

Persistence of efficacy after a brief course of polymerized ragweed allergen: A controlled study

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Immunotherapy with PRW has been demonstrated to be safe and effective. To determine whether the efficacy would remain in successive ragweed seasons without further therapy, a trial was conducted comparing PRW to histamine placebo therapy in patients that had received courses of PRW previously. Those patients were also compared to previously untreated ragweed-sensitive patients. In a double-blind fashion, 21 previously treated patients were treated before the 1982 ragweed season with four weekly injections of PRW therapy, whereas 21 previously treated patients were treated with four injections of placebo therapy. An additional control group of 21 previously untreated ragweed-sensitive patients received no injections. Daily symptom and medication score sheets were completed by patients each day of the ragweed season. Blocking antibody rose elevenfold with treatment ($p < 0.0001$) in the PRW group. There was a statistically significant difference in symptom score mean between untreated patients (1007 ± 174) and previously treated patients whether they received supplemental injections (554 ± 180) ($p < 0.01$) or whether they did not (650 ± 168) ($p < 0.03$). In summary the efficacy of 15 injections of PRW immunotherapy persists at least several years without need for supplemental immunotherapy. (J ALLERGY CLIN IMMUNOL 73:484-489, 1984.)

Immunotherapy with polymerized allergens including antigen E,¹ ragweed,^{2, 3} and grass pollens⁴ has been extensively studied and has demonstrated safety and efficacy. Polymerized allergens have been demonstrated to have reduced allergenicity as compared to unpolymerized allergens and to have retained immunogenicity.^{5, 6} The reduced allergenicity of polymerized preparations allows them to have various advantages over usual aqueous allergens used in standard immunotherapy. There is a lower incidence of systemic reactions per injection and per patient course with polymer than with unaltered extract. Larger doses of polymer can be administered initially than

can be administered of aqueous therapy. An entire course of polymerized immunotherapy can be given in 15 weekly injections, whereas a standard aqueous course of similar immunogenicity would involve approximately 30 weekly injections followed by at least 30 monthly injections. Thus with polymerized extracts, the inconvenience to patients would be reduced.

We sought to determine whether the demonstrated efficacy of PRW therapy^{2, 3} would endure over several years or whether a short course of supplemental PRW injections would be required to maintain efficacy. We conducted a trial of patients treated 12 to 50 mo previously^{2, 3, 7} with PRW therapy. In a double-blind manner, half received four supplemental injections totaling 132 μ g AgE, and half received caramelized glucose-histamine placebo therapy injections. The symptom scores of the two groups were compared to each other, and they were compared to scores of an untreated control group of ragweed-sensitive patients. To further define the length of time that efficacy persists, we compared symptom score means of untreated patients to means of patients with and without supplemental injections treated 12 to 30

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Abbreviations used

PRW:	Polymerized ragweed
PNU:	Protein nitrogen units
IgE-a-AgE:	IgE against antigen E
U:	Untreated
H:	Histamine

mo previously and to the means of patients with and without supplemental injections treated 30 to 50 mo previously.

MATERIAL AND METHODS

Patients

During the spring of 1982, 42 of 89 patients who had each been previously treated with a course of PRW therapy at Northwestern University totaling approximately 50,000 PNU were recruited to participate in the study. They were healthy as determined by history, erythrocyte sedimentation rate, and urinalysis. An additional 21 patients with typical histories of ragweed hayfever and 3+ or 4+ prick reactions to giant and short ragweed extracts (1:20) (Allergy Labs of Ohio [ALO], Columbus, Ohio) were recruited. These patients had not received immunotherapy for at least 5 yr and were otherwise normal as determined by history, physical examination, erythrocyte sedimentation rate, complete blood count with differential leucocyte count, and urinalysis.

Skin test titrations to weight/volume dilutions of crude mixed ragweed (10^{-5} , 10^{-6} , 10^{-7} , 10^{-8} , 10^{-9} , and 10^{-10} ; ALO lot A2091502) and prick tests to mixed Dematiaceae and *Aspergillus* were conducted on each patient. Previously treated patients were divided into two groups (>30 mo and <30 mo) based upon whether they had been treated more than or less than 30 mo ago. Within these temporal groups, patients were randomized into one of two treatment groups on the basis of ragweed skin test titration.

Sixteen of the 89 previously treated patients could not be located as phone numbers and addresses had changed. All other 30 previously treated patients who did not wish to participate in the study responded that their interim health had been good. The most common reasons for patients not participating were that they had difficulties in traveling to Northwestern during working hours for injections (22 patients) and/or that their symptoms had been so reduced by initial PRW therapy that they believed they did not require further injections (six patients). Of the 42 patients who consented to participate in the study, all but two noted that PRW therapy had reduced their pollinosis symptoms.

