

Studies on allergoids from naturally occurring allergens

IV. Efficacy and safety of long-term allergoid treatment of ragweed hay fever

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An allergoid of ragweed was produced by treating dialyzed defatted pollen extract under mild conditions with formaldehyde. This modified antigen was found to have the desirable properties of reduced allergenicity, retained antigenicity, and prolonged stability for IgG antibody production. A series of trials was carried out in ragweed-allergic patients from 1972 to 1978. In 1972 it was demonstrated that on average, allergoid had on the basis of antigen E content about one fiftieth the ability of unaltered whole ragweed extract (allergen) to induce local delayed and systemic allergic reactions when given subcutaneously. IgG antibody responses specific for allergen were readily induced. A blinded comparison in 1973 divided 48 highly sensitive patients into two groups on the basis of basophil sensitivity; one group was treated with allergoid and the other with allergen. In individuals never before treated, allergoid stimulated a significantly more rapid rise in IgG antibodies. Clinical evaluation in 45 of the patients gave a modest advantage (not significant) to allergoid. An open trial employing booster injections every 2 wk except during the ragweed season continued until 1978 in many of these patients and showed no evidence of long-term ill effects of allergoid. IgG antibody titers were maintained at a high level, and IgE antibodies, after a rise in the first year, declined slowly in both groups. Daily symptom-medication scores continued to be lower in the allergoid-treated group each year, and the difference was significant ($p < 0.05$) in 1975, 1976, and 1977. In comparison with allergen, allergoid has the advantages of prolonged shelf life, lowered potential to induce allergic reactions, and more rapid induction of IgG antibody responses. (J ALLERGY CLIN IMMUNOL 68:460, 1981.)

Studies of the properties of ragweed pollen extracts treated with formaldehyde to yield "formallergoids" according to several different formulas revealed a two-step preparation with properties appropriate for further trial as an immunizing agent in man.¹ These properties included (1) greatly reduced allergenicity (100- to 10,000-fold) compared with native ragweed extract as indicated by histamine release from isolated

washed leukocytes from naturally ragweed-sensitive humans, (2) ability to elicit good anti-allergen IgG antibody responses in guinea pigs and rabbits, (3) prolonged stability on storage due to greatly reduced enzymatic activity (unpublished) and intermolecular and intramolecular crosslinking, and (4) enhanced immunogenic properties due to increased size of the modified ragweed antigen molecules (average mol. wt. 260,000 daltons).

Trial of ragweed formallergoid ("allergoid") in ragweed-allergic patients was organized in several phases. Initially, an uncontrolled trial using a series of injections of allergoid at weekly intervals with gradually increasing amounts was performed to determine whether doses of allergoid similar to those employed with native ragweed extract ("allergen") would be safe and would induce IgG blocking antibody responses similar to those observed after injection

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Abbreviations used

PNU:	Protein nitrogen unit
Tr:	Previously treated patients
NTr:	Previously untreated patients
RAST:	Radioallergosorbent test

tion of allergen. After a safe dose was established, a second phase compared allergen and allergoid more strictly over a period of 6 yr. These two phases are reported here. Subsequent phases included development of a simplified treatment regimen followed by a double-blind trial, which will be reported in separate papers.

MATERIALS AND METHODS

Antigens

The allergoid, 9RWF, used for the preliminary study in 1972 was prepared according to a two-step formalinization process described previously for "Prep. E."¹ In brief, lyophilized, dialyzed ragweed allergen was prepared from *Ambrosia elatior* pollen supplied by Greer Laboratories. By radial immunodiffusion analysis² the allergen was found to contain 33.0 μg AgE/mg of non-dialyzable pollen solids. A solution of this extract (10 mg/ml) was incubated at pH 7.5 with 2M formaldehyde for 16 days at 10° C. The solution was diluted fourfold with pH 7.5 buffer and incubated further for 16 days at 32° C. At the end of the incubation, excess formaldehyde and buffer salts were removed by extensive dialysis against deionized water, and the resultant allergoid solution was lyophilized and stored at -20° C until used. For the clinical studies, the allergoid was dissolved in a phosphate-saline-thimerosal buffer (1.15 gm Na_2HPO_4 , 0.221 gm NaH_2PO_4 , 8.10 gm NaCl, and 0.01 gm thimerosal per liter), pH 7.0, at a concentration of 1000 μg AgE equivalents/ml (equiv./ml), which yielded a clear solution. The solution was sterile-filtered and subsequently diluted in the same buffer for injection into the patients. Since AgE could not be measured in the allergoid,¹ the "AgE equiv./ml" was derived from the determination of AgE concentration in the allergen from which the allergoid was derived.

The unmodified allergen used as a control for the double-blind study in 1973 and in most subsequent years was a lyophilized short ragweed extract (Lot No. 16608FD) prepared by Center Laboratories, Port Washington, N. Y. By radial immunodiffusion, it was found to contain 28.0 μg AgE/10,000 PNU. For 1977-78, we switched to a second lot of lyophilized material purchased from Greer Laboratories, Lenoir, N. C. (Lot No. L56-38, 64.2 μg /AgE in a 1/20 w/v solution). The allergoid, 11RWF, used throughout this study was prepared similarly to that used in 1972. Analysis of the dialyzed allergen from which it was derived showed an AgE equivalence of 35.5 μg AgE/mg of pollen solids, a value indistinguishable from that of 9RWF (coefficient variation in this assay is $\pm 10\%$). The allergoid contained 6100 PNU/mg of pollen solids and 58.2 μg AgE equiv./

10,000 PNU. It should be noted that dialysis of the allergen results in a loss of about one half of the phosphotungstic acid-precipitable material detectable by the "PNU" measurement; hence the AgE equivalences of the allergen and allergoid were very similar in terms of milligrams of ragweed protein.

