

Original articles

Skin reactivity to purified pollen allergens in highly ragweed-sensitive individuals

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Five hundred twenty-five atopic patients were skin tested over a 12-mo period to several crude allergens with a puncture skin-testing technique utilizing a bifurcated needle originally developed for smallpox immunization. Of these, 122 were highly allergic to short ragweed pollen and were subsequently tested with a series of purified grass and ragweed allergens. Data of their skin reactivity to these allergens is presented, and the phenomenon that each patient has a unique "allergic fingerprint" to purified pollen allergens is shown. The puncture technique showed good correlation with quantitative intradermal skin titration and offered a definite advantage, because many allergens could be rapidly and accurately assayed with good patient compliance.

In recent years several advances have been made in understanding human immune response to inhaled antigens. One of the better tools used in these investigations¹⁻⁴ has been highly purified pollen allergens isolated from grass and short ragweed pollen extracts. These allergens include rye grass groups I to IV,^{2, 3, 5-7} timothy grass antigen B (AgB),⁸ and short ragweed antigens E (AgE),⁹ Ra3,¹⁰ BPA-R (Ra4),^{11, 12} and Ra5.^{13, 14} Sensitivity to these allergens has usually

been determined quantitatively by intradermal skin titration^{1, 9} and leukocyte histamine release,^{15, 16} both of which are tedious procedures. However, they have proved to be the best available methods to date in determining sensitivity to purified pollen allergens. By quantitative intradermal skin titration with eight different allergens (all the above except Ra4), each atopic individual has been found to possess a characteristic "allergic fingerprint" or "sensitivity spectrum";³ that is, atopic individuals sensitive to crude grass or ragweed extracts have very different patterns of skin reactivity to individual highly purified grass and ragweed allergens. This finding is supported by a number of other studies in which fewer or less pure allergens have been employed.^{1, 4, 5, 7, 9, 11, 17, 18}

This study presents data on skin sensitivity of a large group of unrelated highly atopic subjects to nine purified pollen allergens. The phenomenon of an allergic fingerprint toward the aforementioned allergens was further investigated. All subjects were skin tested using a bifurcated needle, and skin sensitivities to AgE and Ra5 were compared with quantitative intradermal titration to determine the precision and sensitivity of our puncture method in relation to quantitative intradermal testing.

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MATERIALS AND METHODS

Patients

Five hundred twenty-five patients were evaluated over a 12-mo period at Scott Air Force Base Allergy Clinic. All patients were active-duty and retired members of the U.S. Armed Forces and their dependents who had been referred to the allergy clinic by their pediatrician, internist, or general practitioner at the base. All were skin tested by our puncture method (see Skin testing) using crude pollen extracts. Of this group 122 (55 males and 67 females, aged 6 to 45 yr) found to be highly sensitive to ragweed were used for the present study. All had allergy symptoms ranging from mild to severe during the period of ragweed pollination, and all had appreciable skin reactions (5- to 24-mm mean wheal diameter with erythema) to crude short ragweed extract (1:20 w/v) with the puncture tests. Almost all subjects had mild to severe symptoms during grass pollen season, and there was a wide range in relative symptomatology to "ragweed" and "grass." Twenty nonallergic subjects of similar ages and sex served as controls.

Extracts

All 122 subjects were tested with the following crude pollen extracts (1:20 w/v in buffered solution containing 50% w/v glycerine) purchased from Greer Laboratories: short ragweed (*Ambrosia elatior*), timothy (*Phleum pratense*), Bermuda grass (*Cynodon dactylon*), June Kentucky bluegrass (*Poa pratensis*), Johnson grass (*Holcus halepensis*), orchard grass (*Dactylis glomerata*), and perennial rye grass (*Lolium perenne*). The same lot numbers of all crude extracts were used throughout the 12 mo the study was in progress. All extracts were stored at 4° C when not in use.

