasses were tested further by RAST inhibition and representative results from four experiments are shown in Figs. 2 through 5. Bermuda grass was a very poor inhibitor, and addition of 24,000 μg failed to produce 50% inhibition with any of the solid-phase grass allergens. Therefore, inhibition curves with Bermuda extracts are not included in the figures. With June grass as the solid-phase allergen, the pattern of reactiv-

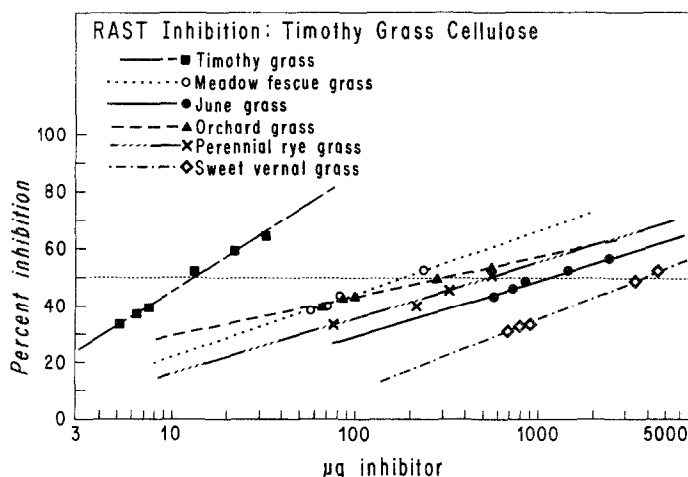


FIG. 5. Comparison of the cross-reactivity of grass allergens by RAST inhibition using solid-phase timothy grass allergen.

ity shown in Fig. 2 was found. Four grass extracts, meadow fescue, June, orchard, and perennial rye, were quite similar in their inhibitory activities, yielding 50% inhibition end points within a factor of two of each other. In contrast, timothy and sweet vernal grasses were considerably less efficient as inhibitors and were approximately 1/10 as active as the weakest of the other extracts. In Fig. 2 the slopes of the inhibition regression lines do not differ significantly. In Fig. 3 the inhibition patterns of the grass extracts competing with the IgE antibodies for solid-phase perennial rye grass allergens are shown. Essentially the same pattern of reactivity as shown in Fig. 2 was seen except that in this case the slopes of the regression lines of timothy and sweet vernal differ ($p < 0.005$) from the others. The 50% inhibition end points of meadow fescue, perennial rye, orchard, and June extracts were within a factor of three of each other in activity, whereas sweet vernal and timothy were 1/10 as active. In Fig. 4, the reactivity of sweet vernal cellulose and the various fluid-phase allergens with IgE antibody is shown. The activities of the same four grasses, namely, meadow fescue, June, orchard, and perennial rye, were quite similar. However, in this case sweet vernal, the homologous allergen, was considerably more efficient as an inhibitor and was comparable to the other four grasses. Timothy now stands alone as the weakest inhibitor of all the six grasses tested in this experiment. Finally, in Fig. 5 we analyzed the inhibitory activities of the extracts with timothy cellulose as the solid-phase antigen. Timothy allergen in this case was the most active inhibitor, and the slope of its regression line was different than the slopes of the regression lines produced by the other soluble allergens. The other five grasses were considerably less active than timothy in inhibiting the reaction between the IgE antibodies and solid-phase timothy.

The results from each of the inhibition experiments are summarized in Figs. 6 and 7. In Fig. 6 the reactions with June grass cellulose, orchard grass cellulose, perennial rye grass cellulose, and meadow fescue grass cellulose are shown. June,

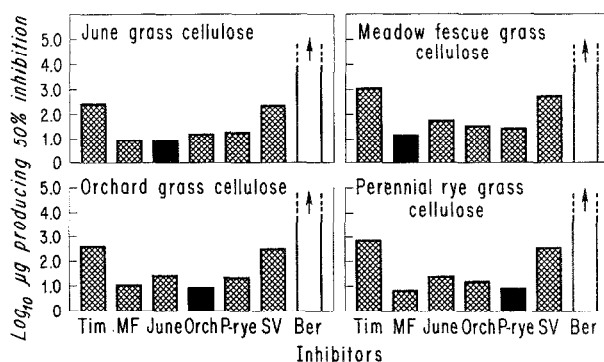


FIG. 6. Inhibitory activities of the various fluid-phase grass extracts competing for IgE antibodies with solid-phase June, orchard, meadow fescue, and perennial rye grass allergens. (Inhibitory activity for this and Fig. 7 is designated as the log of the micrograms of soluble allergen required to produce 50% inhibition in the RAST.)

