

A multi-institutional trial of polymerized whole ragweed for immunotherapy of ragweed allergy

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Eighty ragweed-sensitive patients in four cities were recruited to study the safety and efficacy of partially purified, polymerized whole ragweed (PRW) as an improved form of immunotherapy. Groups of 20 patients in Chicago, Boston, Memphis, and St. Louis had blood drawn for immunologic studies before and after the 1978 and 1979 ragweed seasons and completed detailed daily symptom score sheets each day of the 1978 and 1979 ragweed pollen seasons. Beginning in March, 1979, all patients except one received 15 weekly injections of PRW totaling 50,000 protein nitrogen units (PNU) and containing about 500 µg ragweed AgE. One patient received 25,000 PNU. Symptom score indices of the posttreatment 1979 season were compared with those from the pretreatment 1978 season and also with the scores of similar groups of ragweed-sensitive patients in each city treated only with medication for symptomatic relief during the 1979 season. Local reactions to polymerized ragweed immunotherapy were minimal. No abnormalities in complete blood count, erythrocyte sedimentation rate, chest x-ray film, urinalysis, or rheumatoid factor occurred in the immunotherapy-treated groups. Total serum antibody binding of ragweed AgE increased 12 fold following immunotherapy. When compared either with their 1978 untreated group scores or when compared with scores from the untreated group in each city in 1979 (control group), the symptom score indices of the immunotherapy-treated groups in 1979 were significantly improved. PRW is efficacious in the treatment of ragweed hay fever and can be administered more safely and in higher doses with fewer injections than conventional extracts. It represents an improved form of immunotherapy.

Immunotherapy for pollinosis has been practiced by allergists since 1911.¹ Recent reviews have concluded that immunotherapy is an effective form of

therapy for the atopic patient in selected, defined studies.²⁻⁴ Despite this efficacy, immunotherapy has from the beginning presented major problems. The potential for local and systemic reactions limits immunotherapy to those patients with symptoms uncontrolled by various medications and who are willing to undergo prolonged treatment with gradually increasing amounts of antigens. Although reactions following cautious therapy with aqueous extracts of ragweed are usually not severe, anaphylactic shock does occur.⁵

To improve upon immunotherapy with currently available aqueous extracts, ragweed antigens were prepared as high-molecular weight polymers to retain their immunogenicity with reduced allergenicity.

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Immunogenicity here refers to the induction of IgG antibodies, whereas allergenicity refers to the ability to elicit IgE-mediated reactions. The reduced allergenicity of these polymerized preparations offers improvement over standard aqueous immunotherapy by allowing a larger starting dose for therapy, by permitting the administration of larger doses on maintenance therapy, by decreasing the number of injections for effective therapy, by decreasing the incidence of local and risk of systemic immediate-type reactions, and by decreasing the cost and inconvenience to the patients in terms of physician visits.

We have previously reported the polymerization with glutaraldehyde of ragweed AgE, partially purified whole ragweed, and partially purified grass into polymers with molecular weight ranges of 200,000 to 20 million daltons.⁶⁻⁸ In vitro experiments with polymerized AgE demonstrated that 10 to 100 times as much polymerized antigen on weight basis as monomer AgE was required to release histamine from leukocytes of ragweed-sensitive individuals.⁶ Studies in animals showed the rate of disappearance of polymerized AgE from skin sites was slower than that of monomeric AgE and the radiolabeled polymerized form was localized preferentially to the liver and spleen after intravenous injection in contrast with monomer AgE, which was excreted in the urine.⁹ Studies in animals also showed that polymerized AgE induced the formation of antibody against AgE in concentrations equivalent to that following injection with monomer AgE.⁶

These studies suggested polymerized antigens might be useful for immunotherapy in humans. Results of an initial study with polymerized AgE in humans demonstrated the immunotherapy was safe, reduced the incidence of immediate reactions, was capable of inducing antibody, and resulted in a reduction in allergic symptoms during the ragweed season.¹⁰ Further trials at this institution with partially purified, polymerized whole ragweed (PRW) established a safe dosage range that caused minimal local reactions and appeared to reduce allergic symptoms in treated individuals.¹¹⁻¹³

To further test the safety and efficacy of PRW as an improved form of immunotherapy, a multi-institutional trial was designed. Twenty patients suffering typical symptoms of ragweed hay fever were recruited in Boston, Memphis, St. Louis, and Chicago by independent investigators prior to the 1978 ragweed season. Symptom score diaries were completed by each patient in 1978 prior to treatment with PRW. After PRW immunotherapy, symptom score diaries were completed by each patient and were compared with

