

Controlled evaluation of allergoid in the immunotherapy of ragweed hay fever

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Immunotherapy with ragweed allergoid administered in a clustered regimen, placebos in a clustered regimen, and unaltered ragweed extract (allergen) in a weekly regimen were compared in three groups of hay fever patients carefully matched for ragweed sensitivity. The allergoid and placebo comparison was performed double-blind and the unaltered ragweed extract comparison was single-blind. In terms of antigen E (AgE) equivalents, doses were about 50 times higher in the allergoid-treated group than in the allergen-treated group. Whereas there was no immunologic response to placebos, the allergoid regimen produced a more rapid serum IgG antibody response, with significantly higher posttreatment levels than those in the allergen regimen. Initial IgE antibody rises and subsequent slow declines were similar. Symptom-medication scores were similar in the two specifically treated groups and significantly less than scores reported by the placebo group ($p < 0.01$). Due to an overestimate of the initial allergoid doses, systemic reactions occurred mostly in the early visits with allergoid treatment, whereas systemic reactions appeared late in the allergen treatment. The overall incidence was similar. In a second year, after 8 mo of no injections, a preseasonal booster regimen with both materials produced virtually no untoward reactions. During the period of no injections, IgG antibody declines were modest and, after boosters, IgG antibody levels rose promptly. Clinical results in both groups were again excellent, with an advantage for allergoid. (J ALLERGY CLIN IMMUNOL 70:248, 1982.)

Earlier studies of immunotherapy with allergoid of ragweed in comparison with unaltered ragweed extract (allergen) showed that allergoid, like allergen, could produce a substantial rise in antiallergen IgG antibodies.¹ Furthermore, after an initial rise in antiallergen IgE antibodies, both types of treatment led to a decline in IgE antibodies after prolonged therapy. In a regimen of weekly injections the rises of IgG antibody after allergoid treatment occurred more rapidly and rose to significantly higher levels in comparison with allergen treatment. Clinical relief of symptoms, as

assessed by daily diaries, was better with allergoid, at a level that was significant in 3 out of 6 yr of study.¹ The significant differences were seen when virtually all the treated patients had attained high antigen maintenance doses. At the same time allergoid had a much reduced potential for producing allergic side reactions. Our ability to administer allergoid in doses almost 50 times greater in terms of protein than allergen without an increase in the incidence of local and systemic allergic side effects suggested that it might be possible to give adequate immunizing doses with many fewer visits to the office or clinic and thus reduce the potential for undesired side effects as well as increase the convenience and reduce the cost of treatment.¹

In 1977 a preliminary study of immunization with allergoid showed good IgG antibody responses to ragweed antigens AgE, Ra3, Ra5, and Ra6 after a "clustered" regimen of only five visits to the clinic, each visit usually consisting of two or three injections followed by a wait of 2 to 5 wk for maximum IgG "blocking" antibody responses to develop before giving the next cluster in the series.² When allergen was given in a similar regimen, the rate of allergic reac-

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Abbreviations used

AgE:	Antigen E
PNU:	Protein nitrogen unit
RAST:	Radioallergosorbent test

tions was unacceptably higher and immunologic responses less rapid. Although clinical results with the clustered regimen of allergoid appeared acceptable, the smallness of the groups made it impossible to assess accurately the relative therapeutic merits of the allergoid in the shortened regimen.²

Accordingly, we report here a study organized in 1978 to carry out a double-blind comparison of a clustered regimen of allergoid vs placebo. We also desired a comparison with a more usual program of injections of unaltered extract given at weekly intervals. The differences in the regimens made a double-blind comparison between allergen and allergoid impractical, but all the patients were informed that they might receive allergoid, allergen, or placebo, thus making the comparisons single-blind for allergen.

After a result favorable for the clustered regimen in 1978, we carried out an open trial in 1979 in which a shortened preseasonal booster regimen was administered and immunologic and clinical responses were compared in the remaining allergen and allergoid-treated patients.

MATERIALS AND METHODS

Immunization materials

The allergen used for the 1978 and 1979 studies was a 1/20 w/v 50% glycerinated extract of short ragweed pollen prepared by Greer Laboratories, Lenoir, N. C. In brief, the pollen was extracted at 0° to 5° C with Coca's solution at pH 8.0 at a pollen concentration of 1/5 (w/v) for 23 hr and re-extracted at 1/5 (w/v) for 22 hr. The two extracts were combined and an equal volume of glycerine was added. This material was stored at 4° C until used. Two different allergoids, used in the 1978 and 1979 studies, were prepared by Greer Laboratories from the same lot of pollen by formaldehyde modification of dialyzed aqueous allergen preparations similar to that described elsewhere (Prep. E).³ For the first allergoid preparation, dialyzed ragweed allergen (10 mg/ml) was treated with 2M HCHO for 15 days at 10° C in 0.1M phosphate buffer at pH 7.5, diluted fourfold with phosphate buffer, and incubated for 20 days at 32° C. The respective incubation periods for the second allergoid were 14 days at 10° C and 26 days at 32° C. Excess formaldehyde and buffer salts were removed by extensive dialysis against distilled water and the resultant materials were sterile-filtered and lyophilized.

Analysis of the glycerinated allergen by radial immunodiffusion provided the following data: AgE, 176 ± 30 μ g/ml; Ra3, 40.4 ± 1.4 μ g/ml; Ra5, 20.8 ± 0.7 μ g/ml.

The relative proportions of the antigens (AgE:Ra3:Ra5) were 100:23:12. The allergen contained 239 PNU/ μ g AgE. Similar analyses of the dialyzed, lyophilized allergen preparation from which the 1978 allergoid was derived gave the following results: AgE, 37.0 ± 1.3 μ g/mg; Ra3, 5.1 ± 0.3 μ g/mg; Ra5, 2.19 ± 0.22 μ g/mg (ratios, 100:14:6). As previously noted,³ analysis of AgE, Ra3, and Ra5 could not be performed on the allergoid. Allergoid concentrations were normally expressed in terms of "AgE equivalents/ml" based on the AgE content of the allergen from which the allergoid was derived.¹ The resultant allergoid contained 175 PNU/ μ g AgE equivalent.

Prior to the 1979 study, reanalysis of the allergen used for immunization showed no significant loss of AgE on storage at 4° C. Analysis of the dialyzed allergen used to prepare the allergoid for the 1979 study revealed an AgE content of 32.9 ± 3.0 μ g/mg, a value not significantly different from that of the corresponding allergen used to prepare the 1978 allergoid. The 1979 allergoid contained 202 PNU/ μ g AgE equivalent. This value is not significantly different from that of the 1978 allergoid, since the precision of the PNU measurement is $\pm 20\%$ and the precision of the AgE measurement is $\pm 10\%$ for each allergoid.