Stock solutions of the allergen and allergoid were prepared by dissolving the antigens in the phosphate-saline-thimerosal buffer at concentrations of 10 and 500 μg AgE equiv./ml, respectively, and the allergoid solution was sterile-filtered. Both stock solutions were labeled "1000 U/ml." Note that in terms of AgE equivalents, the allergoid unit was 50 times greater than the allergen unit in order to balance the expected reactivity of the two antigens for both expected immediate and delayed reactions based on our results from the 1972 study.

Fresh dilutions of allergen treatment solutions were prepared every 2 wk from the allergen stock solution, which was replaced every 8 wk. On the other hand, because of the high degree of stability of the allergoid, we found it necessary to prepare dilutions from the stock concentrate only as they became depleted (2 to 3 mo). Also, fresh stock solutions of the allergoid were prepared from lyophilized material as they were depleted (every 2 to 3 yr). All antigen solutions were stored at 4° C when not being used in treatment.

Patient selection

For the preliminary study of allergoid treatment in 1972, 24 patients with histories of hay fever symptoms in the fall and positive 2+ skin tests (wheal 10 mm, erythema 20 mm; intradermal titration injecting 0.05 ml of serial 10-fold dilutions of allergen at a dilution containing 0.1 PNU/ml or less) were selected, with the additional proviso that at least 20% histamine release from isolated washed leukocytes in vitro was obtained to whole ragweed extract.

In 1973, 16 of these allergoid-treated patients were available for restudy, along with 12 patients who had received preseasonal courses of alum-precipitated ragweed allergen (Center Laboratories) for 3 yr and 11 patients who had received placebo treatment for a similar period.³ Additional patients, who had not been treated with ragweed during the preceding 3 yr, were recruited according to the above criteria until 48 patients were available. The entire group underwent new determinations of in vitro leukocyte sensitivity to whole crude ragweed extract and were matched into 24 pairs of patients according to these determinations, so that one member of each pair was assigned to the allergoid group and the other to the allergen group (see Fig. 1). There were no crossovers between previous and new allergen and allergoid treatment. When neither member of a pair had received prior ragweed treatment, assignment to allergen and allergoid was made by the flip of a coin. The final allergoid group contained 13 males and 11 females (including 22 white subjects, one black male, and one Asian Indian male), and the allergen group contained 16 males and nine females (including 21 white subjects, one black male, one black female, and one Oriental male). The age range for the study groups was 20 to 60 yr.

At the end of the first season, there were 22 allergoid-

and 23 allergen-treated patients who turned in symptom diary reports, and 23 allergoid- and 24 allergen-treated patients who donated serum samples. Of these, eight allergoid- and 13 allergen-treated subjects had never been treated previously. The whole group of patients were studied through 1978 without further additions. Several patients dropped out each year, so that at the end of 1978, 13 allergen-treated and 10 allergoid-treated patients remained, of whom 12 allergen-treated and 10 allergoid-treated patients turned in adequate symptom diaries.

Symptom diaries

Patients filled out daily symptom diaries of the type described previously⁴ for 8 wk each year 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 collected and checked for completeness weekly. Any inconsistencies were rectified by discussion with the patient. The symptoms (plus medication) were scored numerically and entered into a computer, which calculated an average daily score for each patient and an average for each group of patients for each day of the season.

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 double-antibody inhibition assay described by Ishizaka et al.⁸ All assays were performed in duplicate in at least two experiments. Assays were repeated if there was a coefficient of variation of greater than 10% within or between experiments.

Specific IgE antibody measurements

Analyses of IgE antibody levels to AgE and to crude ragweed allergen were performed by a RAST technique.⁹ Sepharose-AgE or Sepharose-ragweed beads were prepared with CNBr-activated Sepharose 4B (Pharmacia) according to the manufacturer's instructions. The 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 nanograms per milliliter on the basis of titration against a standard

serum pool (from 10 individuals) having a known high IgE antibody content determined by RAST adsorption.¹⁰

Assay of serum IgG antibody to antigen E

IgG antibody to AgE was measured with a highly sensitive double-antibody radioimmunoassay procedure described in detail elsewhere.¹¹ Briefly, 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 radiobinding 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 on the basis of the antibody content of the standard serum.¹¹

Toxicity studies

Each patient underwent white blood cell count, differential, red blood cell count, hemoglobin, hematocrit, urinalysis, and a standard set of 12 automated blood chemistries before treatment and two or three times during each of the subsequent years. Somewhat different blood chemistries were performed in different years according to the practice of the laboratory of The Good Samaritan Hospital.

RESULTS

Preliminary study of allergoid alone (1972)

Injections were initiated at the end of June 1972 at an allergoid dose of 0.008 μ g AgE equiv. Injections were given two times weekly until Aug. 2, 1972. With the caution required for the use of a new product, doses were generally increased by twofold and not pushed to the limit of tolerance. Reactions to the injections were mostly minor and local. One patient had a mild systemic reaction and responded readily to epinephrine. Local reactions consisted of itching, red soft-tissue swellings at the site of injection, which were maximum in size at 18 to 24 hr and then began to subside. The appearance or timing of such reactions from allergoid was not different from those observed with unaltered allergen. Mild delayed local reactions occurred after 25% of the injections, moderate reactions after 3%, and severe reactions after 0.8% of the injections. Two of the severe reactions occurred in one patient.

