

A double-blind, placebo-controlled trial of polymerized whole ragweed for immunotherapy of ragweed allergy

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Immunotherapy with polymerized ragweed (PRW) has been demonstrated to be safe and effective when compared with monomeric ragweed or untreated controls. To further establish the efficacy of PRW, a trial was conducted comparing PRW, placebo, and no treatment in ragweed-sensitive individuals. In a double-blind manner, 21 patients were treated before the 1981 ragweed season with 15 weekly injections of PRW totaling about 50,000 PNU and 1200 µg antigen E, while 19 patients were treated with 15 weekly injections of a caramelized glucose and histamine placebo. An additional control group received no injections. Blood was drawn for IgE against ragweed antigen E (IgE-a-AgE) and for blocking antibody against AgE before treatment, after treatment (before season), and after season. In the untreated patients, blood was drawn before season and after season. Daily symptom score sheets were completed by patients each day of the ragweed season. Blocking antibody rose more than 40-fold with treatment ($p = 0.0001$) in the PRW group but was unchanged in the placebo group with treatment. IgE-a-AgE rose with PRW therapy. Clinical efficacy of PRW was again confirmed in this study. Symptom score mean in the PRW group was statistically lower than the mean in the placebo group ($p = 0.022$) and in the untreated group ($p = 0.018$). There were no systemic reactions and only minor local reactions during treatment. In summary, PRW is an improved form of immunotherapy. (J ALLERGY CLIN IMMUNOL 69:494, 1982.)

The development of polymerized allergens for therapeutic use in IgE-mediated diseases was based on the premise that equal weights of polymerized allergens and monomeric allergens would have comparable immunogenicity (the ability to induce blocking antibody) but would have decreased allergenicity (the ability to elicit an IgE-mediated reaction). This would allow safe administration of larger doses of allergen in fewer injections to achieve the same maintenance dose and therapeutic effect. Several studies have demonstrated that these results have been achieved and will have therapeutic applicability.

MRW allergens are crosslinked by treatment with glutaraldehyde to form PRW,¹ the allergenicity of

which is decreased by increasing the molecular weight.² Patients treated with PRW have demonstrated rises in IgG antibody levels to ragweed antigens, including antigen E, antigen K, and antigen Ra3.³

In single-blind studies^{4, 5} comparing PRW and MRW in equivalent doses, similar immunogenicity and reduction in symptoms were found. Significantly, although there were seven systemic reactions in nine patients receiving MRW, there were no systemic reactions in the 10 patients treated with PRW.⁴

This demonstration of safety was then further tested by a study of six patients who could not tolerate increase to usual maintenance doses⁶ of conventional aqueous extracts because of large local and systemic reactions to grass and ragweed.⁷ All were converted to therapy with PRW and polymerized grass and advanced to maintenance doses of both with no significant local and no systemic reactions.

A more accelerated dose schedule was developed that allowed the safe administration of 30,000 PNU of PRW in 11 injections.⁸ Finally, to evaluate safety and efficacy in different cities, a multi-institutional trial of

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Abbreviations used

AgE:	Ragweed antigen E
IgE-a-AgE:	IgE against ragweed antigen E
MRW:	Monomeric ragweed
PNU:	Protein nitrogen units
PRW:	Polymerized ragweed

PRW was conducted by four groups of investigators.⁹ Eighty patients in four cities received 50,000 PNU in 15 injections, with no systemic reactions. In comparison with pretreatment values and with those of untreated controls, there was a significant, 12-fold increase in blocking antibody and a statistically significant reduction in symptom scores.

To provide final documentation of the safety and efficacy of PRW as an improved form of immunotherapy, a double-blind, placebo-controlled trial was designed. Forty-two patients with classic ragweed hay fever were recruited prior to the 1981 ragweed season and were randomly assigned to two groups on the basis of skin-test titration to whole ragweed. One group was treated with 50,000 PNU of PRW (1200 μ g AgE) in 15 injections, while the other was to receive 15 injections of a caramelized glucose and histamine placebo in a double-blind fashion in which patients, physicians, and treatment nurses did not know the injected material. A group of 15 additional patients with similar skin-test reactivity was recruited and received no injections. Symptom score diaries were completed by each patient during the 1981 ragweed season. Immediate and delayed reactions to PRW were quantitated, and IgE binding of AgE and total AgE binding by serum samples before and after immunotherapy were measured.