Further tests were performed with the following purified pollen allergens: rye groups I, II, III, and IV,^{2, 3, 5-7} timothy AgB⁸ (prepared in one of our laboratories), AgE described by King et al.⁷ (supplied by the Research Resources Branch of the National Institute of Allergy and Infectious Diseases through Dr. John Nutter and further purified by Sephadex G75 gel filtration), Ra3, BPA-R (Ra4), and Ra5.⁸⁻¹¹ With the exception of rye group IV and AgB, allergens were utilized in a highly purified form according to electrophoretic and immunologic analysis presented in detail elsewhere.^{1-6, 9-13} All allergen solutions were prepared in Tris-buffered physiologic saline at pH 7.7 containing 0.03% (w/v) human serum albumin as a stabilizer.^{1, 9}

Skin testing

Puncture technique. Approximately 0.02 ml of each antigen solution (crude and purified) was placed on the skin of the forearm, which had been cleansed with 70% isopropyl alcohol and allowed to dry. A light puncture (so as not to draw blood) was made at a 90° angle to the skin with a bifurcated needle supplied by Wyeth Laboratories. A new needle was used for each patient and then discarded after testing was completed. This needle was developed for use in vaccination against smallpox in the recent worldwide smallpox eradication program.¹⁹ The design of the needle was such that a reproducible small amount of vaccine could

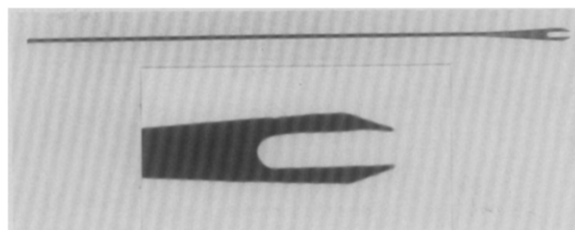


FIG. 1. Photographs of the bifurcated needle (Wyeth Labs) and of the tip (enlarged). Length of needle, 6.6 cm; distance between points, 0.7 mm.

be injected with relatively little training (Fig. 1).

In order to eliminate antigen "carryover," care was taken to wipe the needle carefully between each puncture using a cotton pledget impregnated with 70% isopropyl alcohol. As a further check saline controls were included after application of several of the antigens. Reactions were read at 15 min using a millimeter rule, and the results were recorded as the average of measurements taken in four diameters. Mechanical trauma was not a notable problem when the puncture was properly applied to any nondermographic patient.

We made a detailed examination of variation in the magnitude of puncture skin test reactions arising from possible systematic differences in tests performed on the right versus the left arm, reactivity of different sites of the forearm, measurements made by different readers of reactions induced by the same tester, and the size of reactions induced by different testers (with the same reader) (Table I).

Quantitative intradermal skin titration. Ragweed allergens AgE and Ra5 at concentrations of 10^{-1} μ g/ml were serially diluted 10-fold to 10^{-6} μ g/ml. The skin of the forearm was utilized, and 0.05 ml extract was injected intradermally with a disposable 26-gauge needle on a 1-ml tuberculin syringe. Wheal responses were measured after 15 min. Titration was continued with increasing allergen concentrations until "2-plus" reactions (9- to 10-mm mean wheal diameter with erythema)¹ were obtained. Where necessary the allergen concentrations producing 2-plus reactions were interpolated on a logarithmic scale from concentrations that produced greater and lesser reactions.

Comparative procedure. To compare the results of the puncture method with quantitative intradermal skin titration, 25 patients sensitive to AgE or Ra5 or both were studied by both methods. The forearms of the patients were used, avoiding the uppermost part, and both the intradermal and puncture tests were performed by a single tester (R. L. P.).

RESULTS

Of our total Scott Air Force Base study group (122 subjects), selected on the basis of moderate to high sensitivity (5- to 24-mm wheal diameter by puncture test) to crude short ragweed extract, 118 (97%) showed sensitivity in the same range to all crude grass extracts tested. False-positives did not appear to be a