TABLE I. Immunotherapy schedule

Injection number	Amount injected (PNU)
1	100
2	250
3	500
4	1,000
5	1,500
6	3,000
7	5,000
Injections 8-15	5,000 each

Total dose: 50,000 PNU with 500 μ g AgE.

the symptom score diaries of the previous year. Because the severity of ragweed allergy symptoms may vary from year to year depending on pollen concentrations, a second group of 20 untreated patients (control group) in each of the four cities similarly completed diaries during the 1979 ragweed season. This allowed a comparison of allergic symptoms between immunotherapy-treated and nontreated patients during the same season. Immediate and late reactions to PRW immunotherapy were quantitated and certain laboratory parameters were followed as indices of long-term safety. In addition, measurements were made of IgE binding of AgE and total AgE binding by serum samples before and after immunotherapy.

MATERIALS AND METHODS

Selection of patients

Before the ragweed season of 1978, between 20 and 24 patients with typical histories of ragweed hay fever were recruited in Boston, Memphis, St. Louis, and Chicago. Subjects were men or women between the ages of 25 and 40 yr. No patients had received any form of immunotherapy within 10 yr of this study. Patients with marked skin sensitivity (prick test) to commercial short and giant ragweed (1:20) were chosen to participate in the study. Similar groups of 20 to 24 patients were selected prior to the 1979 ragweed season to serve as untreated (control) groups to minimize the effect of variation in pollen seasons between 1978 and 1979. Skin sensitivity was the same in control patients as in the treated groups and clinical histories were similar.

Completion of symptom score diaries

Daily symptom score sheets were completed by each patient from August to October of 1978. These diaries allowed the patient to quantitate the severity of the following allergic symptoms during the morning, afternoon, and evening; sneezing, nasal congestion, nasal discharge, itchy eyes, cough, and wheezing. In addition, each patient was treated with medications as necessary and the type and amount of these daily medications were also recorded. During the hay fever season of 1979, daily symptom scores were again

TABLE II. Symptom score improvement in PRW-treated patients

	Percentage of patients with improvement of symptom score, 1978-1979		Range of individual score improvement, 1978-1979	Mean of group score improvement, 1978-1979
	Any improvement	30% or more decrease in season symptom score		
Chicago	88%	64%	5% to 79%	51%
St. Louis	82%	70%	12% to 80%	46%
Boston	94%	71%	7% to 100%	49%
Memphis	94%	64%	14% to 90%	61%

recorded by the groups of patients receiving polymerized ragweed immunotherapy prior to that season and by those patients not receiving immunotherapy.

Methods of polymerization of ragweed

These have been described previously in detail.⁶ Briefly, giant and short ragweed pollen were obtained from Hollister-Stier Laboratories (Spokane, Wash.). Following petroleum ether defatting, pollen antigens were extracted with NH_4HCO_3 and whole ragweed protein was precipitated by $(\text{NH}_4)_2\text{SO}_4$, after which monomer ragweed (MRW) was obtained following Sephadex G-15 chromatography. Protein concentration of MRW was determined by Kjeldahl's method. AgE content of MRW was determined by immunodiffusion. For each mole MRW (estimated molecular weight, 38,000), 100 moles glutaraldehyde (25% grade II, Sigma Chemical Co.) were added. The polymerization of gram quantities of MRW was carried out at room temperature for 4 hr and the reaction was stopped with the addition of glycine. By Sepharose 4B and Sephadex G-200 chromatography, PRW with a molecular weight range of 200,000 to 20 million was obtained. Preparations were cultured for sterility at 48 hr and 2 wk of culture. Safety and sterility requirements of the Food and Drug Administration Bureau of Biologics were completed by Allergy Laboratories of Ohio. Solutions of PRW were diluted to 10,000 PNU/ml (1 PNU = 0.01 μg protein nitrogen). One milliliter of PRW was estimated to contain 105 μg of AgE based on the AgE content of the MRW.

Schedule of immunotherapy

Beginning in March, 1979, each patient began a series of 15 weekly injections as outlined in Table I. If a patient developed a significant local reaction to any injection, the dose was repeated but injections would be continued to attempt to reach a total administered dose of 50,000 PNU (which contains about 500 μg AgE as measured in starting monomer).