The average number of injections was 15.7 per patient (range 10 to 18), and the arithmetic mean dose was 130 μ g AgE equiv. in ca. 22,000 PNU (ranges 7.3 to 445 μ g AgE equiv. or ca. 1200 to 77,000

TABLE I. Dosages of allergoid and allergen that first caused a sufficiently large local reaction to require a limitation in dosage increase

Allergoid-treated ($\mu\text{g AgE equiv.}$)		Allergen-treated ($\mu\text{g AgE equiv.}$)	
Tr	NTr	Tr	NTr
2.0	0.5	0.15	0.004
4.0	1.5	0.20	0.04
7.5	2.0	0.30	0.05
10	13	0.35	0.05
13	20	3.0	0.05
20	30	NR	0.1
25	NR	NR	0.4
45	NR	NR	0.4
100		NR	0.8
125		NR	2.0
150		NR	4.0
NR			NR
NR			NR
NR			
NR			

NR = no reaction sufficiently large to cause limitation of dosage increase up to the maintenance level of 500 U (250 $\mu\text{g AgE equiv.}$ for allergoid and 5 $\mu\text{g AgE equiv.}$ for allergen).

PNU). Although this mean dose was significantly higher than that administered in earlier studies with unaltered aqueous extract (an arithmetic mean dose of 23.8 $\mu\text{g AgE equiv.}$, 8800 PNU, in an average of 27.6 injections in 1968 for instance),¹² use of this dosage nevertheless did not approach the theoretical maximum suggested by the geometric mean sensitivity ratio (allergoid/allergen) of 800 obtained by previous histamine-release experiments.¹ Significant posttreatment increases in IgG blocking antibody to ragweed fraction D¹³ were obtained in every patient, and no evidence of toxicity was found in the blood chemistries, blood counts, and urinalysis.

Double-blind study of allergoid and allergen (1973)

Patients were matched into pairs according to their histamine-release sensitivity to ragweed allergen prior to treatment in 1973. Sensitivity data were also redetermined during treatment where earlier experiments were unsatisfactory. The geometric mean sensitivity to allergen (measured at 50% histamine release) was 1.6×10^{-4} $\mu\text{g AgE equiv./ml}$ (range 1.8×10^{-5} to 9.8×10^{-3} $\mu\text{g AgE equiv./ml}$) for the allergen-treated patients and 7.0×10^{-5} $\mu\text{g AgE equiv./ml}$ (range 1.1×10^{-5} to 3.4×10^{-3} $\mu\text{g AgE equiv./ml}$) for the allergoid-treated patients, which illustrates that the groups were well matched. One allergen-treated

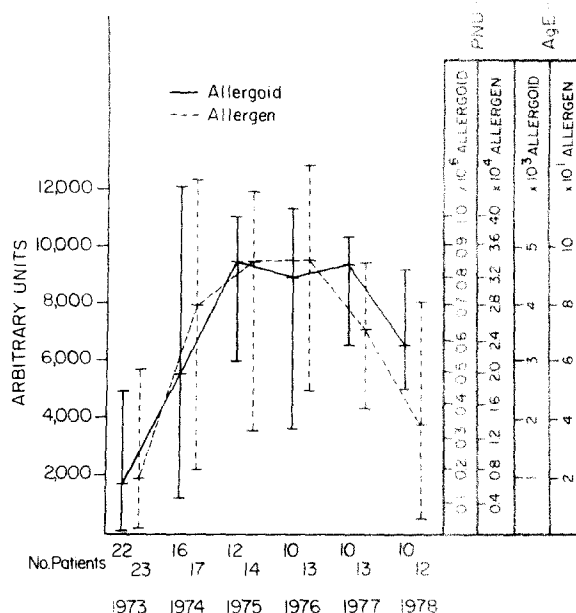


FIG. 1. Annual cumulative doses administered up to the beginning of the ragweed season (August 15) for the years 1973-78. Cross-bars indicate arithmetic mean for the groups; brackets show the entire range of doses. Dosages in arbitrary units, PNU, and AgE equivalents are indicated in the vertical scales.

patient and one allergoid-treated patient failed to release histamine from their basophils; therefore these individuals could not be included in these geometric means. These subjects were, however, highly sensitive to ragweed allergen by skin-test titration. (It so happened that neither of these subjects turned in clinical reports for 1973.)

Allergoid/allergen sensitivity ratios were also determined by leukocyte histamine release assay in 1973. Ratios were determined using antigen concentrations inducing 50% histamine release wherever possible; otherwise ratios were determined at antigen concentrations inducing 20% or 30% histamine release, where low release was obtained with the allergoid. Of 45 patients who gave at least 20% histamine release from their cells with both allergen and allergoid, the overall geometric mean allergoid/allergen sensitivity ratio was 591 (range 10 to 11,500)*, with a geometric mean ratio of 350 for the allergoid-treated group and 1021 for the allergen-treated group.

The geometric mean pretreatment total serum IgE level was 373 ng/ml (range 56 to 3323 ng/ml) for the

*The Oriental male had an unusually low ratio of 10. Only three other persons had ratios less than 100 (55, 94, and 95).