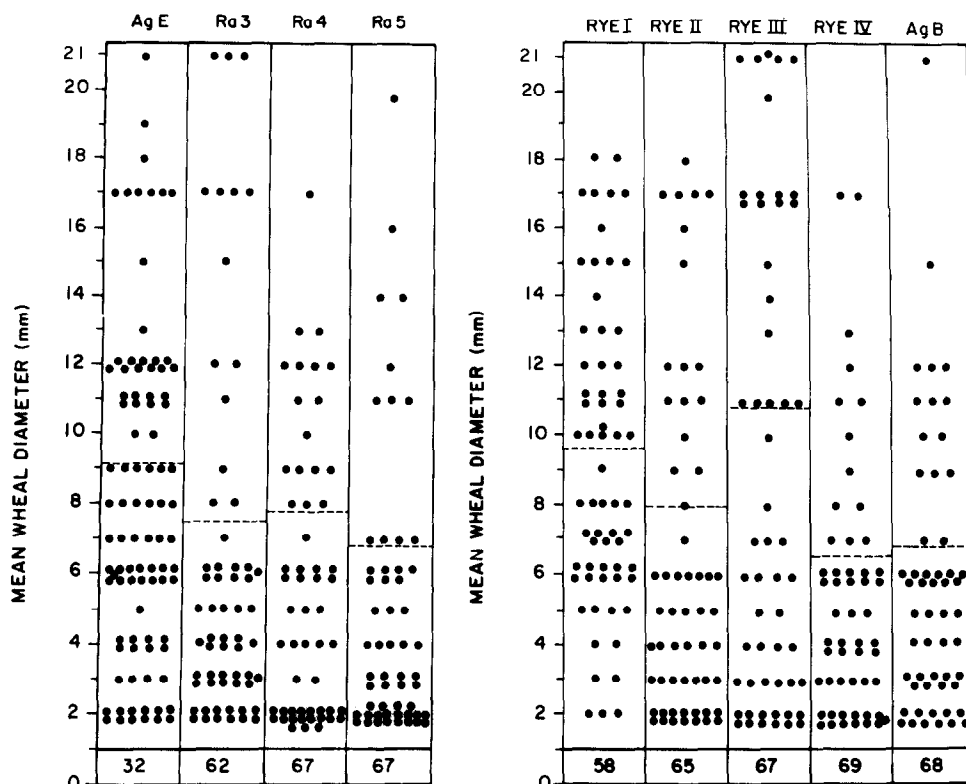


FIG. 2. Scattergrams of wheal reactions in 122 patients using the puncture test with allergens AgE, Ra3, Ra4, and Ra5 of short ragweed rye grass, groups I to IV, and AgB of timothy grass. Allergen concentrations were 30 $\mu\text{g}/\text{ml}$. Dashed lines, mean wheal diameters for people giving significant response (≥ 3 -mm wheal diameter) to the respective allergens. The numbers of patients having reactions of 1-mm wheal or less are given at the bottom of each scattergram.

TABLE I. Analysis of concordance of puncture skin test reactions²⁰

	Number of paired comparisons (N)	Slope of regression line (b)	Regression coefficient (r^*)	Significance of difference of slope from a value of 1
<i>Experiment 1</i>				
Right arm vs left arm				
Reader J. S.	36	0.991	0.993	$0.9 > p > 0.8$
Reader R. L. P.	36	0.988	0.994	$0.6 > p > 0.5$
Reader J. S. vs Reader R. L. P.				
Comparison of all reactions	72	0.992	0.998	$0.7 > p > 0.6$
Site on upper vs lower arm				
Reader J. S.	24	0.919	0.971	$0.2 > p > 0.1$
Reader R. L. P.	24	0.906	0.978	$0.05 > p > 0.025$
Site on upper vs middle arm				
Reader J. S.	24	0.934	0.987	$0.1 > p > 0.05$
Reader R. L. P.	24	0.917	0.987	$0.02 > p > 0.01$
Site on middle vs lower arm				
Reader J. S.	24	0.986	0.989	$0.7 > p > 0.6$
Reader R. L. P.	24	0.990	0.993	$0.7 > p > 0.6$
<i>Experiment 2</i>				
Tester R. L. P. vs Tester J. S.				
Comparison at all sites	18	1.162	0.982	$0.02 > p > 0.01$

*All r values are significant with $p < 0.001$.