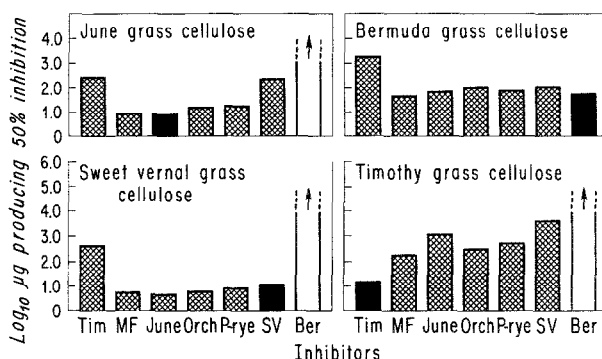


FIG. 7. Inhibitory activities of the various fluid-phase grass extracts competing for IgE antibodies with solid-phase June, sweet vernal, Bermuda, and timothy grass allergens. (June grass solid-phase allergen is included as a representative of the pattern in Fig. 6.)

orchard, perennial rye, and meadow fescue were all very active inhibitors, and their activities were roughly comparable when used as inhibitors in these four systems. In contrast, timothy and sweet vernal were much less active inhibitors, and their inhibition ranges were scattered with greater quantities of extracts required to produce 50% inhibition. As noted previously, we were unable to produce 50% inhibition with Bermuda extract. The other patterns of inhibition are shown in Fig. 7. The prior results with June grass are included for comparison. With sweet vernal cellulose, all of the extracts but timothy and Bermuda were active inhibitors and comparable in their inhibitory activities; timothy and Bermuda were still remarkably poor as inhibitors. Bermuda extract was a good inhibitor when tested in the homologous solid-phase system and was comparable to the other grasses. With timothy cellulose, timothy extract itself was a good inhibitor, but the other extracts were significantly less active. In each of these systems and as shown in earlier figures, June, orchard, meadow fescue, and perennial rye were

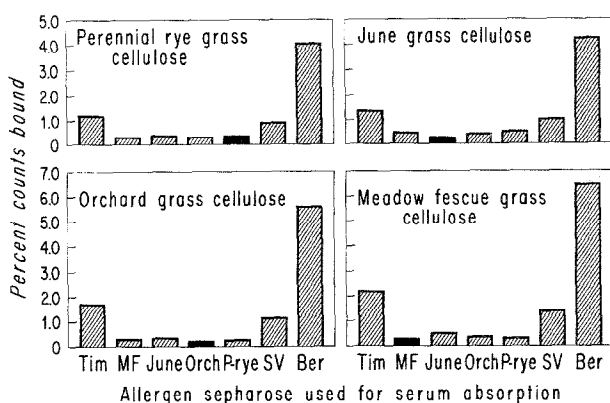


FIG. 8. Reactivity of absorbed serum samples with perennial rye, orchard, June, and meadow fescue solid-phase grass allergens. For this and Fig. 9, samples of serum were individually absorbed with each solid-phase allergen indicated on the abscissa and subsequently tested by RAST with the solid-phase allergens indicated in the panels. RAST results greater than twice that of serum absorbed with the homologous polymer were considered significant.

consistently similar in their inhibitory activities. Timothy, though an excellent inhibitor in its homologous system, was considerably less efficient when competing with any of the other solid-phase allergens for IgE antibodies.

In order to determine whether a given solid-phase grass could remove not only the IgE antibodies reactive with the homologous allergen but also related allergens, we absorbed samples of serum with solid-phase (Sephacel) grass and continued the absorption until the serum had essentially no reactivity with the solid-phase (cellulose) test allergens. The experimental protocol for these experiments is shown in Fig. 1. The absorbed serums were reacted in the usual RAST with the various solid-phase allergens. The results are shown in Figs. 8 and 9. As shown in Fig. 8 (*top right*), when solid-phase June grass cellulose was reacted with samples of serum absorbed with each of the individual Sephacel-grass allergens, the least reactivity was seen with the homologous allergen, June grass. For example, before absorption the serum pool bound 11.8% of counts when reacted with cellulose June grass, and following absorption this reactivity was reduced to 0.2%, a value comparable to nonallergic serum. The reactivity with solid-phase June grass allergen was removed from the serum not only by absorption with the homologous allergen but also almost completely by meadow fescue, orchard, and perennial rye. In contrast, sweet vernal, timothy, and particularly Bermuda grass were poorer immunosorbents and left considerable reactivity with June grass cellulose. The absorbed serums showed essentially the same pattern of reactivity with solid-phase perennial rye grass, orchard, and meadow fescue allergens. In contrast to these results as shown in Fig. 9 (*top right*), when the various absorbed serum samples were reacted with sweet vernal cellulose, five of the grasses absorbed the bulk of the reactivity, while the samples absorbed with timothy and Bermuda grass allergens still retained significant reactivity. In Fig. 9 (*bottom right*) when the absorbed serum samples were reacted with timothy

