

Cross allergenicity among grasses determined by tissue threshold changes

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Twenty-seven volunteers with grass-pollen allergy who had not received prior immunotherapy had titrated prick tests with fivefold dilutions of 10 grasses (timothy, Bermuda, smooth brome, grama, salt, quack, western wheat, Johnson. June, and Bahia) to a final dilution of 1:3,906,250 w/v. Ten subjects served as untreated control subjects. Seventeen subjects received immunotherapy with an extract containing equal parts of timothy and Bermuda. Twelve months later subjects had repeat titrated skin testing with reconstituted aliquots of the same lyophilized extract, at the same time of day, and at the same location on the back as in the initial testing period. Nine treated subjects and eight control subjects completed the study protocol. The skin test areas with all 10 grass extracts were significantly decreased in the treated group compared to the control group ($p < 0.01$), and there was no difference in the extent of the reduction among the 10 grasses by analysis of variance. We conclude that immunotherapy to timothy and Bermuda alone is capable of reducing to a similar degree the clinical sensitivity to all 10 grasses studied. The results of this study suggest that treatment with timothy and Bermuda alone may be sufficient in most grass-sensitive individuals. (J ALLERGY CLIN IMMUNOL 76:789-94, 1985.)

From the results of in vitro studies with the use of gel diffusion¹⁻³ and RAST inhibition⁴ and from in vivo work with skin testing and passive transfer desensitization,⁵⁻⁷ it has long been appreciated that extensive cross-reactivity exists among allergens found in timothy grass (*Phleum pratense*) and many common northern grasses. Bermuda grass (*Cynodon dactylon*), on the other hand, has consistently been demonstrated to be antigenically distinct from the common northern grasses.^{1, 2, 4, 5, 8} Recently Martin et al.⁹ have demonstrated by RAST inhibition that extracts of timothy or Bermuda grass pollens can neutralize most IgE antibodies produced to a group of common northern, western, and southern grasses. These findings suggest

that it may be possible to induce clinically significant responses to multiple grasses by immunotherapy with only timothy and Bermuda grass extracts. The current study was undertaken to test clinically the conclusions from these in vitro studies.

MATERIAL AND METHODS

Patient selection

Seventeen volunteers with grass-pollen allergy and 4+ positive prick skin tests to all of the grass extracts routinely tested in our clinic (June, timothy, Bermuda, brome, grama, and western wheat) were recruited from among patients whose attending physician had, independently from this study, elected to use immunotherapy in the treatment of their seasonal allergic rhinitis. These patients had 12 months of immunotherapy as described below and will subsequently be referred to as the treated group. Similarly, 10 grass-sensitive individuals whose attending physician had elected not to use immunotherapy in the treatment of their seasonal allergic rhinitis were recruited for an untreated control group. No patient in either group had been receiving immunotherapy previously. All patients were more than 18 years of age and gave informed consent before their participation in this study.

Skin testing

Each of the 27 study patients had titrated prick skin testing to 10 common American grasses (timothy, Bermuda, smooth brome, grama, salt, quack, western wheat, Johnson,

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TABLE I. Mean skin test areas for each grass and each patient group at the beginning and the end of the study

Grass	Skin test areas (mm ²)									
	Treated					Control				
	Before		After		Significant Before = After	Before		After		Significant Before = After
	x	SD	x	SD		x	SD	x	SD	
Timothy	618.2	641.9	130.0	70.6	<0.01	528	340.2	512.8	355.1	NS
June	544.5	157.5	66.8	35.3	<0.01	496.5	294.0	372.8	263.9	NS
Smooth brome	451.8	345.6	100.3	62.8	<0.01	383.3	188.2	394.9	269.5	NS
Western wheat	243.3	346.0	88.7	62.74	NS	348.8	207.2	462.0	443.3	NS
Quack	301.8	230.2	93.8	50.2	<0.01	289.4	182.7	347.6	286.3	NS
Johnson	435.0	392.3	84.0	36.9	<0.01	266.0	179.0	262.4	170.6	NS
Bahia	444.0	365.3	93.9	75.7	<0.01	296.4	190.0	337.8	239.2	NS
Grama	74.9	81.0	14.8	39.7	<0.01	123.7	184.8	151.4	242.6	NS
Salt	87.7	55.7	24.1	33.0	<0.01	146.0	73.9	146.4	125.1	NS
Bermuda	167.3	122.6	29.3	44.1	<0.01	172.8	138.5	165.1	167.1	NS
Histamine	137.3	57.2	178.8	50.6	NS	168.1	52.4	212.4	65.6	NS
Unrelated allergen	57.8 ± 42.9		44.7 ± 34.2		NS	ND		ND		

Significant changes during the course of the study in the mean areas are indicated
x = Mean; SD = standard deviation; NS = not significant; ND = not done.