Quantitation of allergic reactions to immunotherapy

Immediate local reactions to injections were observed and total centimeters of erythema or induration were recorded. Late local reactions at the injection site consisted of mild discomfort and swelling occurring 4 to 24 hr after

injection. Any additional injections required to achieve a total dosage of 50,000 PNU were recorded.

Laboratory parameters of treated patients

In addition to a physical examination performed before entry into the study, each patient received the following laboratory examinations: complete blood count, differential leukocyte count, rheumatoid factor, erythrocyte sedimentation rate, chest x-ray film, and urinalysis. These studies were repeated following the 15-wk course of PRW immunotherapy.

Immunologic studies

Blood specimens were drawn from each patient before and after the ragweed season in 1978 and again before and after PRW immunotherapy in 1979. Changes in AgE binding by IgE and changes in total AgE binding by serum samples were determined.

AgE binding by IgE was measured by the polystyrene tube radioimmunoassay technique described previously.¹⁴ Briefly, polystyrene tubes were coated with purified myeloma IgE, and anti-IgE (Behring Diagnostics, Somerville, N. J.) was then added in excess to form an IgE immunoabsorbent. After incubation with a 1:10 dilution of patient's sera, tubes were washed and ^{125}I -AgE was added. The nanograms AgE bound by specific IgE in 1 ml of serum in great antigen excess were determined and the nanograms IgE antibody specific for AgE per milliliter were calculated.

Total serum AgE binding capacity was measured by modification of the ammonium sulfate technique of Lidd and Farr.^{15, 16} Briefly, 0.1-ml aliquots of sera were incubated with 100 and 1,000 ng of ^{125}I -AgE. After appropriate incubation, the antibody-bound AgE was separated from the free AgE by precipitation with 40% saturated ammonium sulfate. The nanograms AgE bound by 1 ml serum in antigen excess were then calculated and the results expressed as nanograms AgE bound per milliliter of serum.

Seasonal pollen counts

Ragweed pollen was quantitated 5 days of each week during the ragweed seasons of 1978 and 1979, by rotating-arm impactor in Memphis and by gravitational method in the other three cities.¹⁷ In St. Louis counts were collected by the St. Louis Department of Community Health. In Chicago counts were collected at the Hinsdale

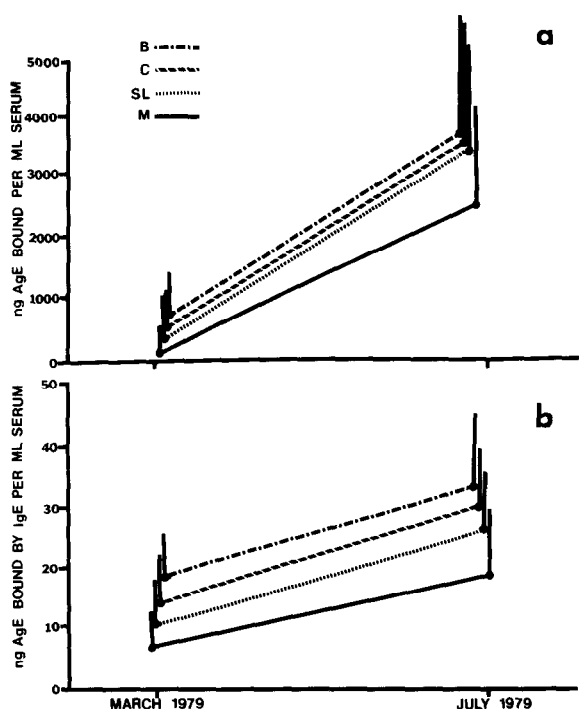


FIG. 1. Changes in serum antibody activity following immunotherapy with PRW. *a*, Increase in total serum binding of AgE for each PRW-treated group; *b*, Increase in AgE binding by IgE for each PRW-treated group.

Hospital and at Grant Hospital in cooperation with Hollister-Stier Laboratories. In the Boston area, counts were made at Chestnut Hill in Chelmsford, courtesy of Jack Farnham, M.D. Pollen counts in the Memphis area were made by the Memphis-Shelby County Health Department.

