

Polymerized whole ragweed: A two-year follow-up of patients treated with an improved method of immunotherapy

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We have previously reported, in a 1-yr study, the effectiveness of polymerized ragweed (PRW) as an improved method of immunotherapy for patients suffering from ragweed hay fever. In that study, treatment with PRW was found to be superior to treatment with monomer ragweed extract (MRW) because of the reduced allergenicity of the PRW. The current study is a 2-yr follow-up of nine patients treated with PRW and nine patients treated with MRW. After 1 yr of immunotherapy, both MRW- and PRW-treated patients received 9,000 protein nitrogen units (PNU) and after a second year of maintenance therapy both groups received a cumulative dose of 15,000 PNU. Serum blocking antibody against ragweed antigen E (AgE) was measured periodically during the study. After 1 yr of immunotherapy, blocking antibody in the PRW- and MRW-treated groups was similar with respective means of 1,300 and 1,500 ng AgE bound per milliliter of serum. At the end of 2 yr of therapy, these serum AgE binding activities rose to 2,700 and 4,100, respectively. No significant local or systemic reactions occurred in the PRW-treated group during the year of maintenance therapy. However, large local reactions prevented three of the nine patients treated with MRW from achieving a monthly maintenance schedule. A significant decrease in rhinitis symptoms was noted in both treated groups as compared with the season before immunotherapy and also to a group of patients untreated with immunotherapy during the 1978 ragweed season.

Ragweed antigens treated with glutaraldehyde can be polymerized and fractionated to form a preparation with molecular weight range of 200,000 to 20,000,000. In previous studies at this institution, we have shown that immunotherapy for ragweed hay fever with glutaraldehyde polymerized ragweed antigen E (AgE) and also polymerized whole ragweed (PRW) is efficacious.^{1, 2} Therapy with PRW results in substantial increases in serum blocking antibody against AgE and also significant improvement in nasal symptoms during the hay fever season. Immunotherapy with polymerized whole ragweed has the advantage of causing few local and systemic reactions.³ Because of this reduced allergenicity associated with

PRW immunotherapy, we have been able to administer to ragweed-sensitive patients at this institution a total of 30,000 protein nitrogen units (PNU) of PRW over an 11-wk period of treatment without significant reactions.* The purpose of this study is to report a 2-yr follow-up of PRW-treated patients.

Nine patients treated with PRW immunotherapy and nine patients treated with monomer ragweed (MRW) therapy in our original study were continued on maintenance injections for a second year. Several parameters were followed. First, blocking antibody against AgE was periodically determined. In addition, records were kept detailing the occurrence of significant local and systemic reactions in both treatment groups by a nurse who was unaware of whether MRW or PRW was being administered. Patients without local reactions were advanced to monthly injections of 500 PNU of PRW or MRW. Finally, daily symptom score indices detailing rhinitis symptoms were collected through the 1978 hay fever season and com-

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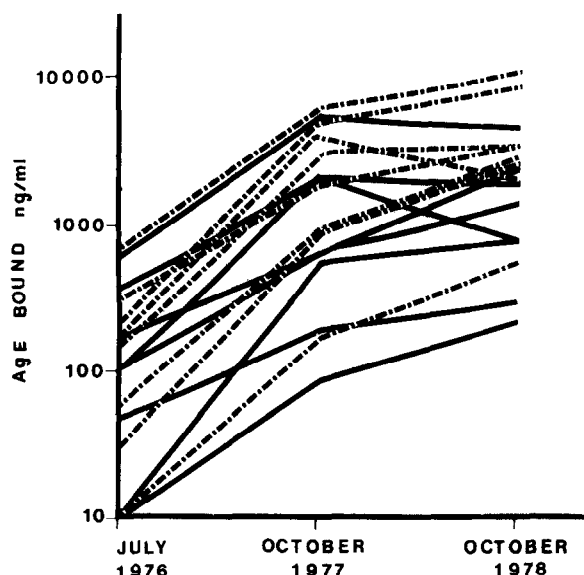