MATERIALS AND METHODS

Patients

During the spring of 1981, 42 patients (ages 21 to 65 yr) with typical histories of ragweed hay fever and 4+ prick test reactions to ragweed extract (1:20; Hollister-Stier Laboratories, Spokane, Wash.) were recruited. They were otherwise normal as determined by history, physical examination, chest x-ray, erythrocyte sedimentation rate, complete blood count, and urinalysis. None had received immunotherapy within 5 yr. Skin test titrations to weight/volume dilutions of crude ragweed (10^{-5} , 10^{-6} , 10^{-7} , 10^{-8} , 10^{-9} , 10^{-10} ; Hollister-Stier) and prick tests to mixed *Dermatophagoides* and *Aspergillus* (1:20; Hollister-Stier) were performed. Patients were randomized to two groups on the basis of ragweed skin-test titration. Fifteen additional patients with similar history, physical examination, laboratory test results, and skin-test titrations were recruited for study and were given no injections prior to the 1981 ragweed season.

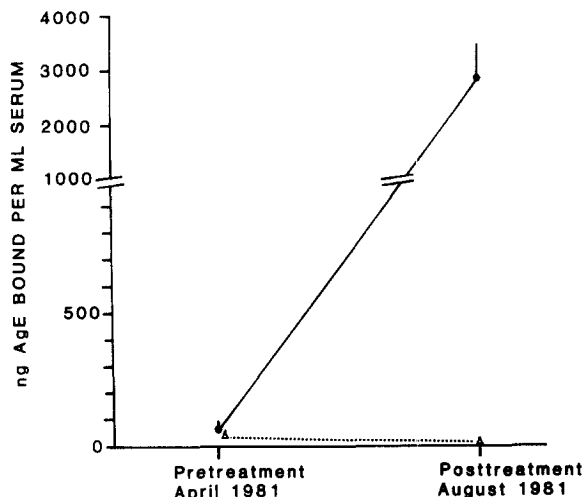


FIG. 1. Change in total serum binding activity of AgE for patients receiving PRW (●) and placebo (△). The vertical bars represent SEM. The rise is highly significant ($p < 0.00001$) in the PRW group, but not significant in the placebo group.

Symptom scores

Daily symptom score sheets were kept by each patient from August to October 1981. This allowed quantitation (0 to 3) three times per day of such symptoms as sneezing, nasal discharge, nasal congestion, pruritus, and wheezing. Patients were also required to list names of medications and numbers of pills taken.

Histamine placebo

The caramelized glucose-histamine placebo, which was indistinguishable by color from the PRW, was prepared as described by Norman et al.¹⁰ The strongest concentration of histamine used was 0.4 mg/ml.

Polymerization of ragweed

In brief, short and giant ragweed pollens (Greer Laboratories, Inc., Lenoir, N. C.) were defatted with ether, extracted with NH_4HCO_3 , precipitated by $(\text{NH}_4)_2\text{SO}_4$, and fractionated by Sephadex G-15 chromatography, resulting in MRW. Protein concentration of MRW was determined by the Kjeldahl method, and the AgE content was determined by immunodiffusion. Polymerization with glutaraldehyde (Polysciences, Inc., Warrington, Pa.) occurred at 25°C for 4 hr and was stopped by addition of glycine. By means of Sepharose 4B and Sephadex G-200 column chromatography, PRW with molecular weights ranging from 200,000 to 20,000,000 was obtained. This method was described in detail elsewhere.⁹ Safety and sterility requirements of the Food and Drug Administration Bureau of Biologics were conducted by Allergy Laboratories of Ohio, Columbus, Ohio. The final PRW concentration used contained 12,500 PNU/ml and approximately 250 μ g/ml of AgE, on the basis of AgE content of the starting monomer.