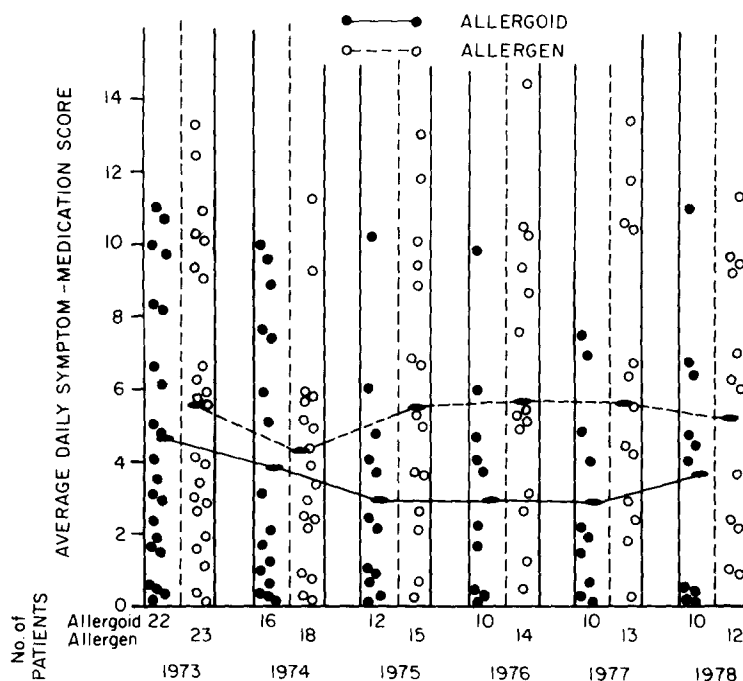


FIG. 2. Mean daily symptom-medication scores for all patients for the 8 wk of ragweed pollination starting the Monday in August nearest August 15 during the period 1973-78. Cross-bars indicate arithmetic mean scores of each group during each pollination season.

allergen-treated group and 375 ng/ml (range 39 to 4598 ng/ml) for the allergoid-treated group. These IgE levels were measured on serum samples prior to any type of treatment, including the 1972 allergoid study and the 1970-72 allergen study, showing that the groups were also well matched in terms of total IgE level.

For the double-blind trial, each patient had a separate vial of treatment material. The color of allergen was matched to the color of allergoid by admixture with caramelized glucose. Arbitrary units were adopted so that no prejudices as to the maximum number of PNU to be given with safety could influence dosage decisions. On the basis of prior experience, units were set as follows: 1 allergoid unit = 50 allergen units = 0.5 μ g AgE equiv. Patients were immunized in about 20 injections at weekly intervals between February 21 and August 20, starting at a dosage of 0.1 U. Fig. 1 shows the arithmetic mean and range of doses in 1973 and subsequent years in terms of arbitrary units. These can be converted from AgE equiv. to PNU with the conversion formula above or the scales on the ordinate of Fig. 1. In 1973, an average of 42.4 times as much allergoid as allergen was administered on an AgE basis and 20.4 times as much on a PNU basis, although similar amounts were administered in terms of arbitrary units. One should note that the mean dose given to the patients receiving allergen was about 6000 PNU or 17 μ g of AgE, a

dose that on the basis of prior studies with aqueous extracts, should be high enough to produce significant symptomatic relief.¹⁴

An important issue in this comparison is whether the increase in dose was pushed to the same degree in terms of the patient's ability to tolerate allergic reactions. Because most of these patients were being treated simultaneously with grass pollen extract in a separate injection, it was impossible to judge whether systemic reactions were caused by grass or ragweed. Dosages were therefore pushed until the occurrence of a local immediate or delayed reaction considered sufficiently large in the physician's judgment to cause a reduction in the planned dosage increase. Such limitation occurred in 17 of the 23 allergoid-treated patients and in 16 of the 24 allergen-treated patients who remained on the study through the 1973 ragweed season.*

Table I shows the doses (μ g AgE equiv.) at which such reactions occurred. Since patients who had previously received treatment might be expected to tolerate larger doses, the table is divided into Tr and NTr groups. The numbers show that much larger doses (AgE equiv.) were tolerated in the allergoid-treated group before cut-back in dosage was indicated.

*Note that one patient in each of the groups failed to turn in adequate symptom reports (which accounts for the smaller numbers in the 1973 clinical data shown in Figs. 1 to 3), but all these patients donated posttreatment blood samples.

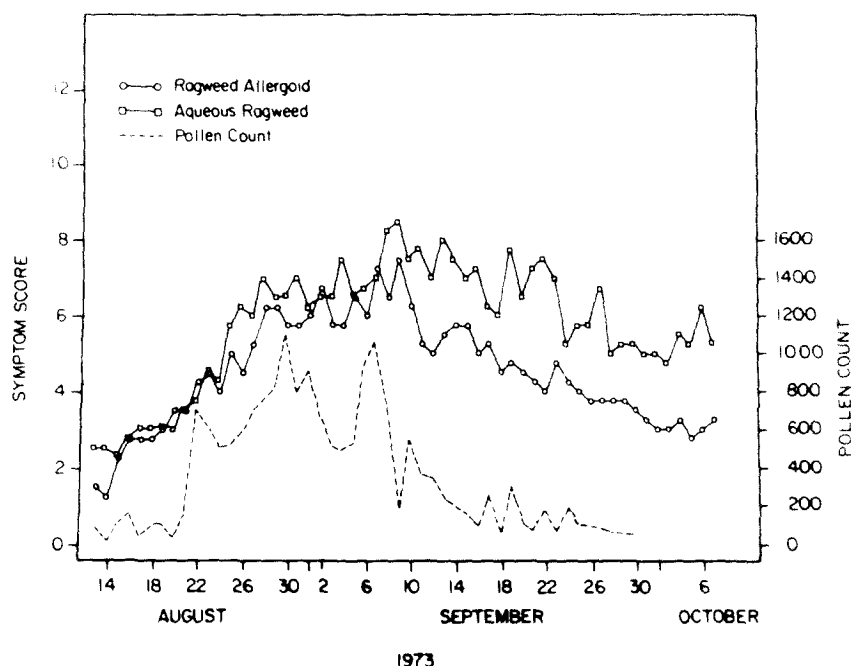


FIG. 3. Mean symptom (including medication) scores for each day during the 1973 ragweed pollination season for the two treated groups of patients.