For immunization purposes, all antigen concentrations were based on the AgE values cited above. In the case of the allergen, 1 unit contained 0.01 μ g AgE; for the allergoid, 1 unit contained 0.5 μ g AgE equivalent (i.e., 50-fold higher). The corresponding values were 2.4 and 88 PNU, respectively, for the allergen and allergoid used in the 1978 study, and 2.4 and 101 PNU for the materials used in the 1979 study. The 50-fold difference in AgE equivalence is based on preliminary studies carried out in 1972,¹ which show this to be an appropriate balance in terms of patient response to the antigen injections.

Stock solutions of allergen and allergoid were prepared at 1000 U/ml in physiologic phosphate-buffered saline at pH 7.2 containing 1:10,000 Merthiolate as a preservative. Subsequent dilutions were made in the same buffer. All solutions were stored at 4° C. The stock solution of the placebo (1000 U/ml) contained 100 μ g/ml histamine base in the phosphate-saline-Merthiolate buffer, to which caramel was added to provide a color similar to that of the allergoid. Dilutions of the placebo containing 100 and 10 U/ml were prepared in diluent containing 100 μ g histamine base/ml, and dilutions of 1 and 0.1 U/ml were prepared in regular diluent (without histamine). Individual sets of dilutions were made and labeled for each patient according to their assignment to a treatment group. With allergen, new sets of the stock solution and further dilutions were made every 2 wk because of the possibility of deterioration of the more dilute solutions (0.1, 1.0, 10, or 100 U/ml). New solutions were not necessary with the more stable allergoid or placebo dilutions.

Study patients

Sixty-six patients were accepted for study on the basis of a history of sneezing, stuffy and runny nose for at least three August and September seasons, relatively minor symptoms at other times of the year, and a 2+ reaction (10 mm wheal,

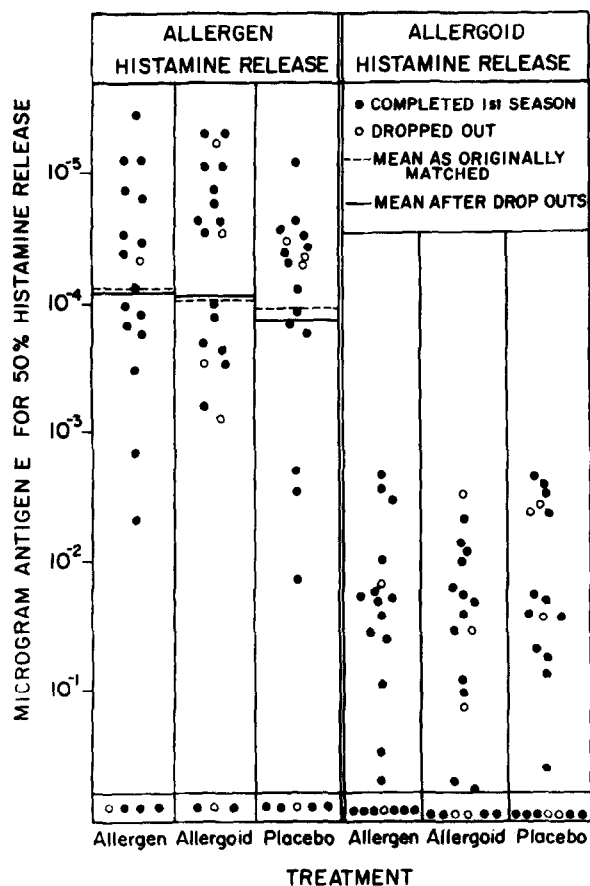


FIG. 1. Histamine release from peripheral blood leukocytes before treatment on all patients admitted to study. Both allergen (see text) and allergoid were tested. The bottom line represents patients whose cells showed poor release of histamine. These patients were nevertheless skin-test positive to allergen.

20 to 30 mm erythema)⁴ to an intradermal skin test with the ragweed allergen subsequently used for immunization at a concentration of 0.001 μ g AgE or less per milliliter. The study patients were matched in 22 sets of triplets using race, pretreatment total IgE level, histamine release sensitivity of peripheral blood basophils to allergen and allergoid, skin-test sensitivity to allergen and allergoid, and allergen/allergoid sensitivity ratios as criteria. Two patients, however, dropped out of the study before the diagnostic testing was complete.

Immunologic methods

Histamine release assays. Leukocyte histamine release experiments were carried out according to the method of Lichtenstein and Osler⁵ as modified by May et al.⁶ Allergen or allergoid were mixed at a series of concentrations with leukocyte preparations from the patients, and the resultant histamine release was analyzed spectrophotometrically. Relative allergenicity (allergoid/allergen) was computed from the concentrations of allergoid and allergen inducing 50% histamine release from the patient's cells.⁷

Total serum IgE. Pretreatment total serum IgE levels were measured by the direct RIST assay developed by Schellenberg and Adkinson,⁸ which utilizes Sepharose-anti-IgE beads as the immunosorbent. All assays were performed in duplicate in at least two experiments and were repeated if there was a coefficient of variation of greater than 10% within or between experiments. Values are cited in nanograms per milliliter (1 IU = 2.42 ng/ml).

Specific IgE antibody measurements. Analyses of IgE antibody levels to dialyzed crude ragweed allergen were performed by a RAST technique as previously described.^{2, 8} Sepharose-ragweed beads were used at a concentration of 2% (v/v) after careful titration against several serum pools having high IgE and IgG antibody titers. Use of this relatively high concentration of beads was necessary to measure accurately IgE antibody levels in the presence of the high levels of IgG antibody found in most treated patients. Each serum was tested in at least two separate assays, and analyses were repeated where there was a coefficient of variation of greater than 10% within or between experiments. All IgE antibody results are expressed in terms of nanograms per milliliter based on titration against a standard serum pool (from 10 individuals) having a known high IgE antibody content determined by RAST adsorption.⁹ The following sera were assayed for samples taken in both 1978 and 1979: pretreatment, 2 to 3 wk after treatment, and 2 to 4 wk after season.

Assay of serum IgG antibody to AgE. IgG antibody to AgE was measured by a highly sensitive double-antibody radioimmunoassay procedure described in detail elsewhere.² In brief, purified AgE was labeled with ¹²⁵I by the chloramine-T procedure and incubated with serial dilutions of a standard human serum of known high IgG antibody content, appropriate dilutions of each patient's serum, or a control serum (containing no antibody). After an initial 4 hr incubation, serum IgG together with antigen-IgG antibody complexes were precipitated by addition of goat anti-IgG (Fc-specific) followed by overnight incubation at 4°C. The resultant precipitates were washed and counted. After subtracting control counts, the radio-binding values for the test sera were compared with those for the various dilutions of the standard serum, and the antibody levels in the test sera were computed. At least two assays were performed on each serum and all results were expressed in nanograms per milliliter based on the determination of the antibody content of the standard serum.² The following serum samples were assayed: immediately before treatment 1978, during immunotherapy 1978 (two specimens), 2 wk after treatment 1978, 2 to 3 wk after season 1978, immediately before treatment 1979, 2 wk after treatment 1979, and 2 to 4 wk after season 1979.