FIG. 1. Serum blocking antibody against AgE for PRW-treated group (—) and MRW-treated group (---). Pre-treatment serum activity is compared with postseasonal activity after 1 and 2 yr of treatment for both PRW- and MRW-treated groups. Both groups were administered 9,000 PNU after 1 yr and 15,000 PNU after 2 yr of therapy. Note log scale.

pared with scores for previous seasons and also with similar indices for a group of ragweed-sensitive patients untreated with immunotherapy during the 1978 ragweed season. This second group of untreated allergic patients was matched for age and sex with our MRW- and PRW-treated groups. Levels of IgE specific for AgE in the untreated group were similar to those of the treated patients prior to immunotherapy.

MATERIALS AND METHODS

Ragweed preparations

We have previously published in detail both the methods of preparation of polymerized ragweed and descriptions of immune reactivity induced by the preparation.^{4, 5} Giant and short ragweed pollen for extraction and polymerization was obtained from Hillister-Stier Laboratories (Spokane, Wash.). Protein nitrogen content of PRW and MRW preparations was determined by the method of Kjeldahl.⁶ In our earlier studies of PRW and MRW, these preparations were standardized for protein content by optical density (O.D.). We have recently found that O.D. methods of measurement overestimated the protein content when compared with the Kjeldahl method. Therefore, the PNU administered to these patients during 2 yr of therapy has been recalculated to reflect Kjeldahl-determined PNU. Solutions of PRW and MRW were diluted to obtain preparations with 0.06 mg ragweed protein/ml (1,000 PNU/ml). Recent analysis of the AgE content of our starting MRW (which has been shown to be totally incorporated into the PRW) demonstrates that 1,000 PNU of the MRW contains 10 μ g AgE/

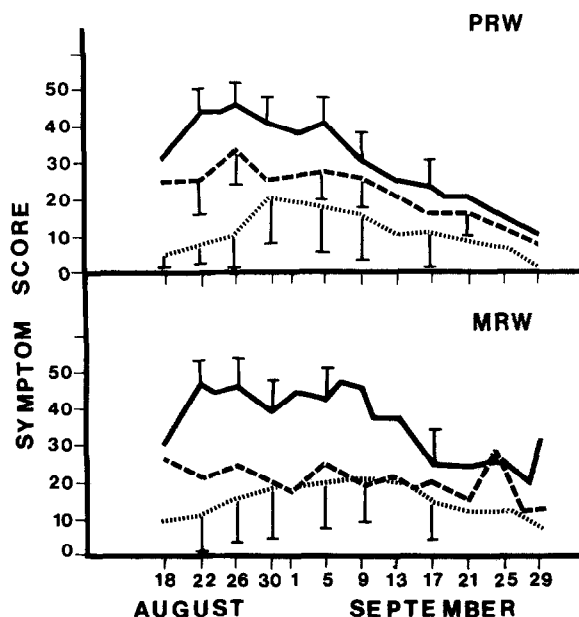


FIG. 2. Mean symptom score indices for PRW- and MRW-treated groups during the 1976 (—), 1977 (---), and 1978 (.....) ragweed seasons. Symptom scores are averaged for 2 days at a time. Means and standard deviations for selected days are shown by vertical lines.

ml. This amount of ragweed AgE is approximately the amount present in a commercial crude ragweed extract equivalent to 2,500 PNU/ml.

Schedule of immunization

During the first year of immunotherapy, PRW- and MRW-treated patients were initially given 0.5 PNU and were advanced weekly by increments until they reached a maintenance dose of 500 PNU. If no significant local reactions occurred with this dosage, patients were advanced to monthly maintenance injections of this dose, usually by the end of the first year of treatment. Those patients who could not tolerate monthly injections because of reactions were continued on weekly injections of 500 PNU. Local reactions at injection sites were determined by measuring the diameters of erythema and wheals by a nurse who did not know which of the two types of immunotherapy was administered. Late local reactions occurring several hours after injection or systemic symptoms were reported and evaluated by the physician. Patients receiving immunotherapy were not told whether they were being treated with MRW or PRW.