RESULTS

Quantitation of local reactions

A total of 240 cm of erythema and 80 cm of induration was recorded at injection sites for the 80 patients during the study. This represented approximately 2 mm of erythema and 0.7 mm of induration per injection. A total of 28 patients had a least one local reaction consisting of erythema and induration. Those immediate-type reactions ranged in diameter from 1 to 6 cm. A total of six patients suffered local, late reactions and required a total of 16 additional injections to reach 50,000 PNU. Three of these patients required one extra injection and three patients required three to seven extra injections. Late local reactions consisted of swelling about the injection area from 4 to 24 hr after injection of PRW. One patient suffered two late local reactions consisting of swelling from the shoulder to mid-arm area lasting for 24 hr and was maintained on weekly injections of 1,500 PNU and received a total of 25,000 PNU in 17 injections. The patient's first late local reaction oc-

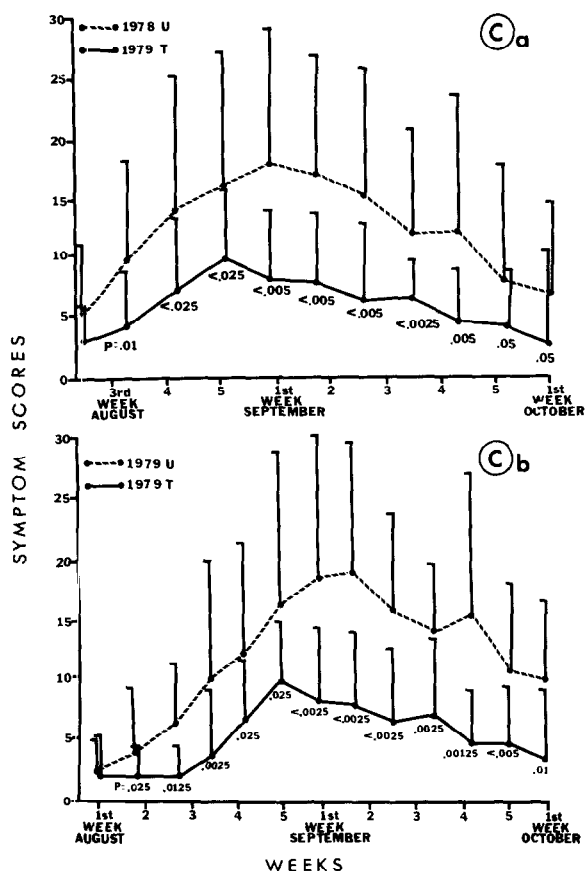


FIG. 2. Daily symptom score indices plotted for 1978 and 1979 for Chicago (C). In *a*, scores of the PRW-treated group (T) in 1979 are compared with the scores of the same untreated group (U) in 1978. In *b*, scores of the PRW-treated group (T) are compared with scores of the untreated control group (U) in 1979. The p value and 1 SD are shown for each point plotted.

curred at 500 PNU and the second occurred at 1,000 PNU. The patient was advanced to 1,500 PNU with diminishing local reactions and finally complete resolution of reactions. It was felt by the staff the patient could then tolerate higher doses of PRW but advancement to these doses was prohibited by time allowed for the study. No systemic reactions to immunotherapy were recorded.

Laboratory parameters of treated patients

No changes in any laboratory parameter accountable to immunotherapy were observed. Two patients had abnormal chest x-ray films before and after therapy. Pleural thickening was noted in the first patient and the second patient had a chest x-ray film suggestive of sarcoidosis. An additional patient was hospitalized for diagnostic work-up of hematuria noted before the start of immunotherapy and no pathology was found. A fourth patient had urinalysis findings positive for trace sugar following immunotherapy.