Histamine placebo. The caramelized glucose-histamine placebo, indistinguishable in color from PRW, was prepared at ALO by use of a method described by Norman et al.⁸ The highest histamine concentration used was 0.025 mg/ml.

Preparation of PRW. PRW was manufactured at ALO by use of methods similar to those described in detail elsewhere.³ Briefly, short and giant ragweed pollens were extracted with aqueous buffer, precipitated with $(\text{NH}_4)_2\text{SO}_4$,

TABLE I. Dosage schedule

Injection no.	μg AgE (starting monomer content)
1	2.5
2	10.0
3	40.0
4	80.0

and fractionated on Sephadex G-15. At this point, the protein concentration and AgE content of the product were determined by the Kjeldahl method and immunodiffusion, respectively. Polymerization with glutaraldehyde was followed by chromatographic fractionation on Sepharose CL-4B and Sephacryl S-200 columns. ALO performed the safety and sterility requirements of the Food and Drug Administration Bureau of Biologics. The final PRW used contained 100 $\mu\text{g}/\text{ml}$ AgE on the basis of AgE content of starting monomer.

Immunotherapy schedule. Beginning in June 1982, each of 42 patients began a series of four weekly injections. The PRW dosage regimen used is shown in Table I. The placebo regimen was identical in volume. If a patient developed a large local reaction, the dose was repeated.

Quantification of allergic reactions to PRW. Immediate local reactions were measured at 20 min and recorded as centimeters of erythema and induration. Delayed local reactions that occurred up to 24 hr later were recorded as the patient described them. If a dose had to be repeated because of a large local reaction, an additional injection would be given in order to reach 132 μg AgE or the corresponding volume of histamine placebo.

Seasonal pollen counts. From the first week in August through the first two days in October, Chicago area ragweed pollen counts were obtained daily by Abbott Laboratories (North Chicago, Ill.) by use of a Durham gravity slide. Rotating arm impactor counts were not available.

Symptom/medication scores. Daily symptom/medication score sheets were kept by each patient from August 1, to October 3, 1982. This permitted a quantification (0 to 3) of such symptoms as sneezing, nasal discharge, nasal congestion, and pruritus. Names and dosages of medications taken were also listed by the patients.

Immunologic studies. Blood samples were drawn from each previously treated patient before the start of injections in June and two wk after the final injection in August. Blood samples were drawn from untreated patients in August.

IgE-a-AgE was measured by the polystyrene tube radioimmunoassay technique.⁹ Briefly, polystyrene tubes were coated with myeloma IgE. Anti-IgE was then added to form an IgE immunosorbent. The patient's serum was incubated in the tube for 48 hr; the tubes were washed and ^{125}I -AgE was added and incubated for 48 hr. The tubes were washed, counted, and ng IgE-a-AgE bound per ml of serum was calculated.

To determine total serum AgE binding capacity of a serum, a modification¹⁰ of the ammonium sulfate method of

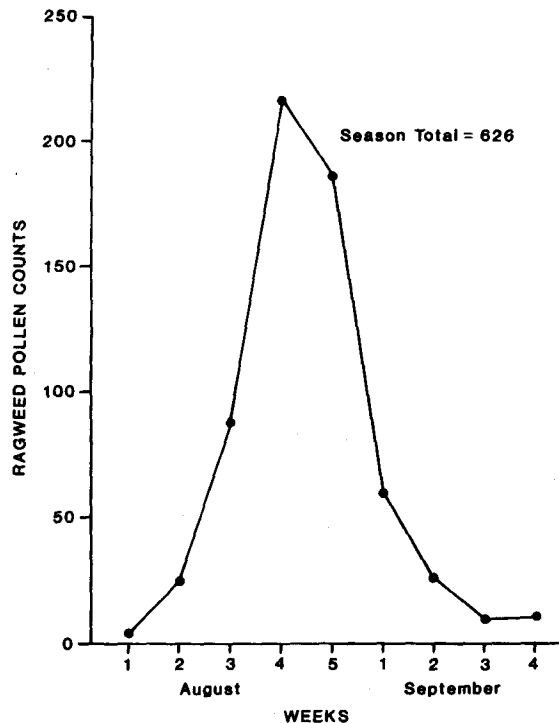


FIG. 1. Pollen counts were obtained daily by gravity method, summed each week, and plotted. Units are grains per square centimeter.