June, and Bahia) at the onset of the study. Aqueous extracts for skin testing (Hollister-Stier Laboratories, Spokane, Wash., 99207) were lyophilized. Aliquots were reconstituted and diluted in 0.03% of human serum albumin in normal saline in fivefold dilutions from 1:50 w/v through 1:3,906,250 w/v. No dilutions were used for skin testing that were more than 4 days old. Patients were instructed not to take antihistamines during the 72 hours before the skin testing sessions. Placement of skin tests at a distance of 5 cm was ensured by the use of a template prepared for each patient. Additionally, the templates allowed the exact site of each skin test to be duplicated at the time of repeat testing. Outlines of the wheal and flare of all positive skin tests were traced with a felt-tip marker and then transferred with transparent tape to a permanent record. In addition to the 10 grasses, each patient in the treated group had similar titrated prick testing to an allergenically unrelated and untreated antigen to which he or she was also 4+ prick positive. All patients were skin tested with histamine and saline controls at each testing session. Because of the number of skin tests applied, two sessions were required to complete the initial skin testing. At the end of the 12-month study period, all patients who successfully completed the study were required to have repeated titrated skin testing with reconstituted aliquots of the same lyophilized extracts. Extract dilutions were of the same age, and testing was accomplished at the same time of the year, at the same time of the day, and on the same area of the back as was done during the initial testing sessions.

Transcription of skin test data

Areas of skin tests were measured by use of an Alvin compensating polar planimeter. Because of the difficulty in

tracing the areas of the flares, only the area of the wheals are reported. For each patient the total area of the pretreatment skin tests for each grass was obtained by measuring the areas of the positive skin tests to all dilutions of that grass and adding them together. Posttreatment total skin test areas were obtained in exactly the same manner. The number of dilutions used to calculate the pretreatment and posttreatment skin test areas was identical for any given patient and grass but varied from patient to patient and grass to grass. Similar procedures were used in measuring pretreatment and posttreatment skin test areas for the unrelated allergens used in the treated group.

Immunotherapy

Patients in the treated group had immunotherapy with a mixture of equal parts of alum-precipitated extracts of timothy (Center-Al timothy, 20,000 PNU/ml, lot 02604AP; Center Laboratories, Port Washington, N. Y.) and Bermuda (Center-Al Bermuda, 20,000 PNU/ml, lot 02704AP). Dilutions were made in buffered saline with phenol and with the strongest concentration containing a total of 20,000 PNU/ml. Patients were treated to a maximum maintenance dose of 0.5 ml of 20,000 PNU/ml or to the highest tolerated dose. No patient who could not tolerate the full strength (although not necessarily the full 0.5 ml) extract was included in the results of this study.

In March 1985 the same lots of alum-precipitated extracts used in this study (which had been maintained since manufacture in 1980 at 4° C) were tested for potency by RAST inhibition. A commercially available serum pool from non-immunized grass-allergic patients was used (Ventrex Laboratories, Portland, Me.). The reference extracts contained 100,000 allergy units per 1 ml (Pharmacia Diagnos-

TABLE II. Levels of specific IgE*

Grass	Treated (n = 9)				Significant Before = After	Control (n = 7)				Significant Before = After
	Before		After			Before		After		
	x	SD	x	SD		x	SD	x	SD	
Timothy	4571	1068	3974	905	$p < 0.05$	3965	987	4270	918	NS
Bermuda	2540	1152	2093	790	NS	2619	1384	2534	1250	NS
Bahia	3139	1023	3097	835	NS	2772	1007	3289	1041	$p < 0.05$
Grama	1183	359	1366	784	NS	1298	600	1361	840	NS
Johnson	3051	1078	2858	822	NS	2768	942	3076	1128	NS
Western wheat	3974	1110	3208	883	$p < 0.01$	3417	1205	3423	723	NS

x = Mean; SD = standard deviation; NS = not significant.