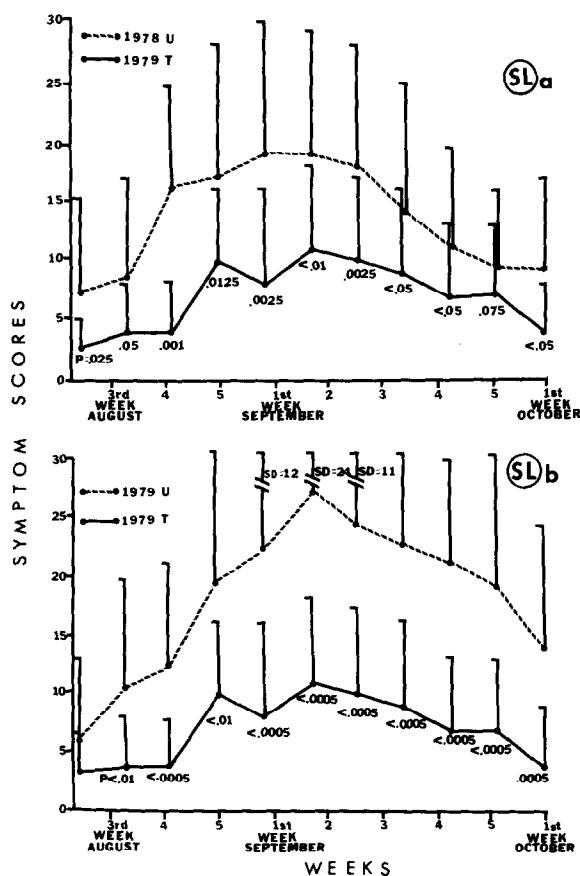


FIG. 3. Daily symptom score indices plotted for 1978 and 1979 for St. Louis (SL). See legend to Fig. 2.

Immunologic studies

Changes in serum immunoglobulins specific for AgE following immunotherapy with PRW are shown in Fig. 1 for each group. A mean 12-fold increase in total AgE binding by serum samples for the groups following immunotherapy with PRW occurred (Fig. 1, a). A mean 1.5-fold increase in IgE binding of AgE occurred following therapy with PRW (Fig. 1, b). Vertical lines indicate 1 SD above the mean for each group.

Improvement in symptom score indices

Graphs of the daily group symptom scores for the four geographic areas are shown in Figs. 2 through 5. Fig. 2 shows graphs prepared for the Chicago area. Fig. 2, a, is a comparison of the average daily indices of the group untreated with immunotherapy during the 1978 ragweed season (U) with the indices of the same group during the 1979 ragweed season following PRW therapy (T). Fig. 2, b, is a comparison of these same 1979 indices of the PRW-treated group (T) with the indices of the control group untreated with immu-

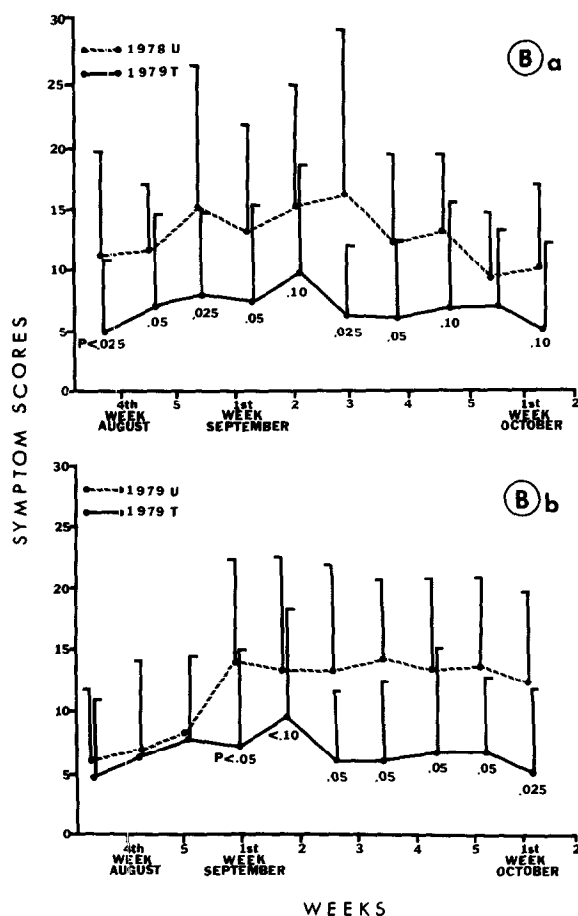


FIG. 4. Daily symptom score indices plotted for 1978 and 1979 for Boston (B). See legend to Fig. 2.

notherapy during the 1979 ragweed season (U). One standard deviation above the mean is shown by the vertical lines. The p values were determined by Student's t test. Fig. 2 shows that symptom score indices of the PRW-treated group were significantly improved during the 1979 ragweed season when compared with the same group's scores during the 1978 season or when compared with the untreated control group during the 1979 ragweed season.

Similarly, symptom score indices graphed for the St. Louis geographic area are shown in Fig. 3. When the symptom score indices of the PRW-treated group in 1979 were compared with the indices of the same group before immunotherapy during the 1978 ragweed season significant improvement was seen (Fig. 3, a). When scores of the PRW-treated group during 1979 were compared with scores of the control group during the 1979 season, significant improvement was again demonstrated (Fig. 3, b).