Symptom diaries

Patients filled out daily symptom diary forms as described previously¹⁰ for 8 wk during the ragweed pollination season. The forms report the duration of sneezing, rhinitis, conjunctivitis, and cough for each 12 hr period, along with antihistamine and other medication use. The forms were mailed in and checked for completeness weekly. Any in-

TABLE I. Treatment groups*

Treatment	Allergen	Allergoid	Placebo
No. Accepted	22	22	22
No completing first year	20	16	17
Mean total IgE (ng/ml)			
Patients starting study†	298.9 (21)	240.3 (22)	242.7 (21)
Patients completing study	266.7 (20)	194.6 (16)	230.6 (17)
Skin-test allergen (mean)			
Patients starting study†	0.80×10^{-5} (21)	1.1×10^{-5} (22)	1.0×10^{-5} (20)
Patients completing first season	0.84×10^{-5} (20)	1.4×10^{-5} (16)	1.2×10^{-5} (16)
Skin-test allergoid (mean)			
Patients starting study‡	1.4×10^{-3} (21)	1.9×10^{-3} (22)	1.7×10^{-3} (19)
Patients completing first season	1.5×10^{-3} (20)	2.5×10^{-3} (16)	2.0×10^{-3} (15)
Allergoid-allergen ratio by skin test (mean)			
Patients starting study§	193 (20)	178 (22)	220 (20)
Patients completing first season	200 (19)	180 (16)	244 (16)
Histamine release allergoid-allergen ratio			
Patients starting study	286 (20)	487 (21)	311 (19)
Patients completing first season	284 (19)	364 (16)	341 (16)

*All means are geometric. Number of patients used to determine means are in parentheses.

†Two patients, no data; one patient, no clear endpoint.

‡Two patients, no data; two patients, no clear endpoint.

§Two patients with no clear endpoint estimated.

||Ratios estimated from less than 50% release data in 13 patients.

consistencies were rectified by telephone discussion with the patient. The symptoms were scored numerically and entered into a computer, which calculated an average daily symptom and medication score for each patient and an average score for each group of patients for each day of the season.

Clinical laboratory analyses

The following tests were performed in the clinical laboratory at the Good Samaritan Hospital before treatment, 2 to 3 wk after treatment, and 2 to 4 wk after the ragweed season in both 1978 and 1979: total white blood cells, total red blood cells, hematocrit, hemoglobin, differential white count, calcium, inorganic phosphorus, uric acid, urea nitrogen, glucose, total protein, albumin, serum glutamic-oxalacetic transaminase, creatinine, total bilirubin, and alkaline phosphatase. In addition, standard urinalyses were performed in our laboratory.

RESULTS

1978 Blinded study

Table I and Fig. 1 show the pretreatment tests employed in matching the patients into three groups with similar characteristics. Listed in Table I are total IgE, skin tests to allergen and allergoid, and ratios of allergoid sensitivity to allergen sensitivity. Data were incomplete or unusable on several patients in several categories, so that the number of patients' data used in

determining each geometric mean is shown. Fig. 1 shows the histamine release data using both allergen and allergoid as antigens. Note that certain patients were insensitive to allergen by histamine release, although they were highly sensitive by skin test. Larger proportions of all three groups were insensitive to the allergoid by leukocyte histamine release; hence it was not appropriate to compute the geometric means in this case. These data show that in the several categories tested, the groups were comparable in the several immunologic tests presumed to relate to either the severity of hay fever or responses to immunotherapy. Furthermore, despite dropouts, the patients who remained at the end of the first year still represented comparable groups according to these pretreatment parameters.

Patients scheduled to receive allergen were divided into an "average" sensitivity group (50% histamine release to allergen at 10^{-4} μ g AgE equivalent/ml or greater concentration) or "higher than average" sensitivity (50% histamine release at less than 10^{-4} μ g AgE equivalent/ml). Average sensitivity patients were put on a weekly schedule starting May 24, 1978, of 17 visits with a single injection per visit, starting at 1.0 allergen unit and progressing to 500 units (1200 PNU) for a projected cumulative dose of 2083 units (5000 PNU). Higher than average sensitivity patients

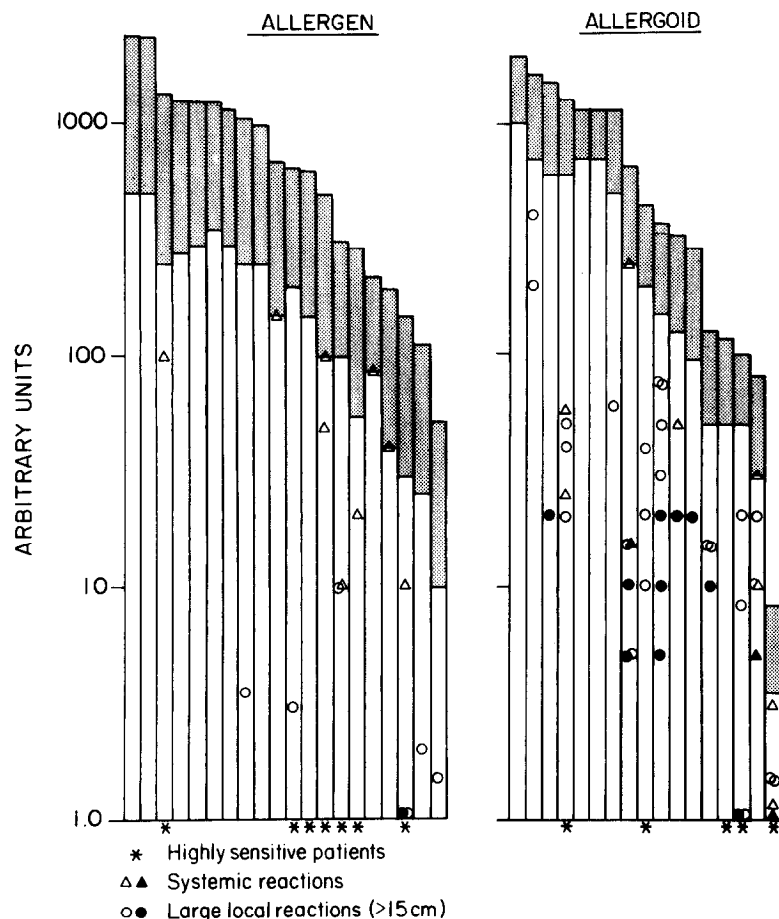


FIG. 2. Doses and reactions to the primary course of immunotherapy. The clear bar represents the largest dose given at a single visit; the total bar (clear plus shaded) represents the total cumulative dose administered. The level at which a reaction symbol appears represents the dose at which that reaction occurred. Solid circles, Local reactions >25 cm; solid triangles, systemic reactions requiring epinephrine.

were put on a schedule starting May 3 of 20 visits, starting at 0.1 allergen unit and progressing to 500 units for a projected cumulative dose of 2084 units.