Lidd and Farr¹¹ was used. In brief, 0.05 ml aliquots of sera were incubated with 10, 100, and 1000 ng of ¹²⁵I-AgE. Antibody-bound immunoglobulin E was precipitated with 40% saturated (NH₄)₂ SO₄, and the ng of AgE bound per ml of serum in antigen excess was calculated. This result is a reasonable estimate of blocking antibody.

RESULTS

Patients

By log rank test of ragweed-antigen skin titration and by chi-square analysis of mold reactivity (5 to 7 mold-reactive patients in each group), there were no statistically significant differences between patients in the three groups: U, previously treated patients receiving H injection, and previously treated patients receiving PRW injections.

Quantification of allergic reactions

Seven of 21 patients receiving PRW therapy had one immediate local reaction consisting of erythema alone, whereas six had erythema and induration. In all, patients receiving PRW therapy had a total of 39.2 cm erythema and 4 cm induration, averaging 4.5 mm erythema and 0.46 mm induration per injection. Two patients receiving PRW therapy had one large immediate or delayed local reaction on the third in-

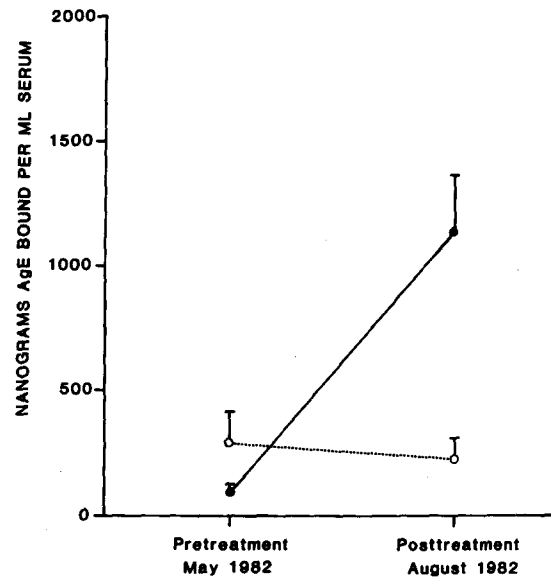


FIG. 2. Change in total serum-binding activity of AgE for PRW-treated (●) and H-treated (○) patients. Vertical bars represent SEM. Rise in PRW-treated group is highly significant ($p < 0.0001$), and there is no significant change in H-treated group.

jection. The fourth injection was administered to these two patients in a divided dose, half in each arm, with no further local reactions. There were no systemic reactions. Seven of the 21 patients receiving histamine placebo therapy had at least one immediate reaction of erythema alone, and four had erythema with induration. In total, H patients had 26.7 cm erythema and 2.8 cm induration, averaging 3.2 mm erythema and 0.3 mm induration per injection.

Seasonal pollen counts

Pollen counts summed weekly are graphed in Fig. 1.

Immunologic studies

Changes of blocking antibody to AgE with injection in the groups receiving PRW and H therapy are depicted in Fig. 2. There was a decrease in blocking antibody in the patients receiving H therapy, and a statistically significant ($p < 0.0001$) elevenfold rise in blocking antibody in patients receiving PRW therapy. There was no change of IgE-a-AgE with injection in either group, as shown in Fig. 3.

Symptom/medication score index comparisons

Average daily symptom/medication score graphs comparing groups receiving PRW and H therapy and U groups are demonstrated in Fig. 4. The figure com-

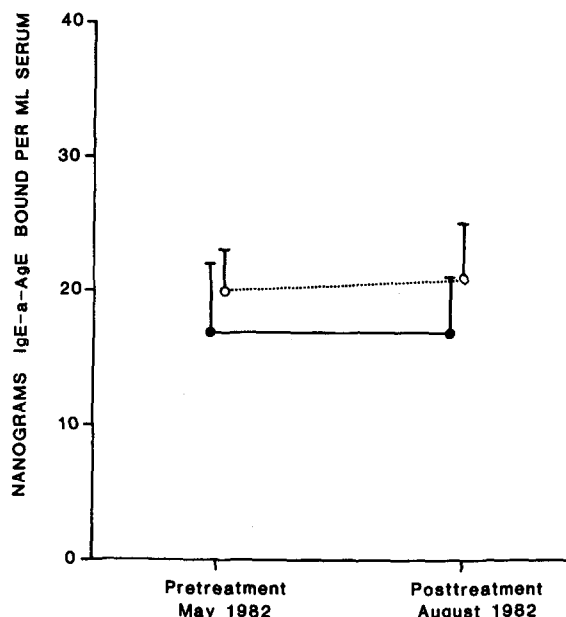


FIG. 3. Change in IgE-a-AgE for PRW-treated (●) and H-treated (○) patients. There is no change in either group with treatment.

compares scores of U and PRW and H therapy-receiving groups. The p value (0.01), determined by one-tailed Student's t test, demonstrates that total scores of the U control group were significantly higher than those of the group receiving PRW therapy and of the group receiving H therapy. There is no significant difference between previously treated patients whether they received PRW or H therapy.