Clinically, both groups fared well. Symptom-plus-medication scores for the 8-wk season starting in mid-August in 1973 are shown in Fig. 2 as a mean for each group, and individual symbols show the score for each patient. The group means for similar groups of patients given placebos have ranged between 8 and 10 consistently in other studies,^{3, 4, 12, 14, 15} so that the means for patients treated with allergoid (4.6) and allergen (5.6) are definitely lower. A statistical comparison between these scores and placebo scores in other studies is not appropriate in view of the lack of matching of the patients and the potential differences in pollen exposure in different years. The allergoid and allergen scores, on the other hand, do arise from matched groups and can be compared by the Mann-Whitney U test, a rank-order test that does not employ the group means. For the entire 8-wk period the scores were not significantly different. Fig. 3 shows mean daily scores for the two groups of patients through the 1973 ragweed season and demonstrates that on 51 of the 56 days, allergoid-treated patients reported lower symptom scores.

A number of serum samples were taken for immunologic study in 1973. IgG antibody levels to AgE on posttreatment sera taken in September during the ragweed season are shown in Fig. 4 for 23 allergoid-treated and 24 allergen-treated subjects. Both groups had IgG antibody responses, but the patients treated with allergoid showed 1.8-fold higher overall geometric mean titers ($p < 0.05$ by two-tailed t test). Since patients who had received prior treatment could

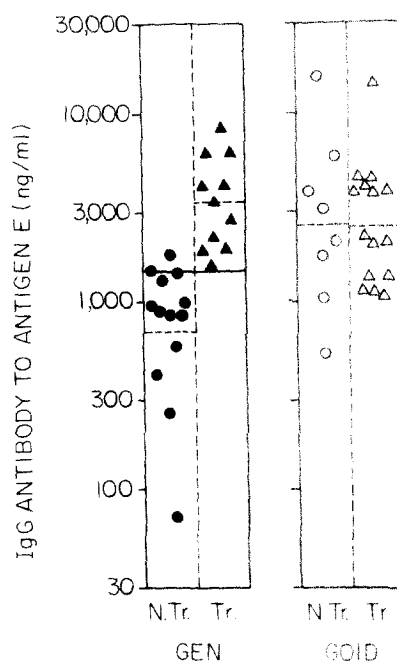


FIG. 4. IgG antibody titers to AgE after treatment but in season (September 1973). Dashed lines, Geometric means for Tr and NTr subgroups; solid line, geometric mean for the entire allergen-treated group.

be expected to have residual antibodies from their previous injections and to respond more readily to new injections, NTr patients are shown separately. The difference between the two NTr subgroups was more striking; eight allergoid-treated subjects had

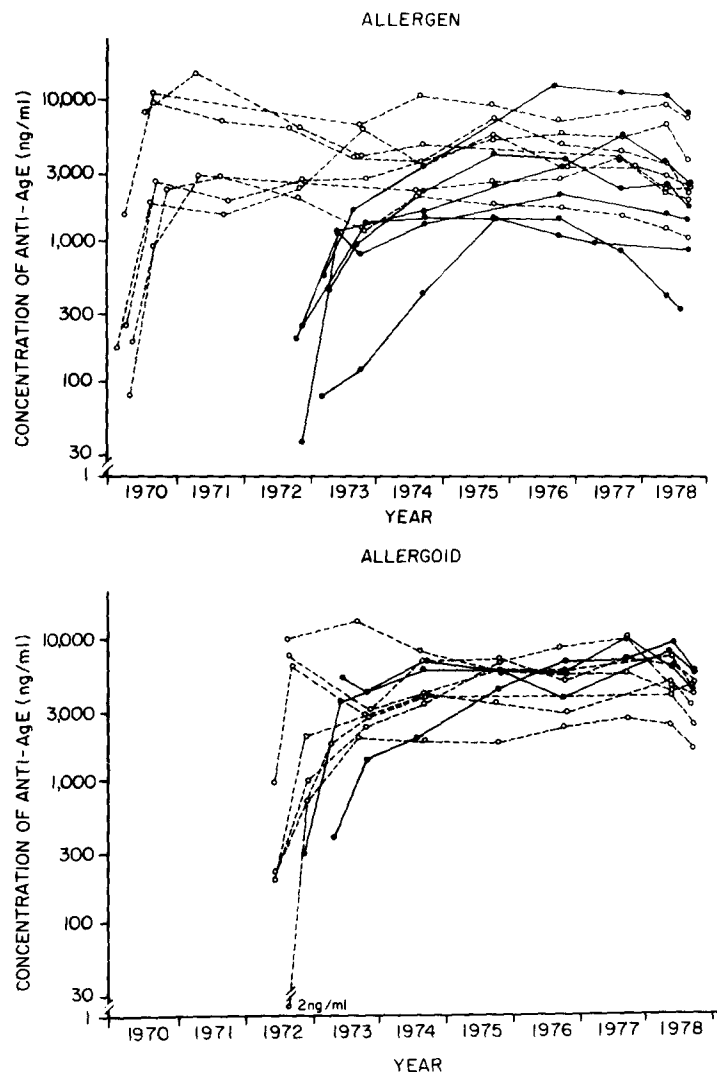


FIG. 5. IgG antibody titers toward AgE in allergen-treated and allergoid-treated patients for the period 1970-78. *Dashed lines*, Tr patients; *solid lines*, NTr patients. See text for further explanation.

