

COMPARISON OF POLYMERIZED AND UNPOLYMERIZED ANTIGEN E FOR IMMUNOTHERAPY OF RAGWEED ALLERGY

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Abstract Polymerization of ragweed antigen into high-molecular-weight polymers could improve immunotherapy for ragweed pollinosis by reducing side effects while retaining immunogenicity. To study this thesis, 23 ragweed-sensitive patients were treated with either ordinary ragweed antigen E or ragweed antigen E polymerized by glutaraldehyde. Four patients received the polymerized antigen, and six controls ordinary antigen according to the standard immunotherapy schedule; two groups of three patients received either form by a "doubling-dose" schedule.

Seven subjects received the polymerized antigen by a schedule in which each successive dose was tripled. Serum antigen E binding capacity (blocking antibody) increased significantly in all subjects ($P < 0.001$ by Student *t*-test). Patients treated with polymerized antigen had fewer local and generalized reactions than those receiving the monomeric preparation.

Polymerized ragweed antigen permits more rapid immunization of atopic persons, with fewer side effects, than standard monomeric preparations. (N Engl J Med 295:1160-1164, 1976)

IMMUNOTHERAPY for ragweed pollinosis is an effective form of therapy for the atopic person as indicated by studies recently reviewed.¹ However, the use of immunotherapy is limited by local and systemic reactions. Efforts to improve immunotherapy have included "repository therapy" using mineral oil and emulsifiers, alum-precipitated extract and the modification of antigen to reduce allergenicity but retain immunogenicity.² The polymerization of antigen into high-molecular-weight polymers represents a further effort to improve immunotherapy by reducing allergenicity while retaining immunogenicity.

Recently, we reported the polymerization of ragweed antigen E with glutaraldehyde into a selected high-molecular-weight polymer with a molecular weight of 200,000 to four million daltons.³ This polymerized ragweed antigen E was found to be slightly more antigenic in animals than antigen E monomer,^{3,4} and it required 10 to 100 times as much polymerized antigen on a weight basis as the monomer to release an equivalent amount of cellular histamine.³ The rate of disappearance of the polymerized antigen from skin sites is less than that of ordinary antigen, and the polymerized form was found to localize to spleen and liver rather than to be excreted in the urine after intravenous injection.⁴ A very-high-molecular-weight polymer (range of four to 20 million daltons) was also prepared but was not used for human studies because in rabbits it was found to induce more IgE antibody than the lower-molecular-weight polymer.³ These studies suggested that polymerized antigen might be useful for immunotherapy.

To show the usefulness of the polymerized antigen for ragweed immunotherapy, clinical studies were undertaken comparing it with regular antigen in 23 un-

treated ragweed-sensitive patients. The first phase of the study was designed to prove its clinical safety, effectiveness and immunogenicity in human beings. Further studies were undertaken to determine if polymerized ragweed antigen E could be given more rapidly, but with less risk to the patient, than antigen E monomer during immunotherapy. This comparison was made with the knowledge that antigen E had already been shown to pose somewhat less risk during immunotherapy than crude ragweed antigen,⁵ but to possess equal clinical efficacy.

The results of the studies suggested that the polymerized antigen was safe, effective and capable of inducing blocking antibody in treated patients. A maintenance dose could be given in as few as nine injections, in less than one-third the time required by crude-ragweed therapy, with little risk to the patient.

MATERIALS AND METHODS

Selection of Subjects

The investigator who was to receive the dose equivalent of a three-year course of polymerized ragweed antigen E to demonstrate safety was nonatopic and had no history of rhinitis or asthma. Subsequently, 23 atopic subjects with marked skin reactivity (prick test) to crude ragweed (1:20, Hollister-Stier, Spokane, Washington) and a seasonal history of ragweed pollinosis were chosen to participate in the study. Two subjects had previously received immunotherapy, each 20 years before this study. Subjects were men or women between the ages of 25 and 40 years and matched as far as possible by cellular reactivity or sensitivity. Women received only standard ragweed antigen E. The project had been approved by the Human Subjects Review Committee of Northwestern University Medical School.