Allergoid and placebo patients were likewise divided on the basis of sensitivity to allergoid. The average sensitivity being considered was 50% histamine release at a concentration of 10^{-2} μ g AgE equivalents/ml, and the higher than average sensitivity was 50% histamine release at less than 10^{-2} μ g AgE equivalents/ml. Average patients were started on a schedule of five visits to the clinic with 11 injections. The first day called for an initial injection of 5 allergoid units, then 10 units, and finally 20 units at 30 min intervals. On each subsequent visit, two injections were planned 30 min apart for a cumulative projected dose of 1925 units (168,000 PNU). Higher than average sensitivity patients were put on a six visit schedule with 13 injections. The first day called for an initial injection of 0.5 units, then 2 units, and finally 3 units, if no reaction. On each subsequent visit, two

injections were likewise planned for a projected cumulative dose of 1175.5 units (103,000 PNU). In either schedule, the interval between the first and second visit was about 3 wk and 2 to 3 wk between subsequent visits. Initially, there were 11 patients in the average and 11 in the higher than average group among the allergen-treated patients. These figures were five and 17 for both the placebo-treated patients and the allergoid-treated patients.

Fig. 2 summarizes doses and reactions in the allergen and allergoid-treated groups. In the allergen group only two patients reached the projected maximum dose of 500 units (5 μ g AgE equivalent, 1200 PNU) and the cumulative dose of 2080 units (21 μ g AgE equivalent, 5000 PNU). All other patients in this group received a lesser dose because local or systemic reactions limited the dose. Of the 20 patients who completed the injection series, nine systemic reactions occurred in eight patients. All patients had at least one instance of local swelling after an injection

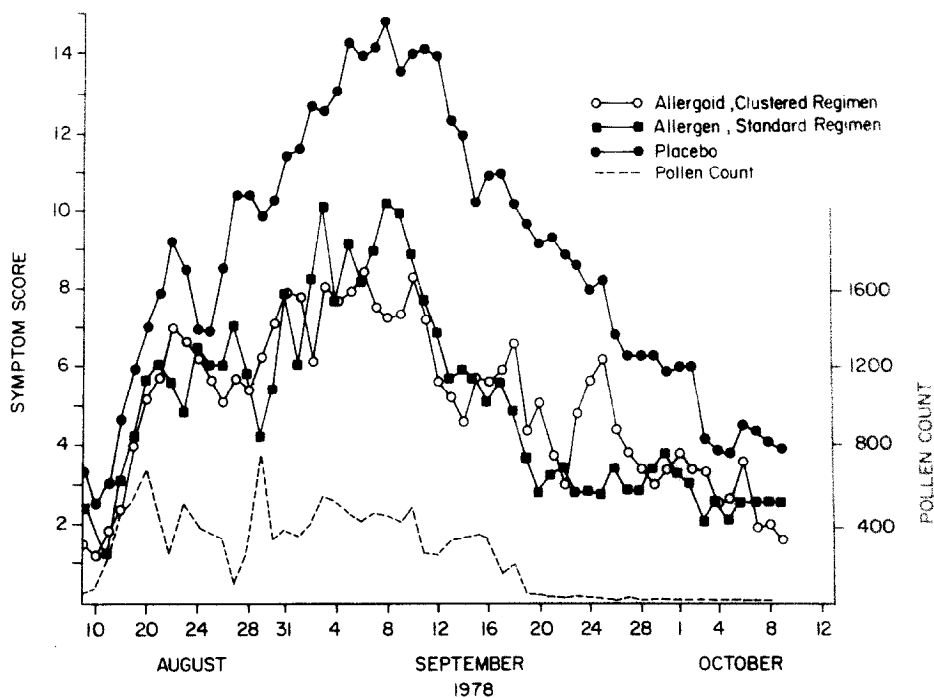


FIG. 3. Mean daily symptom-medication scores for the three groups in 1978.

and most had multiple instances. Large local reactions (>15 cm diameter swelling) occurred in five patients. Among the allergoid patients, one reached the projected maximum of two 500 unit injections (totaling 500 μg AgE equivalent, 87,500 PNU) on one visit and at the same time reached the projected cumulative dose of over 1925 units (963 μg AgE equivalent, 168,000 PNU). Of the 17 patients who completed the injection series, 11 systemic reactions occurred in five patients. The size of local reactions was greater in the allergoid-treated group, although the incidence was only slightly greater. Reactions over 15 cm diameter occurred in 12 patients.

The mean of the preseasonal cumulative dose was 856 units (8.56 μg AgE, 2000 PNU) for allergen and 727 units (364 μg AgE, 63,600 PNU) for allergoid. On the other hand, the mean highest dose on a single visit was 196 units (1.96 μg AgE) for allergen, whereas, due to the clustered regimen, it was 341.4 units (171 μg AgE) (given in two injections) for allergoid.

When one examines where in the series of injections that systemic reactions occurred, an interesting difference between the allergoid and allergen groups emerges. Three injections were planned for the first day of study of allergoid. As a result of a high estimate of the initial safe dose of allergoid, four of the 11 systemic reactions to allergoid occurred on the first day of injections. On the other hand, a conservative

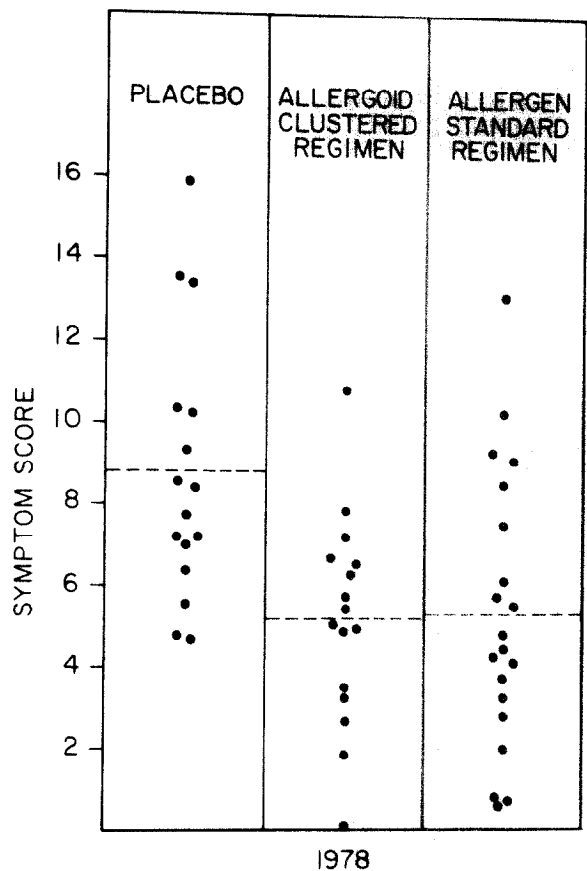


FIG. 4. Mean symptom-medication score for each patient in 1978. Dashed line, Arithmetic mean for each group.