TABLE I. Immunotherapy schedule for PRW or placebo

Injection No.	Dilution (PRW or PL)	Volume (PRW or PL)	PRW patients	
			PNU per injection	AgE per injection (μ g)*
1	1:10	0.10	125	2.5
2	1:10	0.25	312	6
3	1:10	0.50	625	12
4	Full strength	0.10	1250	25
5	Full strength	0.25	3125	62
6-15	Full strength	0.50	6250	125

PL = placebo.

*Based on antigen E (AgE) content of starting monomer.

Schedule of immunotherapy

Beginning in May 1981, each of 42 patients began a series of 15 weekly injections. The PRW schedule is shown in Table I. The placebo schedule was identical. If a patient had a large local reaction, the dose was repeated prior to increasing the next dose.

Quantitation of allergic reactions to immunotherapy

Immediate local reactions were measured and recorded as centimeters of erythema and induration. Late local reactions occurring at 4 to 24 hr were recorded as described by patients. If doses had to be repeated because of a large local reaction, additional injections were given to reach 50,000 PNU or the equivalent volume of placebo.

Laboratory tests

Each patient had a complete blood count, a differential leukocyte count, erythrocyte sedimentation rate, chest radiograph, and urinalysis prior to the study to establish that these were normal. Tests were not repeated after treatment, since the absence of abnormalities had been established in the multi-institutional study of 80 PRW-treated patients.⁹

Immunologic studies

Blood samples were drawn from each placebo- and PRW-treated patient before the start of injections in April 1981, after the final injection in August 1981, and after the season in October 1981. Blood was drawn from untreated patients in August and October 1981. Serum was separated and stored frozen prior to use.

AgE binding by IgE was measured by the polystyrene-tube radioimmunoassay technique described previously.¹¹ In brief, polystyrene tubes were coated with myeloma IgE. Anti-IgE was then added to form an IgE immunosorbent. After a 48 hr incubation with 0.1 ml of patient serum, the tubes were washed and ¹²⁵I-AgE was added in excess. After 48 hr of incubation, tubes were washed and counted and the amount (ng) of specific IgE against AgE in 1 ml of serum was calculated.

Total serum AgE binding capacity was determined by a modification¹² of the ammonium sulfate technique of Lidd and Farr.¹³ Briefly, 0.1 ml aliquots of sera were incubated with 10, 100, and 1000 ng of ¹²⁵I-AgE. Antibody-bound AgE was precipitated with 40% saturated ammonium sulfate and the amount (ng) of AgE bound by 1 ml of serum in antigen excess was calculated. The result is a quantitative estimate of blocking antibody expressed as AgE bound per milliliter of serum.

Seasonal pollen counts

From the first week in August through the first week in October, Chicago area ragweed pollen counts were obtained daily Sunday through Friday by Grant Hospital using a rotating arm impactor.

RESULTS

Patients

By Student's *t* test analysis of IgE-a-AgE, by log rank test of ragweed antigen skin titration, and by chi square analysis of mold reactivity, there were no statistically significant differences between the three groups, demonstrating similarity of the groups in terms of these variables.

Quantitation of allergic reactions

Three of the 21 patients receiving PRW had at least one immediate local reaction consisting of erythema and induration, and seven patients had erythema alone. The size of the immediate local reactions varied from 1 to 6 cm. In all patients receiving PRW a total of 92 cm of erythema and 21 cm of induration were recorded (average, 3 mm of erythema and 0.7 mm of induration per injection). Seven patients receiving PRW had large immediate or delayed local reactions requiring 11 additional injections. Of these, four patients required only one additional injection and three patients required two or three injections. There were no systemic reactions to PRW.