Method of Immunotherapy

Immunotherapy was initiated in a step-wise fashion with either standard ragweed antigen E (polymerized ragweed antigen E, or a polymer of antigen E-IVC, molecular weight of 200,000 to four million daltons) as previously described.³ The polymerized antigen was made in the manner previously described by polymerization of monomer antigen with glutaraldehyde treatment. The amount of standard antigen given per treatment was 0.1 mg; this was equivalent to the amount given during our previous treatment of im-

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Supported by the Ernest S. Bazley Grant and by a grant (AI 11403) from the National Institute of Allergy and Infectious Diseases.

munotherapy with crude ragweed.⁶ The protein content of the antigen was determined from optical-density measurements at 280 nm with a Beckman DU spectrophotometer. The final maintenance dose of either preparation, containing 18.75 μ g of antigen E was considered equivalent to the antigen E in 0.50 ml of 1:50 crude ragweed, a dose used commonly for high-dose therapy.⁷ Each preparation of either antigen was filtered through a 0.20- μ m bacterial filter (Nalge Filter Unit, Nalge Sybron Corporation, Rochester, New York) and cultured for bacteria and fungi. Polymerized ragweed antigen E was prepared every six months. Both forms were diluted in a solution of phosphate-buffered 0.15 M sodium chloride, pH 7.35, containing 0.5 per cent phenol and were stored at 4°C. Both polymer and monomer were renewed for treatment purposes every two to three weeks, although polymerized antigen has been shown to have a shelf life of at least six months.⁷

Treatment Schedule and Clinical Studies

Before its use in patients, one of the physician-investigators received the equivalent of three years' treatment with the polymerized antigen at regular intervals in an accelerated schedule for six weeks. Routine laboratory studies, including complete blood count, differential, sedimentation rate, urinalysis, SMA-12 and chest x-ray examination were performed before treatment and at regular intervals during treatment in this subject and the atopic patients.

Immunotherapy was given to different groups of subjects in three phases. During the first phase (I), the subjects were separated into two groups; one group of four patients received the polymerized antigen, and the other group of six standard antigen, both groups according to a schedule used for standard immunotherapy, which requires 27 weeks to reach maintenance dose. In the second phase (II), two groups of three patients each received the polymer or the monomer, so that each subsequent dose was twice that of the previous dose. Attainment of the maintenance dose required 13 injections. Finally, in phase three (III), a group of seven subjects received only polymerized antigen so that each subsequent dose was tripled. If a local or systemic reaction occurred in either phase II or phase III, the dose at which it occurred was reduced according to a standard protocol and slowly increased until the maintenance dose was tolerated at weekly intervals. Antigen E monomer was not given in phase III because of the potential risk of major local or systemic reactions as evidenced by results of phase II of the study.

In the ragweed season before treatment and during each subsequent ragweed season, the subjects were asked to complete a daily-symptom-score questionnaire from August 15 through October 1. The daily symptom score was derived from weighted total scores recorded by the patient for symptoms experienced and the amount of medication needed to control his symptoms similar to the symptom-score index described previously in the Johns Hopkins studies.¹⁰ Subjects were also questioned and examined weekly by a physician during each season. Local reactions were measured and recorded 30 minutes after an injection by a registered nurse, who did not know which antigen the subjects were receiving. A record of late reactions and systemic symptoms was kept by the physicians and patients.

IMMUNOLOGIC STUDIES

Serum samples. Serum was obtained from each subject when 1, 10, 100, 300, 500, 700 and 1000 μ g monomer or polymerized antigen had been given. These samples were analyzed for antigen-E-specific IgE and total serum antigen E binding capacity. Samples drawn before treatment were labeled pretreatment samples, and those drawn when 10 μ g of AgE had been given were labeled early treatment. Samples drawn when at least 100 μ g of AgE had been given were arbitrarily called late-treatment samples.

The presence of antibodies of other immunoglobulin classes. Antigen-specific IgE. The serum concentration of IgE specific for antigen E was measured with the polystyrene-tube radioimmunoassay technique previously described.¹¹ The advantage of this assay is that the measurement of IgE antibody is not affected by

gen E used for these and the total antibody measurements described below was supplied by Dr. Floyd McIntire.¹²

Measurement of total blocking antibody specific for AgE. Total serum antigen-E-specific binding capacity was measured with a modification of the procedure initially described by Lidd and Farr.¹³ In these studies, antigen E was labeled with ¹²⁵I, and the antibody was precipitated by 40 per cent saturated ammonium sulfate. The antibody was assumed to be largely IgG because of the comparatively small amounts of IgE simultaneously precipitated (unpublished data). In addition, the levels of IgE specific for antigen E simultaneously present were calculated to be very small by a comparison using the techniques described above. In these measurements, total specific antibody (blocking antibody) was estimated by the amount of antigen bound when 800 ng of trace-labeled antigen E was added to 0.1 ml of serum and precipitated by 40 per cent saturated ammonium sulfate. Precipitation occurred near antigen excess and appeared to be a close estimate of total serum specific antibody.