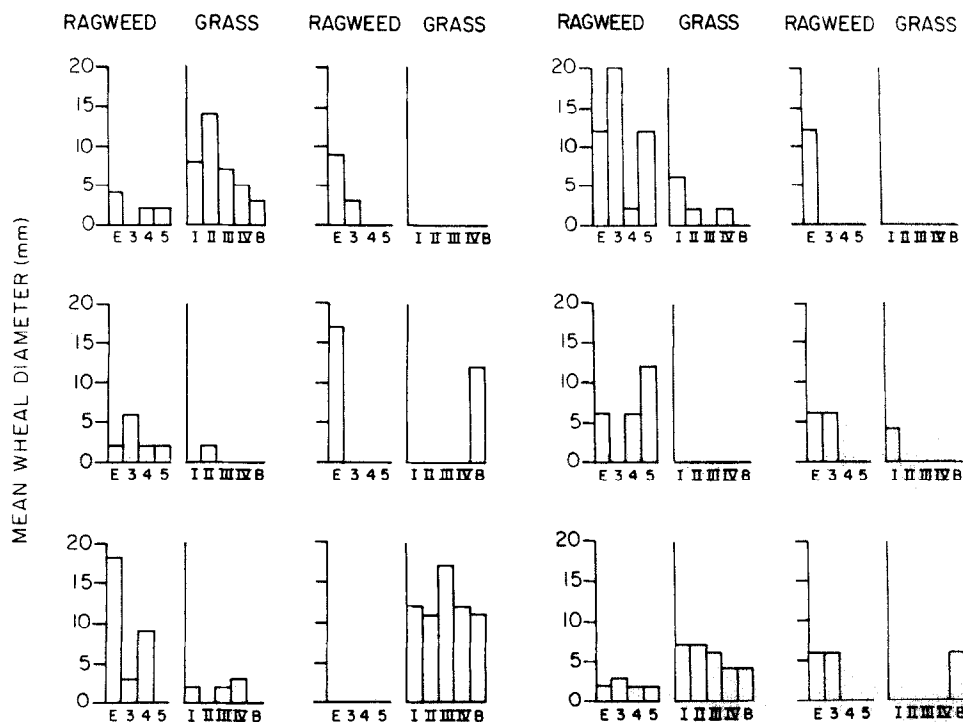


FIG. 3. "Allergic fingerprints" with the four purified ragweed and five purified grass pollen allergens in 12 representative patients.

problem with the puncture technique, because 20 nonatopic controls were all negative 15 and 30 min after testing with the crude and purified pollen allergens.

Reactions of all of our patients to the nine purified pollen allergens varied from 0 to 21-mm wheal diameter with erythema, with the distribution as shown in Fig. 2. Reactions of 2-mm diameter were of questionable significance relative to the control in some subjects, and reactions of 1-mm diameter could not be differentiated from the control. The broken lines represent arithmetic means of the reactions in patients having clearly positive reactions of 3 mm or greater to the respective allergens. The proportions of such positive responders, together with the frequencies of response relative to AgE or to rye I, are shown in Table II.

Fig. 3 illustrates the allergic fingerprints obtained with the purified pollen antigens on 12 patients who are representative of our study group. Although these patients reacted quite similarly to the crude grass and ragweed pollen antigens, each had a characteristic pattern of reactions to the purified antigens.

The puncture method using a single antigen concentration (30 $\mu\text{g}/\text{ml}$) was compared with the 2-plus end point obtained by quantitative intradermal skin test titration at a series of antigen concentrations in 25

TABLE II. Patient responsiveness to purified allergens

Antigen	Percent positives*	Percent relative to AgE or rye I†
<i>Ragweed</i>		
AgE	63.9	100.0
Ra3	39.3	61.5
Ra4	29.5	46.2
Ra5	28.7	44.9
<i>Grass</i>		
Rye I	50.0	100.0
Rye II	35.2	70.5
Rye III	35.2	70.5
Rye IV	32.8	65.6
AgB	35.2	70.5

*People with reactions of ≥ 3 -mm wheal diameter on puncture testing to the antigen (30 $\mu\text{g}/\text{ml}$) divided by total number of people tested.

†Percent of people reacting to each allergen (≥ 3 -mm wheal) relative to those reacting to AgE (in the case of ragweed allergens) and to rye I (in the case of grass allergens).

patients (Fig. 4). Of these patients all were sensitive to AgE and 12 were sensitive to Ra5. Linear regression analysis of mean wheal diameter in the puncture method versus $\log_{10}$ (antigen concentration giving a 2-plus end point) in the intradermal titration proce-