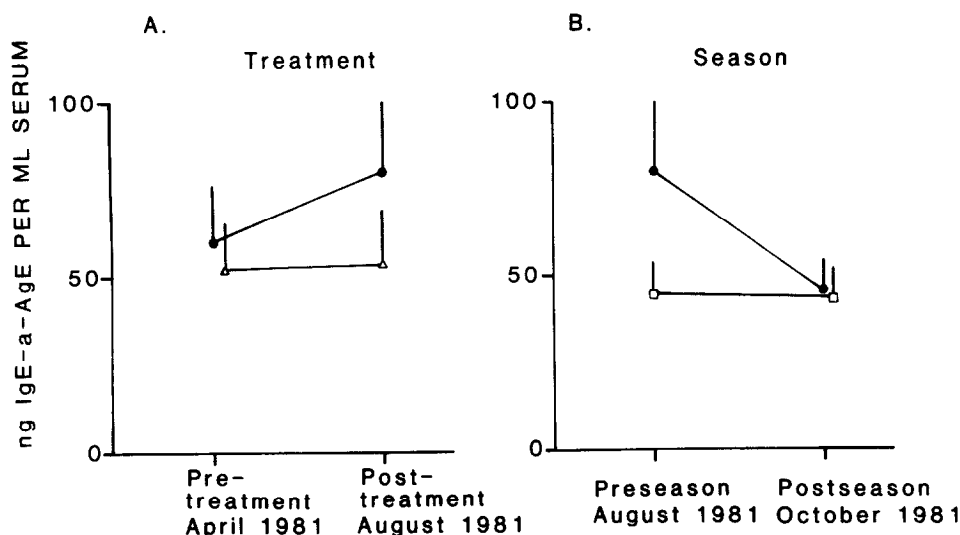


FIG. 2. A, Changes in IgE-a-AgE with treatment in the PRW (●) and placebo groups (△). The vertical bars are SEM. By paired t test, the rise in PRW-treated patients is statistically significant ($p = 0.04$) while the change in the placebo group is not. B, Statistically significant fall (by paired t test) ($p = 0.007$) in the IgE-a-AgE during the ragweed pollen season in the PRW group (●) and no statistical difference in those patients who did not receive PRW (placebo-treated and untreated patients) (□).

Two of the 19 patients receiving histamine placebo had at least one immediate local reaction consisting of erythema and induration, and four patients had erythema alone. In all placebo-treated patients, a total of 24 cm of erythema and 1.4 cm induration were recorded (average, 1 mm of erythema and 0.05 mm of induration per injection). One patient receiving placebo had a large immediate local reaction requiring one additional injection.

Immunologic studies

The changes in total serum antibody against AgE after PRW and placebo injections are shown in Fig. 1. In the PRW patients there was a greater than 40-fold increase in blocking antibody, while there was no change in placebo-treated patients. Fig. 2, A, shows the change with treatment in IgE-a-AgE in placebo and PRW groups. There was a statistically significant (by paired t test) 1.3-fold increase in IgE-a-AgE in PRW-treated patients but no change in placebo-treated patients. Fig. 2, B, demonstrates the change in IgE-a-AgE with ragweed season. The PRW-treated patients had a significant 30% reduction (by paired t test), while those patients who had not received PRW (untreated and placebo-treated) had no change.

Seasonal pollen counts

Pollen counts summed over each week in August and September are shown in Fig. 3. The highest pollen counts occurred during the first 2 wk of September.

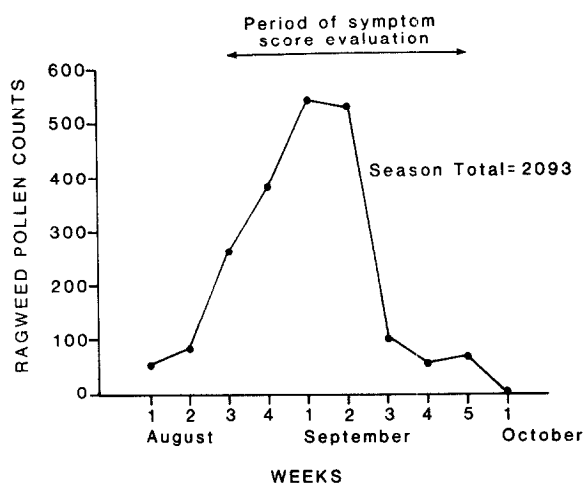


FIG. 3. Pollen counts were obtained by the rotating arm impactor method daily except Saturday, summed each week, and plotted. The units are pollen grains per cubic meter.