Measurement of cellular responsiveness. Cellular responsiveness (reactivity or sensitivity) was measured by histamine release from human peripheral blood leukocytes with use of antigen E. Dose-response histamine-release curves were established as previously described¹³ with use of 30 ml of heparinized blood drawn before treatment and after each ragweed season. Histamine release was measured in most cases in early and late treatment. Cellular reactivity was defined as the maximum percentage of cellular histamine release, regardless of the antigen E concentration effecting release. Cellular sensitivity was defined as the negative log of the micrograms of antigen E required to release 50 per cent or 30 per cent of the cellular histamine. Thus, a numerical decrease in sensitivity indicated that the cells had become less sensitive to the antigenic stimuli.

RESULTS

Clinical Safety

Routine laboratory data obtained on the investigator subsequent to the accelerated course of treatment with polymerized ragweed antigen E were normal. Likewise, all laboratory data obtained from the first 10 subjects treated with monomer or polymer during a year and a half of therapy were normal. There appeared to be no toxic effects from the antigen.

Treatment Schedules

Table 1 summarizes the mean number of injections required to reach maintenance doses with the three treatment schedules and the mean group antibody level attained when 1, 10, 70 (maintenance), 100, 300 and 500 μ g of antigen E had been given. These data show that standard treatment schedule required approximately 29 injections to reach maintenance with either polymer or monomer. When the polymerized antigen was given in doubling doses, the number of injections required to reach maintenance was reduced to less than half that of standard therapy. However, by use of the tripling-dose schedule, maintenance doses of polymer could be given with nine weekly injections. No local reactions were encountered in this reaction three hours after the injection upon receiving the initial maintenance dose. After the maintenance dose was reached, all groups received an equivalent amount of antigen per injection. Thus, in late treatment the savings in time when this regimen was used did not steadily increase.

Table 1. Number of Injections Required to Give Increasing Amounts of Antigen E (AgE) by Three Separate Therapeutic Schedules and Mean Blocking Antibody Level Attained.

AMOUNT OF AGE	MEAN NO. OF INJECTIONS	BLOCKING ANTIBODY*
μg		μg
Monomer standard* (6):		
1	12.5	835
10	22	1,037
Maintenance†	29	ND‡
100	30.5	1,848
300	42.4	ND
500	51	2,117
Polymer standard (4):		
1	12	854
10	22	1,055
Maintenance	29	ND
100	31	1,257
300	39	ND
500	48.7	1,360
Monomer doubling dose (3):		
1	6	560
10	12	733
Maintenance	21	ND
100	23.7	1,233
300	30	2,420
500	40.5	ND
Polymer doubling dose (3):		
1	6.7	597
10	10.7	667
Maintenance	13.3	ND
100	15.3	1,283
300	26	1,967
500	37	2,900
Polymer tripling dose (7):		
1	5	1,023
10	8.1	1,288
Maintenance	9.4	ND
100	13.2	2,760
300	23	3,973
500	34	ND

*AgE bound by 1 ml of post-treatment serum.

†Refers to usual treatment regimen in clinical practice (see text).

‡Figures in parentheses denote no. patients.

§Cumulative dose at maintenance necessarily less with accelerated schedules than with standard immunotherapy.

* Not done.

Therapeutic Safety

Table 2 summarizes the local and systemic reactions encountered in the combined experience with the two antigens. Similar amounts of antigen were given to each group, but 45 times as much erythema and 17 times as much wheal or induration was encountered when the monomer was used. Four times as many generalized reactions were observed with monomer as polymer. The number of injections given each group were nearly the same, but the polymer

Table 2. Comparison of Local and Generalized Reactions Obtained with Polymerized (PRW) and Monomer (RW) Ragweed Antigen E.

ANTIGEN	TOTAL GIVEN	ERYTHEMA*	INDURATION* OR WHEEL	GENERALIZED REACTIONS
μg				
RW (9)†	5,171	4.5	2.8	8
PRW (14)	6,721	0.10	0.17	2

*Expressed as mean cm/100 μg of AgE given.

†Figures in parentheses denote no. of patients.

group received on the average more antigen per injection than those treated with monomer.

All the generalized reactions in both treatment groups were minimal, consisting of mild, generalized urticaria or a feeling of tightness in the chest, that was reversed by epinephrine or antihistamine given by mouth. Although these reactions were not severe, they represented the potential for serious adverse reactions and were considered of potential importance.