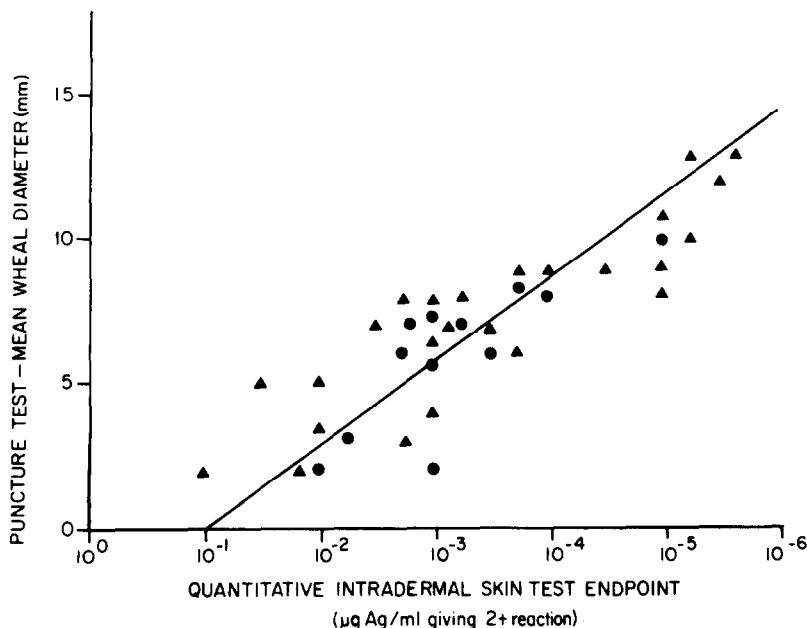


FIG. 4. Comparison of mean wheal diameter from puncture testing (using antigen concentrations 30 $\mu\text{g/ml}$) versus "2-plus" (8- to 10-mm wheal diameter) end point by intradermal titration. Twenty-five ragweed-sensitive patients were tested with AgE (Δ), of which 12 were sensitive to Ra5 ($\bullet$). The regression line is for the pooled data, where $r = 0.86$, $p < 0.001$. For AgE alone, $r = 0.89$, $p < 0.001$; for Ra5 alone, $r = 0.81$, $p < 0.01$.

ture showed that the two methods were significantly correlated. In the comparison of AgE data $r = 0.89$, $p < 0.001$; in the case of the Ra5 data $r = 0.81$, $p < 0.01$. For the comparison of pooled AgE plus Ra5 data $r = 0.86$, $p < 0.001$ (regression line shown in Fig. 2). Overall, the mean error ($1-r^2$) was $\pm 26\%$ for puncture tests in the range of 2- to 13-mm wheal diameter.

We made a detailed examination of variation in the magnitude of puncture skin test reactions arising from possible systematic differences in tests performed on the right versus the left arm, reactivity of different sites of the forearm, measurements made by different readers of reactions induced by the same tester, and the size of reactions induced by different testers (with the same reader). In experiment 1 arm-to-arm, site-to-site, and reader-to-reader variations were investigated in eight ragweed-sensitive and four grass-sensitive subjects utilizing a single tester and two readers. The tester-reader and the additional reader were the same individuals (J. S. and R. L. P.) involved in the main part of the study. Ragweed or grass extract (glycerinated, 1:20 w/v) was applied to three sites on each patient's forearm (upper, middle, and lower), and the resultant reactions were measured independently by the two readers in the manner described in the Methods section.

In a second experiment the tester-to-tester variation

arising from possible differences in the amounts of allergen solution injected by the puncture test was investigated in six ragweed-grass-sensitive patients tested with ragweed and grass extracts on three different sites on the left forearm by one tester (J. S.) and independently in complementary sites on the right forearm by the other tester (R. L. P.). The resultant reactions were read *independently by a single reader* (R. L. P.). The concordance between the various data sets was analyzed by linear regression analysis.²⁰ To test whether there might be significant consistent differences between the data (e.g., one reader versus the other), we examined the slopes of the regression lines to determine whether they were significantly different from a value of 1 by appropriate t test analysis (two-tailed).²⁰

To evaluate possible carryover effect of the puncture technique, 10 randomly selected study subjects were repunctured in an adjacent site with a clean needle using saline control. There was no observable skin reactivity in any of the repunctured sites when the corresponding puncture sites showed normal reaction.