3.7-fold higher geometric mean levels than 13 allergen-treated patients ($p < 0.01$). Furthermore, although the allergen Tr subgroup had a 4.7-fold higher geometric mean IgG antibody titer than the allergen NTr subgroup ($p < 0.0001$), the two subgroups within the allergoid-treated subjects were essentially the same ($p > 0.975$). These findings point up three considerations: (1) 1 yr of allergen treatment was insufficient to bring the patients up to plateau IgG antibody titers such as those found in the allergen Tr subgroup; (2) the presence of Tr patients in the allergen group biased antibody, and possibly symptom, comparisons for 1973; (3) 1 yr of allergoid treatment gave essentially the same IgG antibody levels as 2 yr allergoid treatment (1972 and 1973), illustrating that allergoid-treated patients can be brought to a "plateau

IgG antibody titer" within 1 yr of therapy (see also Discussion).

1974 and subsequent years

After this encouraging result, these patients were continued on the same treatment through 1978. The double-blind method was dropped at the end of 1973, and the remaining work represents a simple open trial to evaluate possible toxicity and the clinical and immunologic results of long-term treatment, comparing allergen and allergoid. In general, booster injections were given every 2 wk, with a goal of maintenance at 500 U per injection. Only one patient receiving allergen was consistently unable to reach this goal because of local reactions in the subsequent years. Because injections were discontinued in mid-August and

usually started again in October or November, the doses at resumption of treatment were about 10-fold lower than those given the previous August, with a plan of building up to a 500-U maintenance dose. The number of injections required to reach this goal varied with the individual, and therefore the cumulative annual dose also varied. Fig. 1 shows the means and ranges of doses by arbitrary units, PNU, and AgE equivalents, along with the number of patients remaining in the study each year.

The use of a 10-mo period of boosters instead of a shorter preseasonal course resulted in an increase in the annual cumulative dose in 1974 and again in 1975. In the 2 yr (1975 and 1976) when annual doses were largest, the allergen group received doses that averaged above 30,000 PNU (nearly 100 μg AgE), whereas allergoid doses averaged about 800,000 PNU (4500 μg AgE). These represent large doses in both treatment groups, although the allergoid/allergen dosage ratio was essentially unchanged throughout. In 1977 supplies of the lyophilized allergen employed originally were exhausted and the substitution of a new batch of lyophilized whole ragweed extract resulted in more cautious initial dosing and general lowering of the annual dose, although the final top doses were similar to those in 1975-76. In 1978 a decision to delay the beginning of boosters until January also resulted in a reduced annual dose. During this time, despite the large antigen dosages, the rates of systemic symptoms related to ragweed allergen and allergoid injections was zero in all except one patient receiving allergen, who had systemic symptoms several times in 1974 and 1975.

Fig. 5 shows the titers for IgG antibody responses to AgE in the 13 allergen-treated and 10 allergoid-treated patients who remained on the study through 1978. The dashed lines show data for Tr patients who had previously been treated with alum-precipitated ragweed allergen during 1970-72 or aqueous allergoid in 1972 in the allergen and allergoid groups, respectively. Pretreatment serum samples on these patients (1970 or 1972, respectively) were analyzed where available. Pretreatment (1972 or 1973) samples were also analyzed for NTr patients who were first treated in 1973 (solid lines). Posttreatment (Sept.-Oct.) sera were analyzed each treatment year for the period 1970-1978.

IgG antibody titers climbed to a maximum in the first or second year of treatment (usually the second year in the case of aqueous allergen treatment). Each antibody level then remained at a steady plateau characteristic for each individual and there was little change thereafter. As noted earlier, in previously untreated patients there was evidence that allergoid-

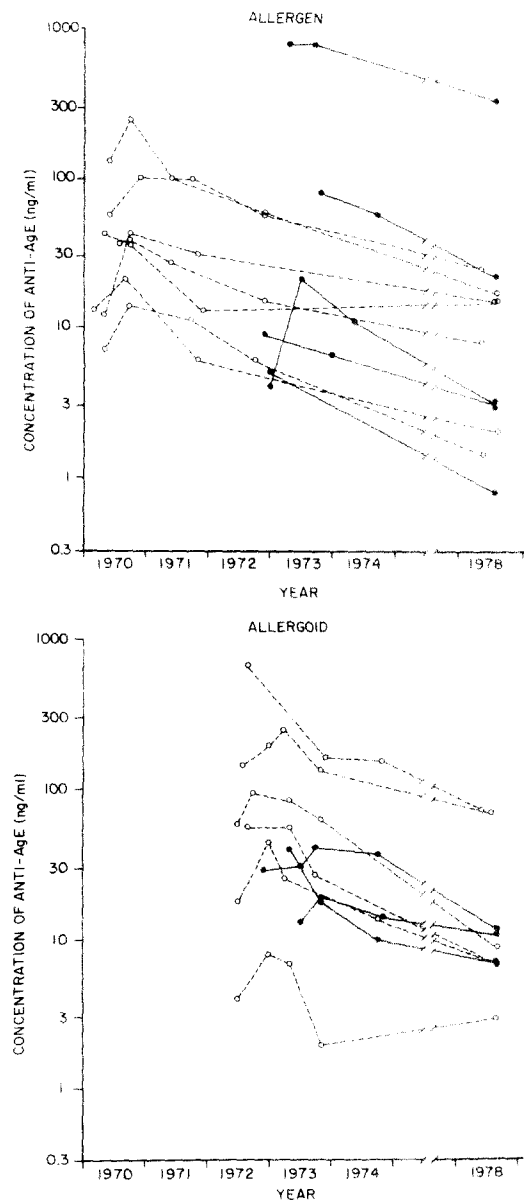


FIG. 6. IgE antibody titers to AgE in allergen-treated and allergoid-treated patients for the period 1970-78. Dashed lines, Tr patients; solid lines, NTr patients. See text for further explanation.