It is important that with polymerized antigen E one of the two systemic reactions was doubtfully due to the polymer, that the other was a mild, late urticarial reaction, that approximately 1500 μg (375,000 protein nitrogen units) more of antigen E polymer were given than monomer and that the total dosage of polymer antigen E given was approximately equivalent to 27 patient-years of standard immunotherapy.

Immunologic Studies

Blocking antibody. Blocking antibody increased from pretreatment levels in all subjects. In some cases, peak levels of blocking antibody in late-treatment samples were more than 10 times pretreatment levels. The mean rise in blocking antibody for each treatment group was approximately fivefold. The mean log ($\pm$ S.D.) for the monomer-treated group rose from 2.7 ± 0.3 to 3.4 ± 0.2 , whereas that for the polymer-treated group rose from 2.6 ± 0.4 to 3.3 ± 0.4 . The levels of blocking antibody in most patients remained stable after reaching peak levels; however, in a few patients the blocking antibody began to fall late in treatment. This phenomenon has been observed by other investigators.^{14,15}

As shown in Table 1, the time required for maximal production of blocking antibody is related to the quantity of antigen given per injection. Thus, the mean level attained in the groups treated by the tripling-dose schedule after 100 μg of antigen E had been given was greater than that in any other group in spite of the fact that only 13 weeks had elapsed since the beginning of treatment.

Specific IgE. The change in serum IgE specific for antigen E from pretreatment to early and late levels was similar in both treatment groups. There was no statistically significant increase or decrease in early or late treatment. However, the mean antibody level for each group decreased in late treatment as compared with early treatment, but because of the large standard deviations and small numbers, this change did not reach statistically significant levels. Serum specific IgE appeared to increase in each patient with the beginning of treatment and with each ragweed season. Because of these complicated changes in IgE levels, we calculated the rise in serum specific IgE after the ragweed season before and after treatment. Figure 1 shows that the post-seasonal rise in specific IgE was significantly reduced after a single season of treatment ($P < 0.01$, with the paired Student t-test). This would support other data, showing that immunotherapy diminished the "boost" in IgE after a ragweed season.^{16,17}

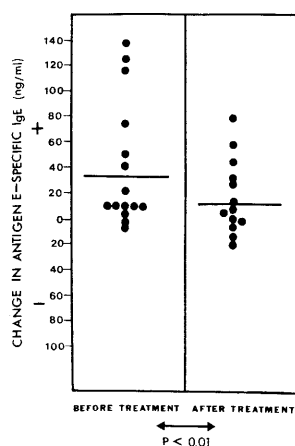


Figure 1. Seasonal Changes in Specific IgE.

The diminished post-seasonal rise in IgE specific for antigen E after one year of treatment with either monomer or polymer is significant at $P < 0.01$ (arrow) by paired Student t-test. The mean change in specific IgE is shown by the solid lines.

Cellular responsiveness. Table 3 demonstrates that in late treatment, cellular responsiveness of most patients, as measured by reactivity or sensitivity, decreased. Several patients' cells were found to become "unreactive" to antigen after therapy. This phenomenon was found in those treated with monomer or polymer.

Clinical efficacy. All subjects were relieved of symptoms during their first season after treatment. Ragweed counts during the 1975 season showed three to four peaks of antigen exposure that were associated with peaks in symptoms experienced by a group of untreated subjects (Fig. 2). The mean symptom scores of the groups treated with monomer and polymer were less than those of the untreated control group.

This untreated group comprised three ragweed-sensitive patients who met all the criteria for entrance into the study. The mean daily symptom scores of these patients are shown in Figure 2. In addition to this control group, 13 or 23 patients collected symptom scores in the ragweed season before therapy and again in the ragweed season after therapy, serving as their own controls. The mean daily symptom scores of

these 13 patients before therapy were almost identical to those of the untreated control group shown in Figure 2.

DISCUSSION

The purpose of this study was to show the clinical safety of polymerized ragweed antigen³ by routine laboratory data, to prove its immunogenicity in spite of a reduced allergenicity, to indicate its effectiveness in symptom control during immunotherapy and to demonstrate its increased safety during immunotherapy with fewer injections to reach maintenance.