A graph of total seasonal symptom scores is shown in Fig. 5. The first bar represents one SEM for the U group, whereas the next bars are for groups receiving H and PRW therapy, respectively. Statistical significance was determined by one-tailed Student's t test. Symptom scores in the groups receiving PRW and H therapy are significantly lower than those scores in the U patients. Symptom scores of groups receiving PRW and H therapy are not statistically different.

All statistical analyses of symptom/medication scores were performed by use of the logarithmic transformations of the raw scores.

Comparison of subgroups of patients treated more than 30 mo and less than 30 mo ago

Another graph of total seasonal symptom scores is shown in Fig. 6. The first vertical bar is one SEM for the U group, whereas the following bars are representations of a group receiving H therapy previously treated more than 30 mo ago ($H > 30$), a group re-

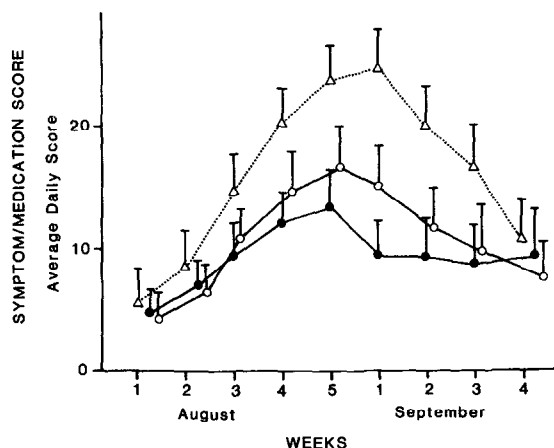


FIG. 4. Average daily symptom/medication score indices are plotted for PRW-treated (●), H-treated (○), and untreated (Δ) patients. Vertical bars represent SEM. There are statistically significant differences between PRW-treated and untreated patients during last week in August and first 3 wk in September.

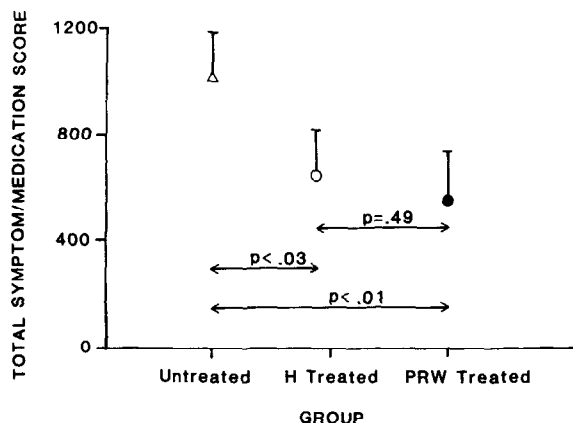


FIG. 5. Total seasonal symptom/medication scores (mean and 1 SEM) are plotted for untreated group (Δ), H-treated group (○), and PRW-treated group (●).

ceiving PRW therapy previously treated more than 30 mo ago ($PRW > 30$), a group receiving H therapy previously treated less than 30 mo ago ($H < 30$), and a group receiving PRW therapy previously treated less than 30 mo ago ($PRW < 30$), respectively. The p values were determined by one-tailed Student's t test. With the exception of $H > 30$ whose p value is 0.06, on the borderline of significance, all other previously treated groups gave statistically lower symptom score means than the U group. There is no statistical difference between the $H > 30$ and $PRW > 30$ group nor is there a statistically significant difference between the $H < 30$ and $PRW < 30$ groups. The p values determined by a one-tailed Mann-Whitney U test all dem-

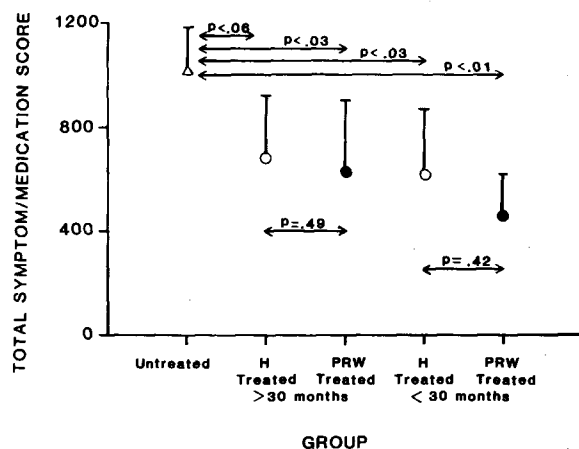


FIG. 6. Total seasonal symptom/medication scores (mean and 1 SEM) are plotted for untreated group (Δ), and for PRW ($\bullet$) and H group ($\circ$) treated more than or less than 30 mo ago.

onstrated statistical significance between the U patients and each of the other four groups.