Measurement of serum blocking antibody against AgE

Blood specimens were drawn from each patient before and after the 1976, 1977, and 1978 ragweed seasons. The sera were analyzed for antibody against AgE by a modification of the ammonium sulfate technique of Lidd and Farr.^{7, 8} Briefly, 0.1-ml aliquots of sera were incubated with 100 and 1000 ng of ¹²⁵I AgE. After appropriate incubation, the antibody-bound AgE was separated from free AgE by

precipitation with 40% saturated ammonium sulfate. The nanograms of AgE bound by 1 ml serum in antigen excess were calculated.

Quantitation of daily symptoms

Symptom score sheets were completed on a daily basis by each patient during the 1976, 1977, and 1978 ragweed seasons. Immunotherapy was begun following the 1976 ragweed season and continued through the 1978 season. Symptom score sheets allowed the patient to quantitate sneezing, congestion, itching, and also the number and type of medications required daily. By assigning a number from zero to three to each symptom and by totaling medications required for relief, a numerical estimate of each patient's daily symptoms was calculated and plotted. Similar sheets were completed by a group of 22 patients followed in our clinic during the 1978 ragweed season who received symptomatic medications for ragweed hay fever but did not receive immunotherapy.

RESULTS

Safety

All patients who received 2 yr of immunotherapy with PRW or MRW had hemograms and urinalyses. No abnormalities were noted in the laboratory tests. At the conclusion of the first year of therapy, both groups received approximately 9,000 PNU and after 2 yr of therapy both groups received a cumulative dose of 15,000 PNU.

Immune response

Blocking antibody against AgE increased in all subjects during the first year of immunotherapy and was maintained at high levels during the second year of maintenance therapy. Fig. 1 illustrates the postseasonal blocking antibody activity in PRW- and MRW-treated patients. Two patients treated with MRW who required weekly injections because of large local reactions had the highest concentrations of blocking antibody (Fig. 1).

Reduced allergenicity

All patients in the PRW-treated group were easily advanced to monthly injections of 500 PNU by the end of the first year of therapy. They were maintained on monthly injections during the second year without difficulty. Three patients in the MRW-treated group failed to reach monthly maintenance because of allergic reactions and thus were continued on weekly injections.

Symptomatic improvement

All patients in both treatment groups reported subjective clinical improvement during the ragweed season on 1978. Fig. 2 compares symptom scores for the 1976, 1977, and 1978 ragweed seasons for both

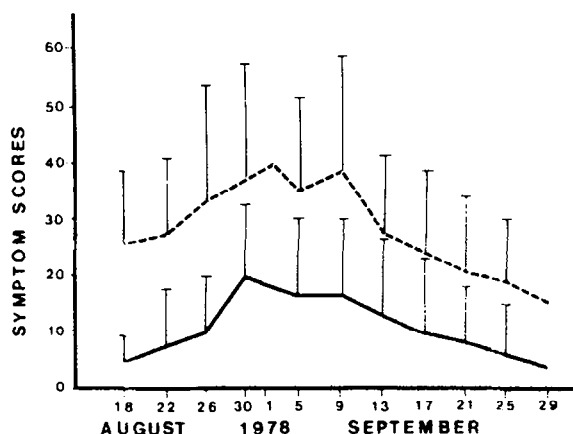


FIG. 3. Mean symptom score indices for the PRW-treated group (—) and 22 adult patients suffering ragweed hay fever untreated with immunotherapy (---) during the 1978 ragweed season. Symptom scores were averaged for 2 days at a time. Means and standard deviations for selected days are shown by vertical lines. PRW-treated patients have statistically lower symptom scores ($p < 0.05$) on all days analyzed. Statistical significance was determined by Student's *t* test.