Symptom score index comparisons

Graphs of average daily symptom scores for comparison of the PRW, placebo, and untreated groups are shown in Figs. 4 through 6. Fig. 4 compares the symptom scores of the PRW and placebo groups. The p values, determined by Student's t test, demonstrate that symptom score indices of the PRW group were significantly lower than those of the placebo control group (Fig. 4).

Similarly, symptom score indices comparing PRW-treated patients with untreated patients are depicted in

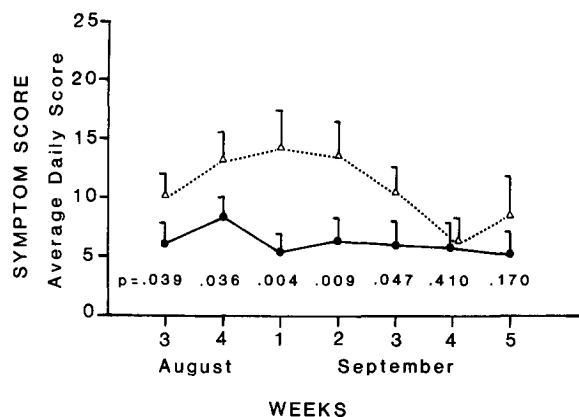


FIG. 4. Average daily symptom score indices are plotted for PRW-treated (●) and placebo treated (Δ) patients. The p value and 1 SEM are shown for each point plotted.

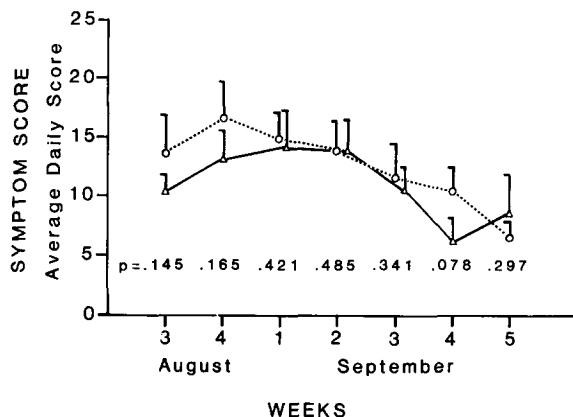


FIG. 6. Average daily symptom score indices are plotted for placebo-treated (Δ) and untreated (○) patients. The p value and 1 SEM are shown for each point plotted.

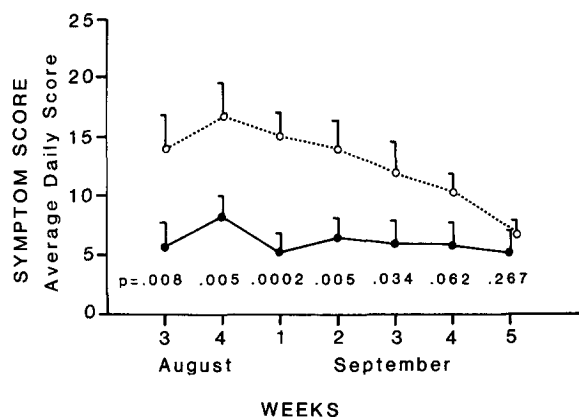


FIG. 5. Average daily symptom score indices are plotted for PRW-treated (●) and untreated (○) patients. The p value and 1 SEM are shown for each point plotted.

Fig. 5. Again, the PRW-treated patients have statistically significantly lower symptom score indices.

As shown in Fig. 6, there were no significant differences between symptom scores of untreated patients and those of placebo-treated patients.

Of interest is the observation that during the period of the highest pollen counts, the first 2 wk of September, the statistical significance of the difference between PRW and placebo ($p = 0.0043$, $p = 0.0090$) and between PRW and untreated groups ($p = 0.0002$, $p = 0.0051$) was at its highest. At that same time, the statistical significance of the difference between placebo and untreated groups ($p = 0.4208$, $p = 0.4855$) was at its lowest.