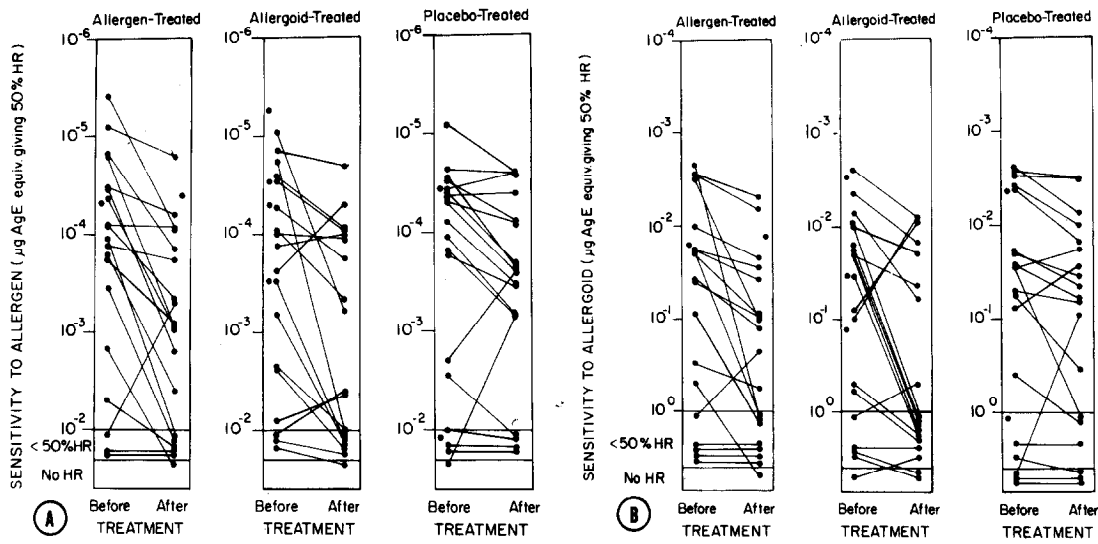


FIG. 5. A, Change in basophil sensitivity to allergen. B, Change in basophil sensitivity to allergoid.

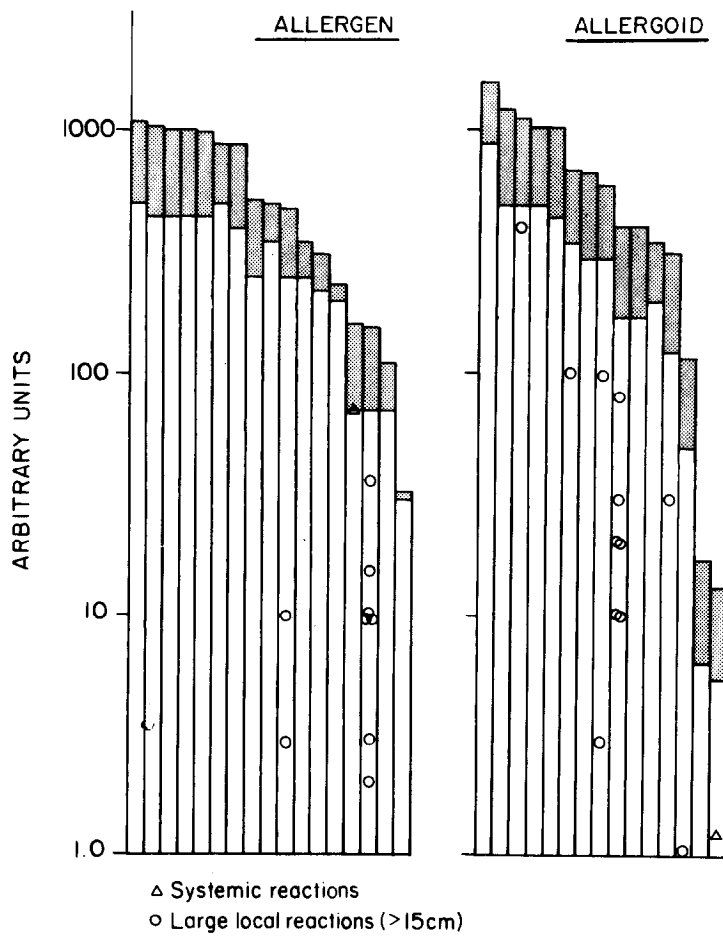


FIG. 6. Doses and reactions to the booster course of immunotherapy in 1979. Symbols are as in Fig. 2.

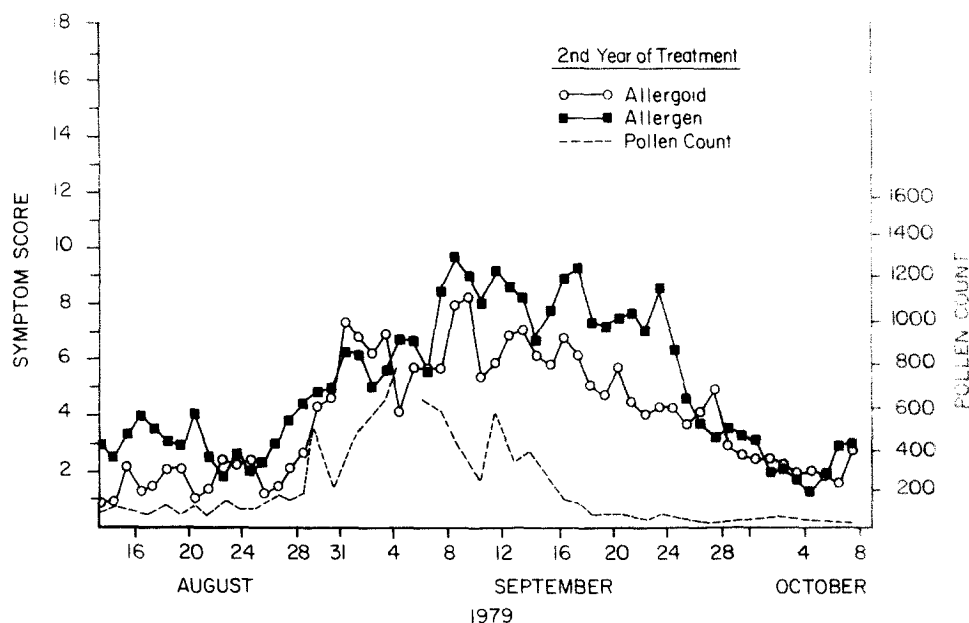


FIG. 7. Mean daily symptom-medication scores for the allergen and allergoid groups treated with boosters in 1979.

estimate of the initial dose of allergen resulted in no systemic reactions with the first three injections (spread over three visits 1 wk apart). As the allergen dose was raised, however, systemic reactions began to occur with increasing frequency. After the initial day of allergoid injection with a high rate of systemic reactions, the planned dosage was reduced in those who had reactions, and the remainder of the immunization program went more smoothly. As noted in Fig. 2, patients designated as above average in sensitivity tended to receive lower doses and have more reactions, but this selection was not entirely successful in identifying in advance all of the patients likely to have reactions.