treated patients reached higher levels at the end of the first preseasonal course because of the higher doses received. The eventual plateaus of geometric mean IgG antibody titers to AgE were 5000 ng/ml for 10 allergoid-treated patients and 3590 ng/ml for 13 allergen-treated patients, calculated for Sept.-Oct. serum samples in 1975-77 for patients remaining on the study. This difference in the geometric means was not, however, significant in these relatively small patient groups. The decrease in both allergen and allergoid dosage in 1978 produced a modest decline in IgG antibody titers to AgE (Fig. 5) but no appreciable

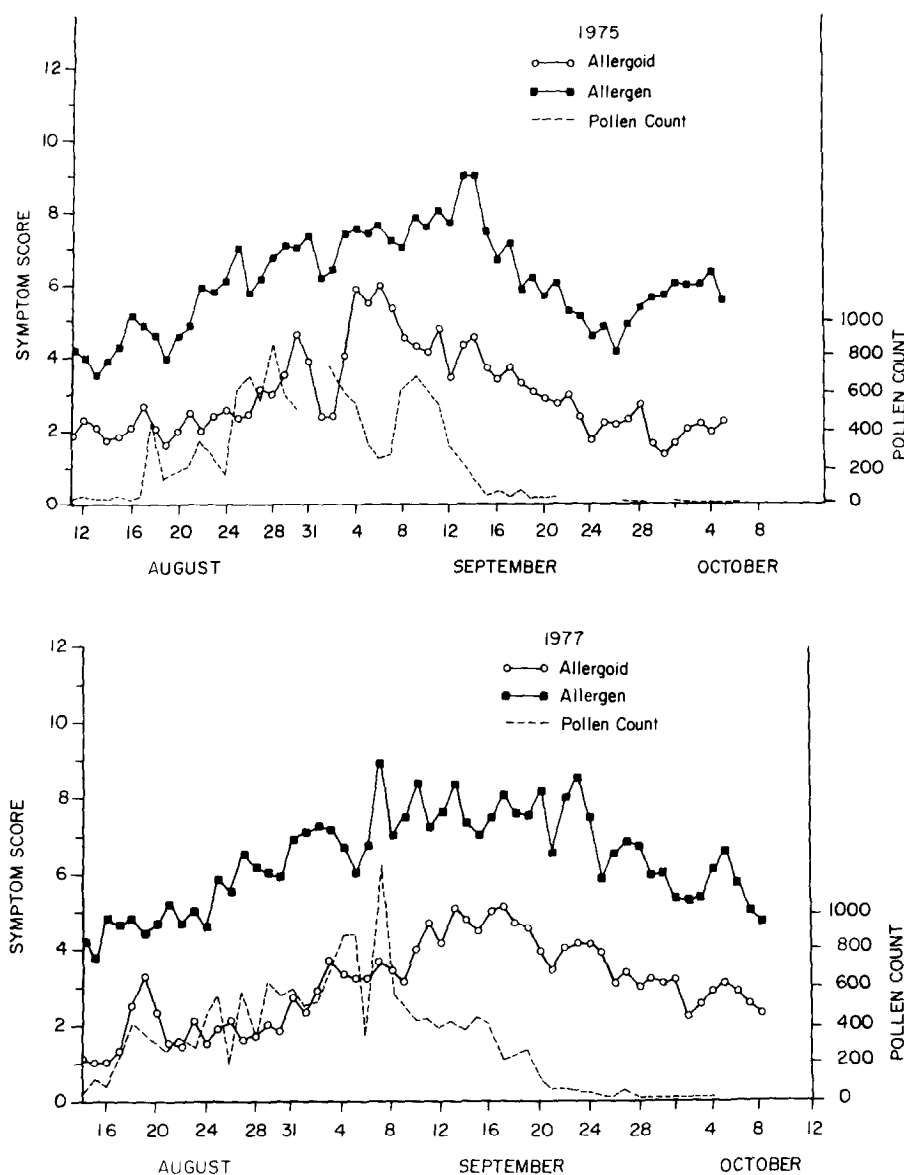


FIG. 7. Mean symptom (including medication) scores for each day during the 1975 and 1977 ragweed pollination seasons.

increase in reported symptom-medication scores (Fig. 2).

Fig. 6 shows the IgE antibody titers to AgE in the patients who continued therapy throughout the study. As in Fig. 5, dashed lines refer to patients who were treated prior to 1973. Where available, samples were analyzed prior to treatment (1970, 1972, or 1973), as well as posttreatment for the years 1970-73 (where applicable) and posttreatment for the year 1978. A few posttreatment sera for 1974 were also analyzed. As in other studies of immunization, IgE antibodies to AgE rose during the first preseasonal course and then began to decline slowly as immunization was continued. Every patient shows an overall IgE antibody

decline, relative to the pretreatment titer, which averaged about threefold by 1978. At the same time, all patients still had significant titers and none had declined to very low or unmeasurable levels. No difference between allergoid and allergen treatment was noted. Besides IgE responses to AgE, we also studied IgE antibodies to dialyzed crude ragweed allergen by the RAST procedure. The data obtained (not shown) showed essentially the same antibody changes as those with responses to AgE.

To show symptom-medication scores in more detail, Fig. 7 shows average scores for the two groups as compiled daily throughout the pollen seasons in 1975 and 1977. The corresponding 1977; on the other

hand, the 1974 data were similar to those for 1973. As in 1973, symptom scores were consistently lower in the allergoid-treated group for the years 1974-78. Our customary statistical test for such scores, the Mann-Whitney U test, showed a $p < 0.05$ for the difference between allergoid and allergen in 1975, 1976, and 1977. In 1974 and 1978 the difference was not great enough to meet this test of significance in groups of the size indicated in Fig. 2.