Symptom score indices were graphed for the Boston area and for the Memphis area in Figs. 4 and 5, respectively. For both areas, significant improvement

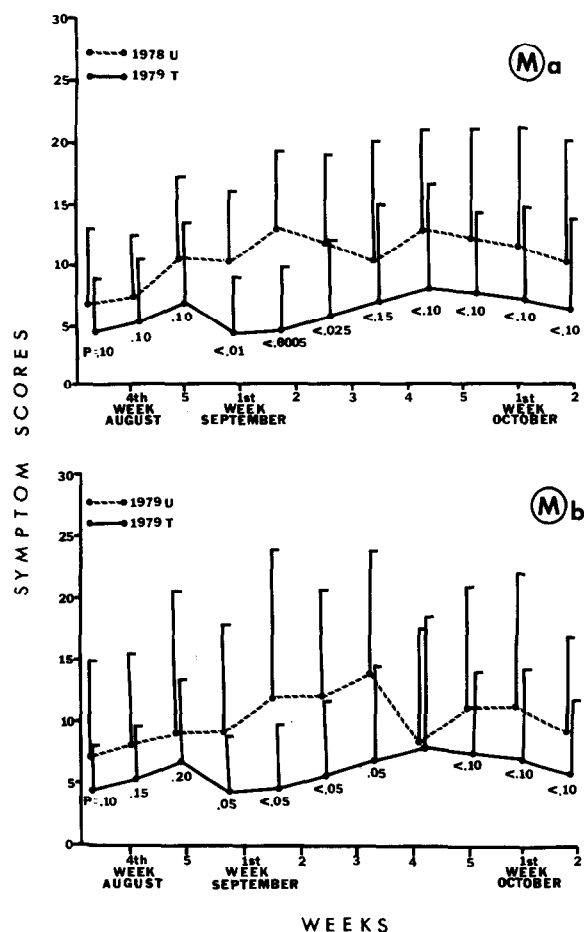


FIG. 5. Daily symptom score indices plotted for 1978 and 1979 for Memphis (M). See legend to Fig. 2.

on most days could be seen for the indices of the PRW-treated group when compared with the scores of the same group during the 1978 season or when compared with scores of the untreated control group during the 1979 season.

Graphs of the total season symptom scores are shown in Fig. 6 for the four geographic areas. For each graph, the first vertical line represents 1 SD from the mean of total season symptom scores for the group during the 1978 ragweed season (U). The second vertical line indicates scores for the same group during the 1979 ragweed season (T) following PRW immunotherapy. The third vertical line indicates scores for the control group (C) during the 1979 ragweed season. The p values were determined by Student's t test. In the Chicago area (C), total season symptom scores of the PRW-treated patients were improved significantly when compared with the same group's total scores for the 1978 season or when compared with scores of the control group during the 1979 season. Similarly, in the St. Louis area (SL), total scores of the PRW-treated group in 1979 were significantly bet-

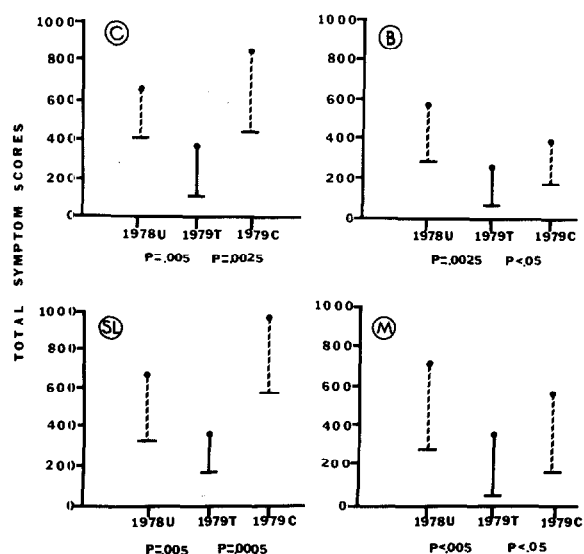


FIG. 6. Total seasonal symptom scores plotted for four geographic areas: Chicago (C), St. Louis (SL), Boston (B), and Memphis (M). For each graph, the first vertical line shows mean and 1 SD from the mean of total scores for the group prior to PRW immunotherapy. The second vertical line shows the mean and 1 SD from the mean of scores for the group following PRW immunotherapy during the 1979 ragweed season. The third vertical line shows the mean and 1 SD from the mean of scores of the control group during the 1979 season.

ter than scores of the same group in 1978 or the control group in 1979. Significant reduction was also noted for total scores of the PRW-treated group in Boston (B) and in Memphis (M).