There were dropouts in all three groups. Among the placebo-treated patients, five did not complete the study: one never began injections, two received the entire series but did not furnish symptom diaries, and two began but did not complete the series. Among the allergen-treated patients, one never began injections and one received only two injections. Among the allergoid-treated patients, two completed the series of six visits but did not furnish symptom diaries, and three dropped out after a single visit at which they had a systemic reaction.

Fig. 3 shows the mean daily symptom-medication scores in the three groups and the ragweed pollen count. Twenty allergen-treated, 17 allergoid-treated, and 17 placebo-treated patients returned adequate diaries. From these, it was necessary to exclude the diaries of one allergoid-treated patient because he re-

ported symptoms from his new home in Pittsburgh, and one placebo patient because he appeared to have received one injection of 200 units of allergoid by mistake (as indicated by IgG antibody studies and an anaphylactic reaction requiring epinephrine and prednisone). Fig. 4 is a scattergram of mean daily symptom-medication scores for the 56 day period of reporting. The arithmetic mean score was 8.8 for the placebo patients and 5.1 and 5.3 for the allergoid and allergen groups, respectively. By the Mann-Whitney U test, the differences between either of the two treated groups and the placebo group were significant ($p < 0.01$, one-tailed test). There was, however, no significant difference between the scores of allergen and allergoid groups.

Fig. 5 shows pretreatment and posttreatment basophil sensitivity by histamine release to both allergen and allergoid in the three treatment groups and indicate that the reduction in sensitivity was greater in both treatment groups than it was in the placebo group. By Mann-Whitney U test the allergen group developed a significantly greater reduction than the placebo group ($0.05 > p > 0.02$ by two-tailed test), whereas the similar trend observed for allergoid vs placebo was not significant ($p > 0.1$). It is worth noting that a trend for reduction of cell sensitivity toward allergoid was found not only in the allergen group but also in the allergoid group, indicating that sensitization to new immunologic specificities present on the allergoid molecules had not occurred to any significant degree.

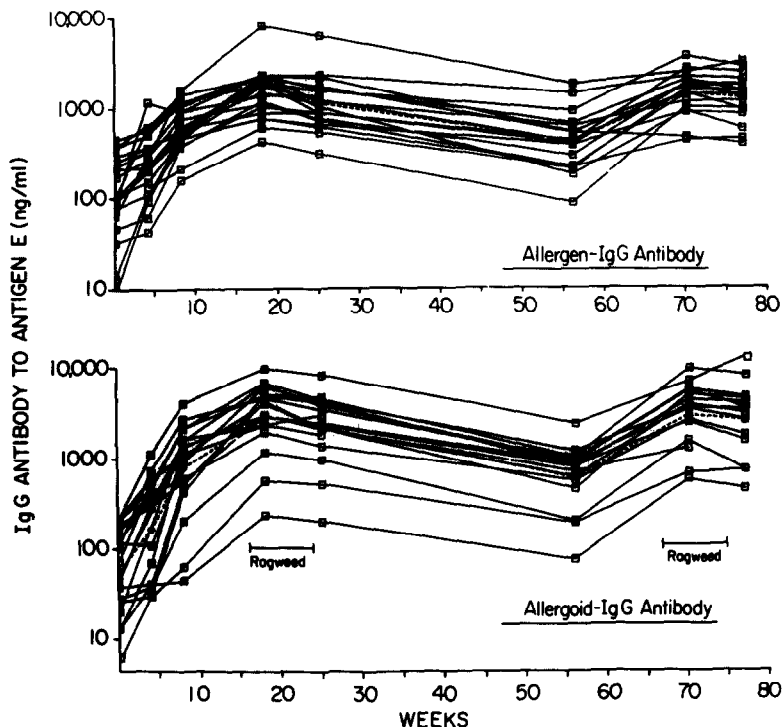


FIG. 8. IgG antibody levels in 1978 and 1979. Horizontal bars, Ragweed pollination season; dashed lines, geometric means.

1979 Open trial

Patients treated with placebos in 1978 were asked to participate in 1979 in a different trial of allergoid to be reported elsewhere. The patients treated with allergoid or allergen were offered the opportunity to continue with booster injections of the same materials in 1979. Essentially the same program of injections was planned in both groups and the trial was open with no attempt to blind the patients.

There were two classes of patients: (1) Those who had previously received a top dose of less than 100 units of antigen received their first injections the first or second week of May. There were seven patients previously on allergen and seven patients previously on allergoid in this category. (2) The remaining patients received their first injection about 2 wk later. There were 10 allergen patients and eight allergoid patients in this category.

At the first visit, 1/100 of the largest single dose given in the previous year was administered. If no significant reaction occurred within 30 min, a second dose 10-fold higher was then administered. If there was no significant reaction after an additional 30 min, a third dose two-fold higher than the second was given. Subsequent injections were given about once every 4 wk. Doses were generally doubled to a limit of 500 units in a total of four to 13 injections administered during three to seven visits to the clinic.

Seventeen patients completed the course of allergen injections and 15 patients completed the course of allergoid injections.

Fig. 6 shows doses and reactions in the booster study. In contrast to the first year of treatment, administration of the booster injections produced little in the way of systemic reactions. One allergen patient and one allergoid patient had mild systemic reactions treated with antihistamine only. Both patients had received low doses the previous year. The incidence and size of local 24 hr swellings was greater in the allergoid group. The mean cumulative preseasonal dose was 574 units ($5.74 \mu\text{g AgE}$, 1400 PNU) in the allergen group and 637 units ($319 \mu\text{g AgE}$, 64,300 PNU) in the allergoid group. Because we gave more than one injection of allergen per visit this year, the mean of the maximum dose per visit was similar in the two groups: 292 units ($2.92 \mu\text{g AgE}$, 700 PNU) for allergen and 302 units ($151 \mu\text{g AgE}$, 30,500 PNU) for allergoid.