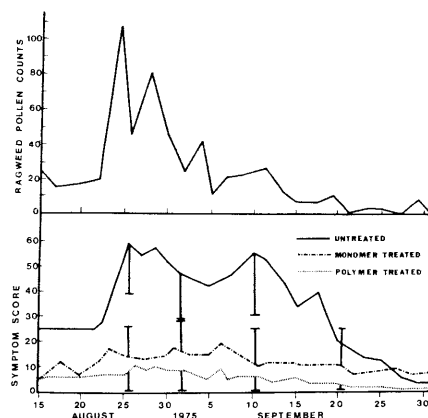


Figure 2. Mean Daily Ragweed Pollen Counts Averaged Two Days at a Time (Upper Graph) and Mean Symptom Scores Recorded during the 1975 Ragweed Season by a Group of Untreated Patients and the Groups Treated with Polymer or Monomer (Lower Graph).

Representative mean ($\pm$ S.D.) symptom scores of the treated and untreated groups are significantly different ($P < 0.05$, by Student t-test), as are those for the intercurrent days, except for day 20 and thereafter.

During the first phase of the study, one investigator who received the equivalent of three years of therapy (approximately 775 μ g of antigen E) and those treated with either monomer or polymer underwent 1 1/2 years of therapy without any abnormal laboratory data. Because of the absence of any evidence of toxicity the polymerized antigen was considered safe for clinical trials of immunotherapy in patients in the same manner as standard therapy.

In the third phase, seven patients treated with the polymer were able to reach the full maintenance dose in less than one-third the time required for standard therapy. The safety of the polymerized antigen may be a result of its slower absorption from the injection site and the fact that there are fewer molecules per unit weight in a polymer, so that less molecular bridg-

Table 3. Changes in Cellular Responsiveness in Late Treatment with Monomer (RW) or Polymerized (PRW) Antigen E.

ANTIGEN	NO. OF PATIENTS	PATIENTS WITH DECREASE	PATIENTS WITH INCREASE	PATIENTS WITH NO CHANGE
RW	6	4	1	1
PRW	8	8	0	0
Totals	14	12 (86%)	1 (7%)	1 (7%)

ing of surface-bound IgE molecules is possible. Furthermore, some antigenic determinants may be inside the large polymer that cannot attach to cell-bound IgE.

In these studies, polymerized antigen E was found to be as immunogenic as the monomer, producing comparable levels of blocking antibody during therapy (Table 1). The production of antibody was greater in the subjects treated with polymerized antigen in tripling doses than in the other groups (Table 1). Of special interest is the fact that after 100 μ g of antigen E had been given, these antibody levels appeared to be greater than in the other groups treated at a slower pace. This form of accelerated immunotherapy also appeared to be associated with rapidly falling serum IgE specific for antigen E in a few cases, in spite of current ragweed seasons. These results suggest there may be a correlation between rapidly rising IgG blocking antibody and decreasing antigen-specific IgE.

Cellular responsiveness fell in late treatment regardless of which type of antigen was used for therapy (Table 3). Although cellular sensitivity may be increased soon after treatment is begun, a finding probably related to an early treatment boost in specific IgE, histamine release decreased in late treatment in a majority of patients.

Clinical benefit was seen in all patients during their first post-treatment season, suggesting that the polymerized antigen is as clinically efficacious as the monomer or crude ragweed.

In the light of these studies, polymerized ragweed was found to be a safe and effective form of immunotherapy. It is immunogenic and results in clinical improvement comparable to that obtained with monomer antigen E, the major antigenic component of ragweed.⁶ Immunologic changes found with polymerized ragweed were similar to those found with standard therapy as reviewed recently.¹⁸ Contrary to other attempts at reducing allergenicity, such as formalin treatment to modify antigenic determinants,^{2,19} the antigenic determinants of the polymerized antigen are not destroyed or modified, but retain their native ability to induce antibody. It is important that the equivalent maintenance dose of either monomer or polymer produced similar levels of blocking antibody. Much more formalized antigen (allergoid) appears to be required to induce an antibody response similar to that obtained with unaltered antigen.

In conclusion, we believe that polymerized antigen represents an improved form of immunotherapy that is as effective as standard immunotherapy but offers the important advantage of rapidly administered doses with greater safety. Polymerized antigen would

allow a rapidly effective dose pre-seasonally and offer the advantages of immunotherapy to a greater number of patients with less risk and less expenditure of time and money. This concept should be applied to whole ragweed and other seasonal inhalant antigens. A clinical trial using polymerized grass antigen is being evaluated.

We are indebted to Mr. Herbert Peak, Hinsdale Sanitarium and Hospital, Hinsdale, Illinois, who kindly supplied the ragweed counts for the 1974 and 1975 seasons.

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