DISCUSSION

PRW therapy has been demonstrated to be a safe, effective form of immunotherapy for ragweed pollinosis. This study was undertaken to ascertain how long that efficacy would persist and if supplemental injections would be necessary to maintain efficacy after a period of time. Overall, previously treated patients, whether or not they received supplemental injections, had statistically significantly lower symptom/medication scores than untreated patients. We considered the possibility that more recently treated patients might have lower symptom scores, and thus separately we considered previously treated patients H > 30 mo, PRW > 30 mo, H < 30 mo, and PRW < 30 mo. We still found in comparing patients' scores that each of the four groups of previously treated patients had significantly ($p < 0.05$) lower symptom scores than untreated patients as determined by one-tailed Mann-Whitney U test. Thus efficacy of PRW therapy persists up to 50 mo without need for supplemental injections.

When we undertook our study, we determined the power of our test in detecting a difference between the groups receiving PRW therapy and H therapy to be 0.90 with the assumptions that the standard deviation of the population symptom scores would be that observed in our previous ragweed immunotherapy studies and that the change we wished to detect would be a return to baseline symptom scores. In addition to total symptom scores, we also analyzed the groups receiving H and PRW therapy during the peak wk, but no statistically significant difference could be found by

use of one-tailed Student's t test on log transformed scores.

We consider previously untreated patients to be an adequate control group as a result of our study² and one study done at another institution¹² demonstrating no statistical difference in symptom score means between placebo-treated controls and untreated controls. The placebo-treated effect of 30% cited in immunotherapy studies refers only to those studies in which data answering the question "Were you better?" is analyzed by chi square.

Although we did not study all previously treated patients, we do not believe that our selection process introduced a favorable bias into the sample. Irrespective of whether they consented to be in the supplemental-injection study or not, similar numbers of patients believed themselves to be improved. In fact, the selection process may have introduced an unfavorable bias in that six patients were so improved that they were certain they did not need supplemental injections. In all probability their symptom scores would have lowered the mean, increased the n, and thus increased the statistical significance of the study.

In addition to determining persistence of efficacy of PRW therapy, this study also afforded us an opportunity to check the health of patients treated with PRW therapy over the past 4 yr. Except for one 62-year-old woman who died of a myocardial infarction (unrelated to PRW therapy), other patients reported no change in their state of health since being treated with PRW therapy. Of the patients who consented to be in the study, all had normal erythrocyte sedimentation rates and urinalyses except one 55-year-old woman who had a urinary tract infection.

In addition to the above confirmation of long-term safety in patients treated with PRW therapy, the immediate safety of PRW therapy in terms of allergic reactions was again demonstrated. There were no systemic reactions and only two significant locals requiring splitting of the next dose. Thus far we have treated 283 patients with PRW injections, and there have been three systemic reactions early in the studies. That is an incidence of 0.08% per injection and 1.0% per patient course that compares favorably to the usual incidence of 7% to 29% systemic reaction rate per patient course of standard aqueous therapy.¹³

The dose of our four injection, preseasonal booster course, 132 μ g AgE, was chosen because that approximated the lowest dose that had demonstrated efficacy in previous studies of initial treatment of ragweed pollinosis.¹⁴

There was a significant elevenfold rise in blocking antibody in the group receiving PRW therapy but no rise in the group receiving H therapy. This elevenfold

anamnesic rise is similar in magnitude to that observed originally: twelvefold in the multi-institutional trial (500 mg AgE)³ and fortyfold in the double-blind trial (1200 µg AgE).² There were no significant changes in IgE-a-AgE in either group. This demonstrates the immunogenicity of ALO-produced PRW that has been demonstrated by various methods including Sephadex G 200 chromatography, cutaneous end point titration, and radioimmunoassay inhibitory capacity¹⁵ to be comparable to PRW produced by the Northwestern Allergy Section.

In summation, this double-blind placebo-controlled trial of supplemental PRW injections demonstrates that the efficacy of PRW therapy is maintained without need for additional therapy even several years after a 15-week treatment course. Moreover, it further demonstrates both immunogenicity and immediate and long-term safety of PRW therapy in that there were no systemic allergic reactions and that general good health prevailed in patients followed 12 to 50 mo after a course of PRW therapy, respectively.

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