Clinically, the patients again fared well, as shown by mean daily symptom scores (Fig. 7). Fig. 7 shows mean daily symptom scores for each of the groups. The allergoid-treated patients reported lower scores at the height of the season but the difference was not significant. The mean daily symptom-medication score was 3.9 for 12 allergoid patients who returned satisfactory diaries and 5.1 for the 17 allergen pa-

TABLE II. Comparison of IgG antibody responses to AgE in allergen- and allergoid-treated patients

	Treatment		
	Allergen	Allergoid	p value*
Posttreatment IgG ab/pretreatment IgG ab in 1978†	14.5	48.7	0.004
Geometric mean posttreatment IgG ab in 1978 (ng/ml)	1547	2952	0.01
Posttreatment IgG ab/pretreatment IgG ab in 1979†	3.45	4.97	0.10
Geometric mean posttreatment IgG ab in 1979 (ng/ml)	1429	3010	0.006

ab = antibody.

*Two-tailed t test on log [IgG].

†Computed from difference in log [IgG] after treatment minus log [IgG] before treatment in 1978 or 1979, as appropriate.

TABLE III. Correlation between IgG antibody response and dose

Treatment	Comparison	No. of patients	Correlation coefficient	
1978				
Allergen	Log posttreatment IgG vs	log final dose	20	0.398
		log cumulative dose	20	0.375
Allergoid		log final dose	17	0.842†
		log cumulative dose	17	0.807†
Allergen	Rise in IgG* vs	log final dose	19	0.703†
		log cumulative dose	19	0.718†
Allergoid		log final dose	17	0.710†
		log cumulative dose	17	0.725†
Allergen	Log posttreatment IgG vs	symptom score	20	-0.336
Allergoid		symptom score	16	-0.378
Allergen	Log final dose vs	symptom score	20	-0.369
Allergoid		symptom score	16	-0.528§
1979				
Allergen	Log posttreatment IgG vs	log final dose	17	0.471
		log cumulative dose	17	0.653‡
Allergoid		log final dose	15	0.733‡
		log cumulative dose	15	0.784†
Allergen	Rise in IgG* vs	log final dose	17	0.159
		log cumulative dose	17	0.355
Allergoid		log final dose	15	0.449
		log cumulative dose	15	0.376
Allergen	Log posttreatment IgG vs	symptom score	17	-0.225
Allergoid		symptom score	12	-0.529
Allergen	Log final dose vs	symptom score	17	-0.357
Allergoid		symptom score	12	-0.704†

Similar p values were obtained by calculating r_s (Spearman rank correlation coefficient).

*Log [posttreatment IgG] minus log [pretreatment IgG].

‡ $p < 0.01$ (two-tailed tests).

§ $0.01 < p < 0.05$ (two-tailed tests).

§ $0.05 < p < 0.1$ (significant by one-tailed test).

tients. There were no placebo-treated patients for comparison.

Immunologic studies

Fig. 8 shows serum IgG antibody levels taken before treatment, twice during the course of treatment, 2 wk after the final preseasonal injection, and after the season during 1978, and before treatment, after treat-

ment, and afterseason in 1979. Placebo-treated patients (with the exception of the one patient who inadvertently may have received active allergoid on one occasion) had no rise of antibody during the period of injections but had modest rises in season as a result of natural exposure (not shown). In 1978, both treated groups had prompt and distinct rises in serum antibody: 14-fold in the case of allergen treatment and

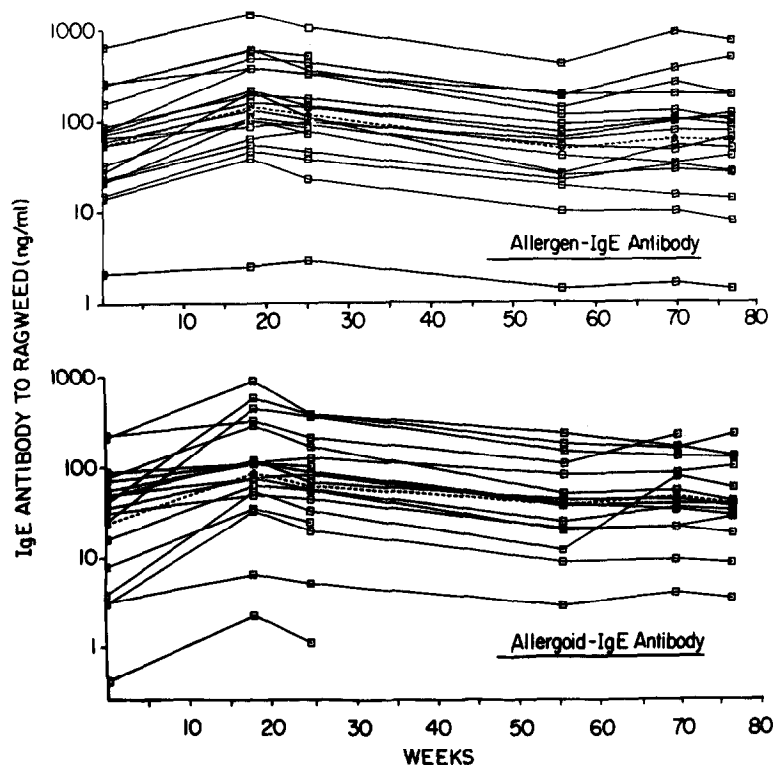


FIG. 9. IgE antibody levels in 1978 and 1979. *Dashed lines*, Geometric means.

49-fold in the case of allergoid treatment (computed from the ratios of the geometric mean titers). The trend for larger and more rapid IgG antibody responses in the allergoid-treated group was apparent in the first posttreatment specimen taken about 1 wk after four injections to the allergen patients and about 3 wk after the first visit (three injections) to the allergoid patients. The increase in log [IgG antibody] across the whole treatment period in 1978 were significantly greater in the allergoid-treated patients than in the allergen-treated patients by unpaired *t* test ($p = 0.004$); the actual log [IgG] titers for 1978 posttreatment sera were also significantly greater in the allergoid-treated group ($p = 0.01$) (see Table II). Subsequently, the increases in log [IgG] values as a result of the 1979 treatment were not significantly different between the two treated groups. This is perhaps not surprising in view of the high pretreatment titers persisting as a result of treatment in 1978 (see below). On the other hand, the final 1979 posttreatment titers were significantly higher in the allergoid-treated than in the allergen-treated patients, showing that the difference found after treatment in 1978 persisted through 1979. In fact, the 1978 and 1979 posttreatment titers for each of the two groups were almost the same, showing that the 1978 and 1979 treatment regimens led to similar results.