DISCUSSION

We were surprised to find that 97% of our ragweed-sensitive patients were skin reactive to the crude grass extracts. This rather high figure is prob-

ably a reflection of both the high concentrations of crude extracts (see below) and of the varied history of exposure of our patient group to the pollens of many different grass species. With the exception of Ra5, the prevalences of positive responsiveness to the various highly purified allergens (Table II) are much lower than previously reported in ragweed- and grass-allergic subjects.² We believe that the principal reason for this is the different criteria for selecting the allergic subjects. Studies in Baltimore have utilized exquisitely ragweed-sensitive subjects. In the present study subjects were selected on the basis of a significantly positive (≥ 3 -mm mean wheal diameter) response to a puncture test using glycerinated pollen extracts at high (1:20 w/v) concentrations. Glycerinated ragweed and rye extracts should contain (in addition to high concentrations of other allergens) about three to ten times as much AgE or rye I as was subsequently used in skin testing with the highly purified materials.* It is therefore not surprising to find a relatively low prevalence of significantly positive reactors to AgE and rye I at 30 $\mu\text{g/ml}$.

The people who responded with wheal diameters of 3 mm or more by puncture test correspond to individuals having intradermal end points at 10^{-2} $\mu\text{g/ml}$ or lower (Fig. 2), which is the range of sensitivity previously studied in Baltimore subjects. It would therefore be expected that the prevalence of response to minor ragweed allergens relative to AgE and of minor rye allergens relative to rye I would be similar to the Baltimore data. This proved generally to be the case (Table II). The proportion of subjects sensitive to Ra3 relative to AgE (61.5%) is almost the same as that recently found in a study of 126 white subjects in Baltimore (60.3%).²⁴ Also the rye II:rye I and rye III:rye I proportions found in the present study are similar to the values of 66% and 70% previously reported.² However, the prevalence of Ra5 sensitivity relative to AgE (45%) is much higher than the value of 22% reported previously.⁴ This probably reflects an initial bias in the present study toward collecting Ra5-sensitive subjects for a concomitant genetic study.^{4, 21} No previous data are available on Ra4, and previous data on rye group IV and AgB² are not directly comparable, because different partially purified materials were used in the present study.

In a recent investigation of the question of allergic uniqueness, Marsh³ found that highly atopic indi-

viduals have quite different patterns of skin sensitivity to rye groups I, II, III, and IV, to timothy AgB, and to ragweed allergens AgE, Ra3, and Ra5. Our study confirms the observation that each allergic subject has his own allergic fingerprint to these purified allergens and to ragweed allergen Ra4.

In past quantitative studies of skin sensitivity to purified pollen antigens the procedures used have hindered convenient observation of large patient populations. While quantitative intradermal skin titration is widely recognized as the most precise method of allergy skin testing, it is limited in the number of antigens that can be used at a given time, because of the large skin area required for several titration dilutions. Also the procedure is tedious and drawn out, factors that render it difficult to obtain patient cooperation. The leukocyte histamine-release assay is an excellent *in vitro* test requiring minimal patient cooperation.¹ However, the assay requires skilled researchers to obtain consistently accurate and definitive results^{15, 16} and, like intradermal skin titration, is tedious and limited in the number of antigens that can be tested at a given time. It therefore does not lend itself to widespread use in a clinical setting.

Norman et al.^{22, 23} have demonstrated a good correlation between patients' allergic sensitivities in terms of clinical symptom score, quantitative intradermal skin sensitivity, and leukocyte histamine-release sensitivity. The establishment of these correlations has reassured the clinician of the value of quantitative intradermal skin titration and has thus encouraged its use as an acceptable clinical tool despite the aforementioned disadvantages.

Routine scratch and certain prick techniques are usually considered to be more qualitative than quantitative; one exception was the recent study of Belin and Norman,²⁴ who demonstrated that titration with a series of antigen dilutions by conventional prick test (using 26-gauge needles) correlated well with intradermal skin titration. In two separate comparisons of these methods, in patients sensitive to enzymes used in the detergent industry, they obtained correlation coefficients of r of 0.84 and 0.85. We have shown that the puncture test at a *single* antigen concentration is a good semiquantitative technique that correlates with quantitative intradermal skin titration just as well ($r = 0.86$) as the more laborious prick test titration. The main advantage of our method is that it lends itself to the rapid assay of large numbers of allergens at a time. We were able to screen large patient populations easily and observe their reactivity to purified pollen allergens used in the study.

In conclusion, data on a large group of ragweed-sensitive individuals show their sensitivity to nine dif-

*This statement is based on many studies of ragweed and rye extracts in our laboratories. Regrettably, the extracts used in this experiment were discarded in error before AgE and group I contents could be measured.

ferent purified pollen allergens, illustrating an earlier observation of allergic uniqueness:

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