Most patients treated with PRW had a reduction in their total season symptom score for the 1979 ragweed season when compared with their score for the 1978 ragweed season. Table II lists the percentages of PRW-treated patients for each geographic area who had some reduction in the 1979 score from the 1978 score. The degree of improvement in symptoms varied among patients. For each area, the range of individual reduction in symptoms along with the mean reduction for the group is shown in Table II.

Pollen counts for the 1978 and 1979 ragweed seasons for the four geographic areas are shown in Fig. 7. For Chicago (C), St. Louis (SL), and Boston (B), the pollen counts are plotted as total grains per square centimeter counted for Monday through Friday of each week. For Memphis (M), pollen counts are plotted as grains per cubic meter air counted from Monday through Friday of each week. There was a rough correlation between total season pollen counts in 1978 and 1979 (Fig. 7) and the total season symptom scores of the patients untreated with immunotherapy in 1978 and 1979 (Fig. 6).

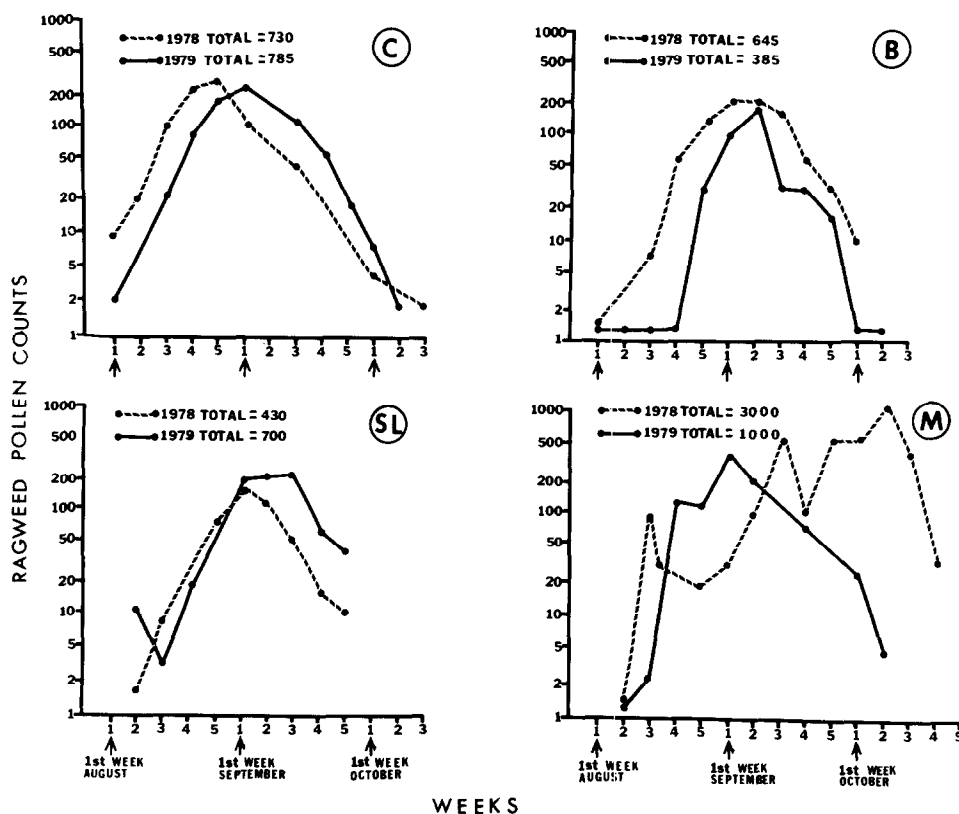


FIG. 7. Pollen counts collected in four geographic areas in 1978 and 1979 ragweed seasons: Chicago (C), St. Louis (SL), Boston (B), and Memphis (M). Counts obtained Monday through Friday of each week were added and plotted on a semilogarithmic scale. For Chicago, St. Louis, and Boston, pollen counts are plotted as total grains per square centimeter counted for Monday through Friday of each week. For Memphis, pollen counts are plotted as grains per cubic meter of air counted from Monday through Friday of each week.

DISCUSSION

Using conventional immunotherapy in the treatment of ragweed pollinosis, the estimated incidence of systemic reactions is 1/307 patient visits.¹⁸ Avoidance of severe local and particularly systemic reactions to allergen injections requires immunotherapy to be a prolonged and expensive form of treatment of allergic symptoms. The purpose of this trial was to demonstrate the safety and effectiveness of PRW as an improved form of immunotherapy by investigators in different geographic areas.