*Counts per 2 minutes in blood obtained before and at the conclusion of the study in the two groups. Paired specimens were tested on the same day by use of the same reagents.

tics, Piscataway, N. J.). The Center-AI timothy, 20,000 PNU/ml, was 13% as potent as the 100,000 allergy unit per milliliter of timothy, and the Center-AI Bermuda, 20,000 PNU/ml, was 7% as potent as the 100,000 allergy unit per milliliter of Bermuda control. Given the estimated loss of 30% of potency in 18 months for Center-AI pollen extracts under these conditions of storage, the data suggest that the extracts were between two and three times this potent when the study was performed in 1980 to 1981.

Blocking antibody and specific IgE

Serum was collected initially and at the close of the 12-month study period. Specimens were stored at -70° until determinations of blocking antibody and specific IgE could be performed.

IgG (blocking) antibody levels were determined by the method described by Sobotka et al.¹⁰ Rye group I antigen (National Institutes of Health, Bethesda, Md.) was labeled with ¹²⁵I. Serial dilutions of patient and control sera were reacted with ¹²⁵I rye group I antigen. Bound ¹²⁵I rye group I antigen was precipitated with goat antihuman IgG (Antibodies, Inc., Davis, Calif.). The serum titer was the reciprocal of that dilution binding 20% of the total rye group I antigen added. Specific IgE levels for six of the grasses (timothy, Bermuda, Bahia, grama, western wheat, and Johnson) were measured by the radioallergosorbent method.¹¹ Cellulose discs were activated by cyanogen bromide and sensitized by exposure to a 1:10 w/v dilution of the respective grasses. Monospecific IgE was obtained from Pharmacia Diagnostics.

Statistics

Comparisons of the initial and final total skin test areas were performed by use of the Student's t test for paired samples. Data were expressed as a percent change from baseline values. Comparisons of the mean change in skin test area between the treated and control groups for each grass during the study period expressed as percent change from baseline were performed by use of the Student's t test for means. Data for all treated patients and for nine of the 10 grasses (June excluded because several patients were not

tested to this grass) were compared by use of a two-way analysis of variance to detect any difference in the degree of skin test suppression that might be present after treatment.

RESULTS

Nine treated subjects and eight untreated control subjects completed the study protocol. Of the 17 treated subjects, six had moved, one could not tolerate the 20,000 PNU/ml concentration as a result of local reactions, and one patient declined to return for repeat skin testing. One patient in the control group had moved during the course of the study.

Initial skin test areas for grasses and histamine did not differ significantly between the treated and control groups (Table I). There were no significant differences in pretreatment levels of blocking antibody, and specific IgE levels were similar in the two groups (Table II). Posttreatment histamine skin test areas were not different from pretreatment values in either group (Table I). Skin test areas of the untreated-unrelated antigens used in the treated group did not change significantly during the course of the study period (Table I).

Post-treatment skin test areas in control subjects for all the grasses and histamine in both study groups are illustrated in Table I. A comparison of the percent change from baseline for each of the grasses in both study groups is presented in Fig. 1. A significant decrease from baseline ($p < 0.01$) in the posttreatment skin test areas was noted for all grasses except for western wheat in the patients receiving active treatment. The change in the mean skin test area to western wheat in the treated group, however, was significantly decreased in comparison to that of the untreated control group ($p < 0.01$).

Levels of blocking antibodies were significantly increased after treatment in the treated group ($p < 0.05$) but were unchanged in the untreated control group.

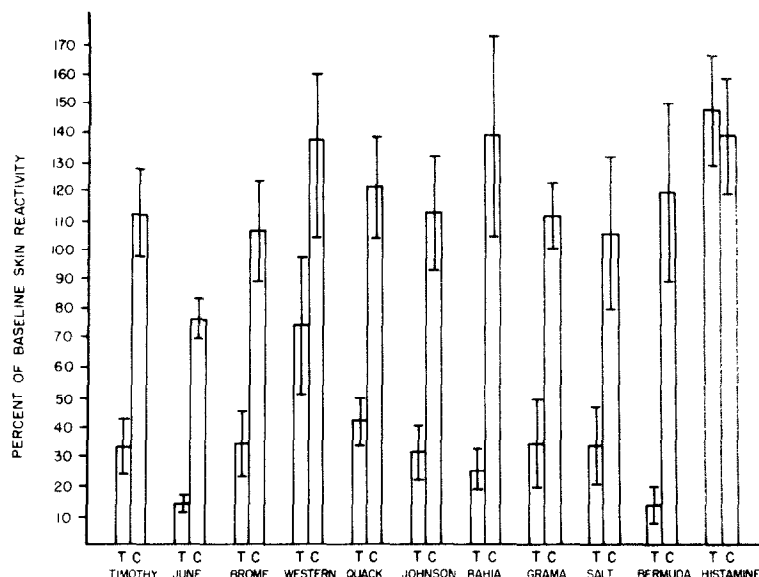


FIG 1. Change in skin test reactivity to grasses is depicted for treated (T) and control (C) patients. The results are expressed as the total area of skin tests after 1 year of treatment divided by the total area of baseline skin tests to the same dilutions of extract.