As determined by telephone interviews, no dropouts occurred because of untoward side effects. It should be noted that within any given group, the patients who dropped out report no more severe symptoms than those who continued in the study. Furthermore, investigation of the geometric mean leukocyte sensitivities to allergen determined in 1973 showed that the dropouts from the allergoid group had a sensitivity of 1.8×10^{-4} μg AgE equiv./ml and those from the allergen-treated group had sensitivity of 6.9×10^{-5} μg AgE equiv./ml. These geometric mean sensitivities are in both cases almost identical to those of patients starting the study. Hence the dropouts cannot account for the difference in symptom scores between the two groups in the latter years of the study.

During the period 1973 to 1978, specimens were taken from allergen- and allergoid-treated patients at least two times annually for blood counts, blood chemistries, and urinalysis. Although occasional blood chemistries showed values slightly outside the usual 2 SD from the mean customarily considered as the normal range, these values were in the normal range when next tested. Intercurrent acute infections produced abnormal white counts in two patients, which subsequently returned to normal. Three menstruating women developed slightly low hematocrit and hemoglobin determinations, which returned to normal when iron was administered. No urine abnormalities were noted.

DISCUSSION

After our initial studies showing reduced allergenicity of ragweed allergoid,¹ this study was undertaken to explore the short- and long-term safety and efficacy of ragweed allergoid when given in a manner comparable to the initial graduated increase in dose and perennial maintenance commonly employed with unaltered ragweed pollen extract. This seemed to be a prudent course, since the potential side effects of allergoid were unknown. As it developed, no side effects were observed except for some local allergic reactions mainly in the first year (one mild systemic reaction occurred in 1972), which were indistinguishable in timing or appearance from reactions to unal-

tered extracts. Despite the high dosages of antigen administered over a long period, especially of allergoid, there was no evidence of untoward toxicity as assessed by blood chemistry, hematology, and urinalysis. When both allergoid and allergen treatments were pushed to tolerance, it was found that 50-fold higher doses of ragweed protein could be maintained with allergoid. The immunologic and clinical changes observed under these conditions suggest certain important advantages of allergoid over allergen treatment: (1) significantly higher IgG antibodies after 1 yr of treatment (Fig. 4) and (2) significantly better clinical efficacy after 3 or more years of treatment (Figs. 2 and 7).

A further advantage of allergoid is its excellent stability relative to allergen. Thus it was necessary to prepare fresh stocks of allergen (particularly dilute solutions) on a very regular basis because of the well-known instability of aqueous ragweed allergen solutions (see Materials and Methods). On the other hand, allergoid stocks were only reprepared from lyophilized material as they became depleted (every 2 to 3 yr). Similarly, fresh dilutions of the stocks were prepared infrequently as needed. Despite prolonged storage of at 4° C (and at ambient room temperature during treatment sessions), the allergoid solutions remained effective both in terms of immunogenicity and clinical efficacy.

With continued treatment, geometric mean IgG antibody levels to AgE of the two groups tended to come together, although the levels in the allergoid-treated patients were still 1.4-fold higher during the 1975-77 period of "plateau response." The 50-fold ratio in annual cumulative dose between allergoid and allergen was maintained throughout most of the study (Fig. 1). With regard to IgG titers, it seemed unlikely that larger maintenance doses would have pushed titers higher. Indeed, from our more recent studies (to be reported) it appears probable that we greatly exceeded the dose necessary for maintenance of plateau titer in the allergoid group. Presumably, control mechanisms come into play, which limit the maximum IgG response to AgE to a level characteristic for each patient. On the other hand, symptoms of hay fever were 2 to 2½ units less in the allergoid group during most of the study (Figs. 2, 3, and 7).

Since changes in IgE and IgG antibodies to AgE were similar, the lower symptom-medication scores in the allergoid group may reflect differences either in patient selection or in immunologic changes such as IgG responses to minor ragweed allergens or other responses not susceptible to measurement. Two potential sources of unequal matching during selection are sensitivity to allergens such as molds or other fall

weed pollens and sensitivity to allergens in ragweed other than AgE. Random selection should distribute these differences equally in the two groups. The two treatment materials could differ in their ability to induce immunologic responses to minor allergens, for which tests are being developed. Furthermore, the immunologic responses to immunotherapy described to date do not correlate well with clinical improvement, leaving open the possibility that still other changes are important for clinical relief.¹⁴

After the early rises in serum IgE antibody titers during the first year of treatment with allergen or allergoid, levels of IgE antibody generally fell during prolonged allergen or allergoid therapy (Fig. 6). These findings are similar to those noted in previous studies of allergen immunotherapy.¹⁵ Such rises and falls in IgE titer are, respectively, consistent with (1) initial stimulation of IgE-producing cells by parenterally administered allergen when levels of IgG antibody are insufficient to prevent allergen from reaching these cells and (2) absence of restimulation of IgE-producing cells by antigen (parenterally administered or naturally inhaled) in the presence of high levels of IgG antibody. It is also possible that regulation of IgE and IgG antibody synthesis are to some extent independent of each other and that during immunotherapy inducer and suppressor T cells for specific antibody of each class govern the changes in antibody synthesis. Two recent papers present evidence that peripheral blood cells identifiable as suppressor cells (either by surface markers or effects on lymphocyte proliferation) are increased during immunotherapy.^{16, 17}

These studies suggest that a relatively brief course of primary immunization with allergoid would take best advantage of its relatively low reactivity and result in rapid attainment of increased IgG antibodies and clinical relief. This hypothesis was tested from 1977 to 1979 and, as will be reported in subsequent papers, short courses of allergoid induce favorable clinical and immunologic responses similar to those reported here.

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