Based upon our experience from earlier studies, a safe and effective schedule of PRW immunotherapy was arrived at.^{11, 12} In the multi-institutional study, 50,000 PNU of PRW were injected preseasonally. A total of 1,200 injections were given during the study without systemic reactions and with minimal immediate local reactions. Late local reactions interrupted the schedule of immunotherapy in only seven patients during the study. Patients began immunotherapy with 100 PNU and advanced to maintenance immunother-

apy with seven injections. This is in contrast to one standard mode of immunotherapy with aqueous ragweed in which therapy is begun with 2.5 PNU (0.05 ml of 1:10,000 w/v dilution) and 30 or more injections are required to reach maintenance.³

There were no changes in any laboratory parameters done to detect toxicity in PRW-treated patients attributable to immunotherapy. Although abnormalities on chest x-ray films of two patients were noted, these were present before the study and were stable throughout the study.

In this study only a modest increase in IgE binding of AgE was noted following PRW therapy. However, a marked increase in total serum binding of AgE was seen. The 12-fold increase in total AgE binding of serum of PRW-treated patients was similar to increases noted in a group of patients treated with standard ragweed extracts reported by Irons et al.¹⁹ In this study, 24 patients suffering ragweed pollinosis received 50,000 to 80,000 PNU ragweed extract over a 2-yr period with a mean 10-fold increase in blocking

antibody. At the end of therapy in the present study, the ratio of total serum binding of AgE to IgE binding of AgE was 100:1. Immunologic parameters known to change with standard immunotherapy include an initial increase in specific IgE followed by a decrease after prolonged therapy; a rise in total serum binding of injected antigens ("blocking antibody"); and a decreased histamine release in certain treated patients.²⁰⁻²² Although no immunologic changes have been consistently shown to predict clinical remission of symptoms, an increase in "blocking antibody" is associated with clinical improvement.²³

Improvement in symptoms of ragweed pollinosis was demonstrated in the four PRW-treated groups when symptoms for the 1979 ragweed season were compared with their symptoms during the 1978 untreated season or when compared with the 1979 symptoms of untreated control groups. Ninety percent of all patients treated with PRW reported improved symptom scores with an overall mean reduction of 1979 symptom scores of 52% from 1978 scores (Table II).

Placebo-treated patients were not included in the study because in our 6 yr of experience with PRW immunotherapy, we have found that late, 6- to 24-hr mild discomfort and swelling at the injection site is not uncommon. These reactions cannot be simulated with histamine placebo materials. In a survey of the literature, Irons and Patterson reported that an average 29% of placebo-treated individuals reported improvement in at least one clinical parameter.³ It is unlikely that the consistency and magnitude of the symptom score reduction in this study is the result of a placebo effect. In addition, when daily symptom score diaries were kept by a group of 20 patients receiving placebo injections and a group of 21 untreated ragweed-sensitive patients during a ragweed season, it was found there was no statistically significant decrease in severity of symptom scores of the placebo-injected group when compared with the scores of the untreated group.²⁴

Other investigators have made efforts to improve current aqueous extract immunotherapy. Efforts have included use of a repository form of immunotherapy using alum-precipitated materials.²⁵ Modification of ragweed with formaldehyde to produce an "allergoid" with reduced allergenicity has been attempted and brief reports of small clinical trials have appeared.²⁶ Incorporation of allergens in tyrosine crystals has produced a depot-type material that is slowly released from the injection site and has been used in Europe to reduce the incidence of allergic reactions to immunotherapy.²⁷ New immunologic studies in animals suggest that in the future it might be

possible to specifically suppress IgE against certain allergens and thereby control clinical symptoms of atopic disease.^{28, 29}

Both the previous trials of PRW at Northwestern University and the current multi-institutional study demonstrate that PRW is an improved form of immunotherapy. We believe the safety and reduced incidence of allergic reactions to PRW is a result of conversion of ragweed antigens into larger molecular weight antigens. Allergenic proteins of ragweed such as AgE are incorporated into the matrix of the polymer reducing the availability of antigenic determinants to IgE on tissue mast cells while the antigens are still available to stimulate a blocking antibody response. Polymerization also results in a reduction of the molecular concentration of allergens relative to the monomers.³⁰

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