PRW- and MRW-treated groups. Although the PRW-treated group had higher but not statistically different scores than the MRW-treated group in 1977, both groups showed statistically significant improvement during the 1978 ragweed season compared with the 1976 control season.

Fig. 3 compares the 1978 symptom scores of the PRW-treated group with the symptom scores of 22 ragweed-sensitive patients followed in our clinic during the 1978 season who were treated only symptomatically. A statistically significant improvement in symptom scores for the PRW-treated group is demonstrated on all days of the ragweed season. Our treated groups were matched for sex and were of similar ages to our untreated group. The symptom scores of the untreated group during the 1978 season were similar to those of the treated group during 1976. Determinations in our laboratory of IgE specific for AgE in the treated group before immunotherapy were similar to those in the untreated group.

DISCUSSION

The purpose of this study was to provide long-term follow-up of a group of nine patients receiving PRW immunotherapy for ragweed hay fever over a 2-yr period.

Serum blocking antibody against AgE increased during the first year of immunotherapy in PRW- and MRW-treated groups. During the second year of monthly maintenance immunotherapy, blocking antibody activity remained significantly elevated in both groups. The highest blocking antibody activity was

seen in two patients treated with MRW who required weekly injections because of large local reactions and who therefore received a higher total dose of ragweed antigen.

During the second year of maintenance immunotherapy, all PRW-treated patients were easily advanced to monthly injections. However, three patients in the MRW treated group could not be advanced to monthly injections because of large local reactions and thus were maintained on weekly injections.

The rhinitis symptoms of both PRW- and MRW-treated patients improved over the 2-yr study. Although the symptom scores of the PRW-treated group were slightly greater than those of the MRW-treated group during the 1977 ragweed season, they were slightly lower than those of the MRW-treated group during the 1978 ragweed season. When the symptom scores of the PRW-treated group were compared with those of a similar group of adult patients followed in our clinic during the 1978 ragweed season who were treated only with symptomatic medications, significantly lower scores were reported by the treated group.

We therefore conclude that immunotherapy for ragweed hay fever with polymerized whole ragweed represents an improved form of immunotherapy. Patients maintain high levels of blocking antibody against AgE with monthly maintenance injections. They tolerate this form of immunotherapy well with substantially fewer allergic reactions resulting in the

unhampered attainment of maintenance injections. Therapy with relatively low PNU dosage of PRW over a 2-yr period results in a significant decrease in rhinitis symptoms and symptomatic medications compared with years before immunotherapy in this treated group and also compared with a current group of patients not treated with immunotherapy.

REFERENCES

1. Metzger WJ, Patterson R, Zeiss CR, et al: Comparison of polymerized and unpolymerized antigen E in immunotherapy of ragweed allergy. *N Engl J Med* **295**:1160, 1976.
2. Bacal E, Zeiss CR, et al: Polymerized whole ragweed: An improved method of immunotherapy. *J ALLERGY CLIN IMMUNOL* **62**:289, 1978.
3. Patterson R, Suszko I, et al: Reduced allergenicity of high-molecular weight ragweed polymers. *J ALLERGY CLIN IMMUNOL* **63**:47, 1979.
4. Patterson R, Suszko I, McIntire F: Polymerized ragweed antigen E. *J Immunol* **110**:1402, 1973.
5. Patterson R, Suszko I: Polymerized ragweed antigen E. III. Differences in immune response to three molecular weight ranges of monomer and polymer. *J Immunol* **112**:1855, 1974.
6. Williams CA, Chase MW: *Methods in immunology and immunochemistry*. New York, 1968, Academic Press, Inc., vol. II, p. 257.
7. Lidd D, Farr RS: Primary interaction between ¹³¹I labeled ragweed pollen and antibodies in the sera of humans and rabbits. *J ALLERGY* **33**:45, 1962.
8. Zeiss CR, Metzger WJ, Levitz D: Quantitative relationships between IgE antibody and blocking antibodies specific for antigen E in patients given immunotherapy with ragweed antigen E. *Clin Exp Immunol* **28**:250, 1977.