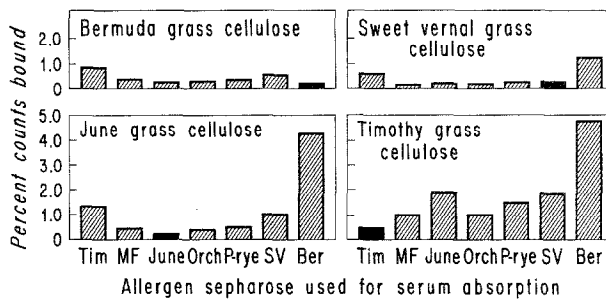


FIG. 9. Reactivity of absorbed serum samples with Bermuda, June, sweet vernal, and timothy solid-phase grass allergens. (June grass solid-phase allergen is included as a representative of the pattern in Fig. 8.)

cellulose, absorption with timothy itself removed reactivity, but the other allergens were not as efficient as timothy, and, in particular, serum absorbed with June grass still retained considerable activity. Orchard, perennial rye, sweet vernal, and meadow fescue were roughly comparable, while Bermuda was a less efficient immunosorbent for IgE antibodies reactive with solid-phase timothy cellulose. Finally, in the absorption experiments, testing the ability of Bermuda grass solid-phase allergen to react with the various absorbed samples of serum (Fig. 9, top left), almost all of the solid-phase allergens were quite efficient in removing antibodies reactive with Bermuda grass with the exceptions of timothy and sweet vernal.

DISCUSSION

These results with grass pollens indicated that meadow fescue, June, orchard, and perennial rye were remarkably similar in terms of their allergenic makeup when they were reacted with a pool of serums from individuals sensitive to northern grasses from the Minnesota area. Sweet vernal and Bermuda were antigenically deficient to these grasses and did not possess unique antigenic determinants as recognized by antibodies in our serum pool. Timothy grass, though antigenically deficient compared to the other grasses, contained unique determinants reactive with IgE antibodies; these antigenic determinants were not possessed by any of the other grasses that we tested.

Our results agree remarkably well with the studies of previous investigators. In particular, they confirm those of Marsh, Haddad and Campbell,⁴ who found that comparable skin test reactions were provoked by the principal antigens of rye grass (*Lolium perenne*), fescue grass (*Festuca elatior*), and orchard grass (*Dactylis glomerata*). These grasses all possessed representatives of the Group I, II, and III antigens initially found in perennial rye grass. They also found Group I, II, and III components in sweet vernal and timothy, but they concluded that the antigens from these pollens possessed less cross-reactivity with the group antigens of rye grass than did those of fescue, orchard, and velvet. Bermuda pollen did not contain any of the classified group antigens and did not display appreciable cross-reactivity with the other grass pollens. AgB¹³ was detected only in timothy pollen and may account in part for its antigenic distinc-

tiveness. Baer and associates¹⁴ found that the Group I antigens from rye and timothy did not cross-react sufficiently to permit standardization of these extracts on the basis of their Group I content. In contrast, the cross-reactivity among orchard, June, fescue, and sweet vernal Group I antigens was sufficiently strong to allow standardization of these extracts. Thus, our findings confirm the *in vivo* and *in vitro* data of other investigators concerning the chemical identification of the various cross-reacting antigens in grass pollen.

Knowledge of the patterns of cross-allergenicity has application for *in vitro* standardization of allergy extracts, diagnostic testings, and treatment of sensitive patients. A standardized solid-phase allergen prepared with any of the similarly reacting grasses, June, orchard, meadow fescue, and perennial rye, or a mixture of these four grasses reacting with a pool of IgE antibodies, could be utilized for measuring the potency of allergy extracts by RAST inhibition¹⁵ or in diagnosing grass sensitivity by the RAST technique.⁹ Because of the allergenic distinctiveness of timothy grass, another solid-phase antigen would be needed for standardization of extracts and diagnostic testing. Because neither sweet vernal nor Bermuda grasses are indigenous to our area and the serum pool we used in these experiments could have been deficient in specific reactivity with these allergens, we are reluctant to apply our results to the question of standardization of these grasses. However, it seems likely that because of the antigenic differences of sweet vernal and Bermuda, additional solid-phase allergens would be necessary for standardization and detection of sensitivity to these pollens.

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