Across the 1978 ragweed season (8 to 9 wk) there were geometric mean declines in anti-AgE IgG titers of 1.4-fold in both the allergen- and allergoid-treated groups. There were surprisingly modest declines in IgG antibody titers between post-treatment (August) 1978 levels and pretreatment (May) 1979 levels; geometric mean ratios had declined 3.8-fold in the allergen-treated patients and 5.0-fold in the allergoid-treated patients who continued through 1979. Of interest, only one treated patient exhibited more than a 10-fold decline during this 9 mo period. Declines in IgG titer across the 1979 season were only 1.1-fold for the allergen and 1.2-fold for the allergoid-treated patients.

The correlation between IgG antibodies and dose of allergen or allergoid is examined in Table III, employing log values for both antibody levels and dose. In 1978, posttreatment antibody levels in the allergen-treated patients failed to correlate significantly with dosage, whereas such levels showed a high correlation with dosage in allergoid-treated patients. The rise in antibody levels did, however, correlate with dose in both groups. In 1979, when boosters were given, correlations between posttreatment antibody level and dose were better in the allergoid-treated group. The rise in IgG antibody failed to correlate well with dosage in both groups due to the high pretreatment titers

discussed earlier. The trend for larger dose or higher posttreatment antibody to correlate with lower symptom score was not significant. In 1978-1979, the negative correlation between dose and symptom score was highly significant.

Fig. 9 shows serum IgE antibodies to dialyzed crude ragweed allergen in the two treated groups in 1978 and 1979. The data reflect rises occurring during the first year of treatment, a fall across the 1978 season, and a fall between the postseason level in 1978 and the pretreatment level in 1979. The geometric mean rises in IgE antibody as a result of treatment in 1978 were somewhat greater (3.6-fold) in the allergoid-treated than in the allergen-treated group (2.6-fold), although this difference was not statistically significant. Subsequent changes were similar in the two groups. It is of some interest that after a period of more than 8 mo with no injections, resumption of treatment resulted in much smaller (or no) rises in IgE antibody than the initial series in both groups.

During the 2 yr of study reported here, no abnormalities of blood chemistries, liver function tests, blood counts, or urine were detected except transient changes that could be accounted for by acute intercurrent infections.

DISCUSSION

These studies confirm that in previously untreated patients allergoid in a clustered regimen (given in 12 to 15 wk) can be given in higher dose (42.5-fold) and results in significantly higher serum IgG antibodies than unaltered extract in a weekly program. For allergoid, there was a 49-fold rise in IgG antibody to AgE, as compared with a 15-fold rise with allergen. In addition, the final IgG level was twofold higher with allergoid. Antibody titers seem to be more clearly related to dose with allergoid than allergen for reasons that are not readily apparent. *Increases* in IgG antibody, however, were significantly related to dose in *both* groups for the first year of treatment.

During the 9 mo before booster injections were given in 1979, the decline in IgG antibody was quite modest (about one half of a 1 log₁₀) and the persistence of these antibodies may account for the low rate of untoward reactions in both groups in the second year of treatment. Furthermore, the second series resulted in little, if any, rise in IgE antibodies (Fig. 9). These findings raise questions about the need for frequent booster injections after the primary course of immunotherapy and we plan further investigation of the safety and efficacy of less frequent boosters.

As in previous studies, just after the initiation of therapy we observed a rise in IgE antibodies followed by a slow decline thereafter. It is of interest that the

preseasonal booster course in 1979 failed to induce a second rise in IgE antibodies in most patients, further indicating the safety of a relatively long period between boosters.

The clustered program of administering ragweed allergoid in a single pretreatment course in 1978 was accompanied by a clinical response not only significantly better than placebo but equal to the response obtained from many more visits for injections of standard ragweed extract. Unfortunately, our estimate of the appropriate dose for the first day's injections of allergoid was overoptimistic and resulted in a number of systemic reactions early on. Subsequently, we have been exploring the optimum regimen for allergoid administration with several different pollen allergens and will report that immunization can be initiated in a short course with few or no systemic reactions. Both the more leisurely regimen reported earlier¹ and the rapid program reported here show differences in the allergic reactions to allergen and allergoid. With allergen, systemic symptoms are more likely to occur at or near the highest dose administered. With allergoid, only the overambitious schedule attempted here brought out the ability of high doses to produce systemic symptoms in the early phase of immunization (before a sufficient IgG response had developed). The reduced tendency of allergoids to produce systemic reactions comes at the expense of larger local reactions. In general these local swellings are not tender and subside rapidly after 24 hr. Our patients' chief complaint about them was itching, which often could be controlled with antihistamines.

It is important to make a distinction between the advantages of a clustered regimen per se, no matter what form the allergen, and the advantages of allergoids. Clustered programs with insect venoms have been superior to weekly programs, both with respect to prompt IgG antibody response and the incidence of allergic reactions.¹¹ On the other hand, with unaltered ragweed extract, members of our group have reported a comparison between a clustered regimen and a weekly program and found that the clustered regimen gave neither fewer allergic reactions or larger antibody responses.¹² In the latter study,¹² as in a previous smaller study,² ragweed allergen administered in a clustered regimen gave an unacceptable number of systemic reactions. It seems likely that the unique characteristics of allergoid are important to the success of a clustered regimen. Allergens that can be given in sizable doses without an allergic reaction seem likely to work well in a clustered regimen.

The reaction data emphasize that most of the allergic reactions to immunotherapy occur during the early period of injections. Our previous experience with

allergen and allergoid in a more conventional regimen was similar.¹ We continue to find allergic reactions to immunotherapy largely unpredictable by prior testing of the degree of sensitivity by skin tests, histamine release from blood basophils, or specific IgE antibodies. Although highly sensitive patients are at somewhat greater risk, what determines risk at the time of any particular injection remains obscure. Since IgG and IgE antibody levels change rapidly after immunization is started, it may be that the levels at the time of each injection are important determinants. In any event, it is clear that most patients become less susceptible to such reactions with continued immunization and eventually tolerate doses large enough to induce significant IgG immunologic responses. A few are persistently prone either to large local swellings or systemic reactions and end up with a dose much lower than we consider optimum. It may be reiterated that these are not necessarily the patients with the greatest sensitivity to the allergen by objective measurements. Nevertheless, it is nearly always possible to reach full immunizing doses after several years, if physician and patient are willing to persist with immunotherapy.

Allergoid in this study again showed an advantage in clinical scores over allergen in the second year. In our study of a conventional weekly dosage regimen, we obtained similar data.¹ Clinical scores were not different at year 1, were lower in allergoid-treated patients by year 2, and were significantly lower by year 3. This suggests that a build-up to high allergoid dosages, with persistent maintenance, eventually leads to significantly greater efficacy than with optimally tolerated dosages of allergen. These findings, plus the significantly higher IgG antibody levels consistently stimulated in allergoid-treated patients, make us believe that the higher doses of protein possible with allergoid have favorable therapeutic implications. Although there are likely immunologic parameters in addition to serum IgG antibodies that determine clinical response, IgG antibodies do represent an easily measured indicator of immunologic adequacy.

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