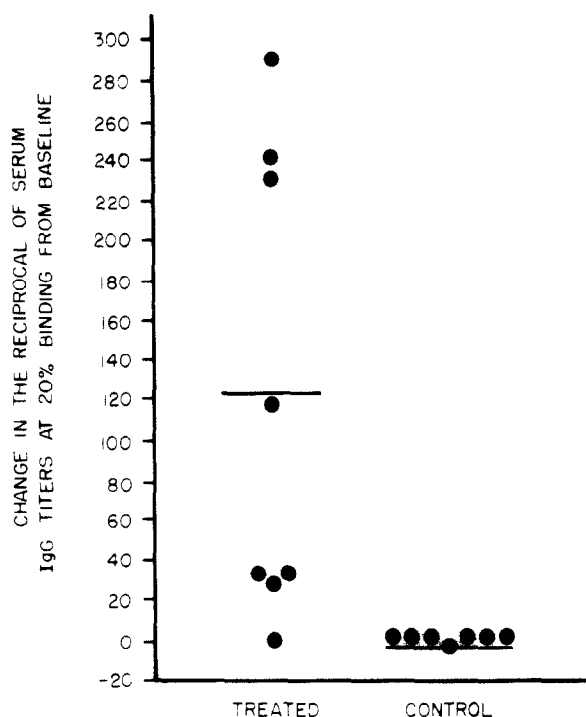


FIG 2. Changes in individual titers of IgG antibodies to rye group I antigen during the 12-month study period in both treated and untreated control groups.

Results of the blocking antibody determinations in eight treated patients and seven control patients are illustrated in Fig. 2. (One specimen from one member of each group was not available.)

The one patient in the treated group with no rise in blocking antibody had less than 20% binding at 1:20

serum dilution both before and after immunotherapy. The patient also had only a 3% reduction in skin test areas to the four grasses that would be most cross-reactive with the group I antigen used for assessing blocking antibody. In comparison, the patient had an 85% reduction in skin test areas to the grasses cross-reacting with Bermuda.

To detect any significant differences in the degree of suppression of skin test reactivity among the various grasses in the treated group, a two-way analysis of variance was performed. No significant difference could be detected.

Comparison of changes in specific IgE levels during the course of the study (Table II) revealed an increase in the mean antibody level for all six grasses in the control population. This change was significant ($p < 0.05$) only for Bahia. In the treated group the mean level of specific IgE declined for four of the six grasses, significantly for timothy ($p < 0.05$) and western wheat ($p < 0.01$). The change in specific IgE for the six grasses in the treated subjects was compared by analysis of variance. There was no significant difference in the changes in specific IgE toward the different grasses.

DISCUSSION

Nine patients sensitive to multiple grasses had immunotherapy to timothy and Bermuda grass extracts. Significant depression of skin test reactivity was noted in the treated group for all of the grasses tested when these were compared to the control group. Since skin reactivity to histamine and an unrelated allergen extract did not change in the treated group nor did grass

skin tests in the control group change during the course of the study, the changes in skin test reactivity noted in the treated group cannot be ascribed to nonspecific factors. Despite the fact that treatment was with timothy and Bermuda alone, the reactivity of all the grasses analyzed was found to be depressed to a similar degree. Specific IgE declined during treatment for four of six grasses in the treated group compared to a rise in specific IgE for all six grasses in the control group. The reduction in specific IgE was significant only for timothy and western wheat, but overall there was no significant difference in the degree of reduction of specific IgE for the six grasses tested. As in previous studies^{12,13} in which decline of skin reactivity has been observed early in the course of allergy immunotherapy, it was not believed that the reduction in titrated prick tests could be accounted for by the decline in specific IgE.