A graph of the total seasonal symptom scores is shown in Fig. 7. The first vertical line represents one standard error of the mean for the PRW group, while the second and third bars represent standard errors of the mean for placebo and untreated groups, respec-

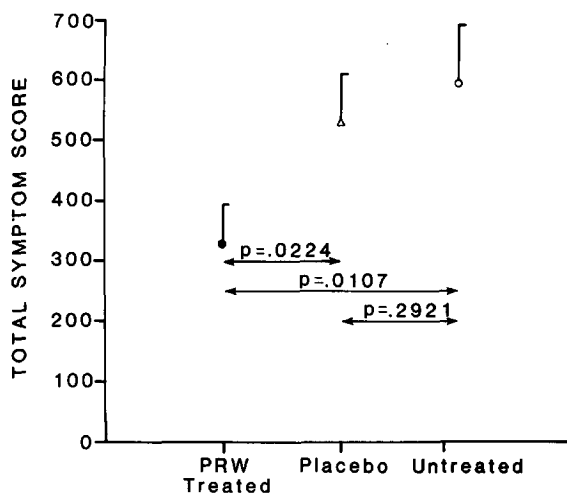


FIG. 7. Total seasonal symptom scores (mean and 1 SEM) are plotted for the PRW group (●), the placebo group (Δ), and the untreated patients (○).

tively. The p values were determined by Student's t test. Symptom scores in the PRW group are significantly lower than those of either the placebo or untreated group. There is no statistically significant difference between the symptom score indices of the placebo and untreated groups.

DISCUSSION

Utilizing a PRW immunotherapy schedule similar to that of the multi-institutional trial, we sought to further demonstrate the efficacy and safety of PRW. Although the general incidence of systemic reactions per injection approximates 0.3%¹⁴ and 12% per patient¹⁵ with conventional immunotherapy, there were no systemic reactions in any of the 21 patients treated with PRW. Late local reactions did occur in seven

patients. These did not prevent any of the patients from reaching maintenance dose and the therapeutic goal of 50,000 PNU. The cumulative number of patients treated with PRW is now 242 with 3500 injections. There have been two very mild systemic reactions, giving a reaction rate of 0.004% per injection and 0.8% per patient.

There was a highly significant, 40-fold increase in blocking antibody in PRW-treated patients, whereas placebo-treated patients demonstrated no rise. There was also a very small but statistically significant increase in specific IgE after PRW therapy. There was no change in placebo-treated patients.

With the ragweed season, there was a small decrease in IgE-a-AgE in the PRW-treated patients, while there was no change with season in patients who had not received PRW. Although Yunginger and Gleich¹⁶ found an overall seasonal rise of IgE-a-AgE in untreated patients, there was variability among patients. Our group did not demonstrate such an overall rise, although there were individual patients whose values did increase.

Significantly lower symptom scores overall were reported by PRW-treated patients as compared with placebo-treated (Fig. 4) or untreated patients (Fig. 5). When placebo effect was ascertained by asking the patient whether he had improved,¹⁷ the placebo effect was estimated at 29%. However, when placebo effect was ascertained by comparison of symptom score indices of untreated and placebo-treated patients, there was no statistically significant placebo effect.¹⁸ The current study also clearly demonstrated that when symptom score indices are used to evaluate placebo and untreated groups, no statistically significant difference is observed (Figs. 6 and 7). It is important to note that the placebo and untreated groups are the most similar (Fig. 6) at the height of the pollen season (see Fig. 3) when those two groups are most statistically different from the PRW group (Figs. 4 and 5). This demonstration of the absence of placebo effect on symptom score indices validates the establishment of efficacy in the earlier multi-institutional study in which the differences between PRW and untreated groups were statistically significant in all four cities.

This double-blind, placebo-controlled trial further demonstrates the efficacy, safety, and immunogenicity of PRW. Patients developed high levels of blocking antibody and tolerated PRW well, with fewer systemic reactions and fewer injections than with conventional immunotherapy. Finally, symptom scores of PRW-treated patients were statistically significantly less than those of placebo-treated or untreated patients.

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