For many years there has been controversy concerning the specificity of grass immunotherapy. As early as 1911 Freeman¹⁴ suggested that extracts of timothy grass alone would suffice for diagnosis and treatment of grass allergy in Great Britain. In 1916 Cooke and Vander Veer¹⁵ skin tested several hundred patients to multiple northern grasses and found total cross-reactivity among the grasses tested. At that time they recommended that treatment in grass-sensitive patients be accomplished with timothy extracts alone. In 1932 Stull et al.⁷ demonstrated that multiple northern grass-pollen extracts were identical in their neutralizing and desensitizing capacity. Other investigators such as Feinberg³ have pointed to the antigenic diversity that exists even among such closely related groups as the northern grasses and called for a more specific approach to immunotherapy.

Recently RAST inhibition techniques have been used in the study of cross allergenicity among grasses. With the use of this method, Martin et al.⁹ studied five common western grasses (smooth brome, grama, western wheat, quack, and salt), five common northern grasses (June, rye, redtop, meadow fescue, and sweet vernal), and the three southern grasses (Bahia, Johnson, and Bermuda) for their immunologic relationship to each other. Brome, quack, and western wheat grasses were found to neutralize a similar set of IgE antibodies as did the common northern grasses. Salt and grama grasses were found to be closely related to Bermuda grass. Johnson grass was allergenically related to both the northern grasses and to Bermuda grass. Bahia grass, although not as closely related to any of the other grasses as were the grasses mentioned above, demonstrated cross allergenicity with both timothy and Bermuda. Their findings suggested that a combination of timothy and Bermuda grass could be expected to neutralize most IgE anti-

bodies produced to the grasses tested in their study. A possible exception would be Bahia grass. If their findings are correct, it would be expected that clinically significant suppression of in vivo sensitivity to multiple grasses could be accomplished by immunotherapy with extracts of timothy and Bermuda grass alone. Again, Bahia grass might be expected to be an exception to this prediction.

Our findings confirm these predictions completely. Additionally, the possible uniqueness of Bahia grass suggested by in vitro studies was not borne out. We conclude that immunotherapy with timothy and Bermuda grass alone should be sufficient in many grass-sensitive patients. Our results do not preclude the possibility that some patients may be sensitive to relatively minor but unique antigens in one of the grasses and require specific therapy with that grass extract. Since multiple grasses are available as aqueous extracts, one could question the advantage of treating with only timothy and Bermuda grasses. One advantage with this treatment strategy is that it does not require a knowledge of the relative contribution of each species of grass to the local airborne pollen. Furthermore, as new modified antigen preparations become available, the practical advantage of being able to treat grass allergy with only two grass-pollen extracts increases.

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The diversity of reaginic immune responses to platinum and palladium metallic salts

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*As part of a National Institute for Occupational Safety and Health health hazards evaluation, workers employed in a precious metal refinery exposed to platinum (Pt), palladium (Pd), and other group VIII metallic salts were evaluated for direct skin test sensitivity to Pt. Current (107) and former (30) workers who quit or were discharged because of Pt-related health problems were prick tested with ammonium hexachloroplatinate ($[\text{NH}_4]_2\text{PtCl}_6$). Of the 107 currently exposed workers, 15 (14%) exhibited positive skin tests, as indexed by immediate reactivity at a dose of 10^{-4} gm/ml or less. Eight (27%) of the 30 former workers no longer exposed to Pt also demonstrated positive Pt skin tests. Sera obtained from the workers were assessed for transferable antibodies to Pt and Pd salts by monkey passive cutaneous anaphylaxis. In addition, Pt-specific antibodies were evaluated by RAST. Results of these studies suggested that short- and long-term passive cutaneous anaphylaxis immune responses occur after exposure to both Pt and Pd compounds. Results of RAST analysis for Pt-specific antibodies indicated that significantly higher ($p < 0.001$) levels were present in the sera of skin test-positive workers as compared to control sera from Pt-exposed, skin test-negative workers or nonexposed control subjects. Evidence was also obtained that Pt or Pt-protein adducts present in the sera of exposed workers may compete for IgE-binding sites in the RAST assay. The specificity of the Pt-specific RAST system was proved by inhibition experiments. (*J ALLERGY CLIN IMMUNOL* 76:794-802, 1985.)*

Refining of the Pt group precious metals (Pt, Pd, iridium, rhodium, ruthenium, and osmium) is accomplished by sequential solubilization and precipitation

in order to obtain purified metal salts. These metal salts may be further processed to yield the respective elemental metals. Because of the nature of this process, workers employed in precious metal refineries

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