

Polymerized whole ragweed: Human safety and immune response

Joseph F. Kelly, M.D., C. Raymond Zeiss, M.D., Roy Patterson, M.D.,
Doris Levitz, B.S., and Irena M. Suszko, B.S. Chicago, Ill.

Polymerized ragweed (PRW) has been shown to have reduced allergenicity while maintaining immunogenicity. In order to evaluate whether the reduced allergenicity would permit high initial doses and rapid progression to maintenance, two groups of subjects with ragweed pollenosis were placed on immunotherapy with PRW standardized for antigen E (AgE) content. Group I, 28 subjects, received an initial dose of 100 protein nitrogen units (PNU) (1 µg AgE) and reached a maintenance dose of 1,000 PNU (10 µg AgE) in four weekly injections. In the first 10 wk each subject received 7,850 PNU (78.5 µg AgE). Group II, six subjects, received an initial dose of 100 PNU (1 µg AgE) and after 11 weekly injections received 30,000 PNU (300 µg AgE). No anaphylactic reactions occurred in the study groups. In group I, three large immediate-type local reactions and nine large late-type reactions occurred in 110 injections. Anti-AgE antibody activity in group I rose from a pretreatment mean value of 264 ng AgE bound per milliliter serum to a posttreatment value of 3,182. The major rise occurred after only 4 wk of therapy. In group II, anti-AgE antibody activity rose from 66 to 2,123 after the 11 wk of therapy. IgE antibody to AgE did not change to any extent in either group, and histamine release in response to AgE measured in group II patients did not change. Symptom score evaluation of group I patients revealed a marked decrease in symptoms after therapy with PRW. Immunotherapy with PRW can be initiated at higher doses, with rapid attainment of maintenance dosage with safety resulting in a brisk immune response and symptomatic improvement.

Polymerization of ragweed proteins by glutaraldehyde treatment has been extensively studied in this laboratory.¹⁻¹⁰ The primary purpose of these studies was to prepare and evaluate the use of polymerized preparations as an improved form of immunotherapy for ragweed pollenosis. Two significant clinical observations were made. First, the polymerized ragweed (PRW) is at least as clinically effective as the standard aqueous preparation.^{5, 9} And second, PRW has reduced allergenicity while retaining immunogenicity.⁵⁻⁸ Allergenicity here refers to the ability to elicit IgE-mediated reactions, whereas immunogenicity refers to the induction of IgG antibodies.

The reduced allergenicity of these polymerized preparations offers areas of significant potential 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 and risk of local and systemic immediate-type reactions, and by decreasing the cost and inconvenience to the patients in terms of physician visits. The decreased allergenicity has been demonstrated in humans by decreased in vitro leukocyte histamine release,² decreased reactivity with specific IgE in vitro,² decreased ability to elicit cutaneous reactivity in both direct and passively sensitized skin test sites in man,^{2, 7, 8} decreased local and systemic reactions in clinical trials with conventional doses,^{5, 9} and the ability to rapidly increase antigen doses in clinical trials without adverse effect.^{5, 9} Despite this decrease in allergenicity, immunogenicity has been maintained, indicating no major loss of antigenic determinants.^{5, 10} This study was therefore designed to determine the clinical ef-

From the Section of Allergy-Immunology, Department of Medicine, Northwestern University Medical School.

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Reprint requests to: Dr. Roy Patterson, 303 E. Chicago Ave., Chicago, IL 60611.

TABLE I. Immunotherapy protocol for polymerized ragweed—group I

Week	No. of injections	Dose* (ml)	Dose (PNU)	Cumulative dose (PNU)	Cumulative dose AgE (μ g)	Cumulative dose PRW protein (μ g)
1	1	0.10	100	100	1.0	6
2	2	0.25	250	350	3.5	21
3	3	0.50	500	850	8.5	51
4	4	1.00	1,000	1,850	18.5	111
5	5	1.00	1,000	2,850	28.5	171
6	6	1.00	1,000	3,850	38.5	231
7	7	1.00	1,000	4,850	48.5	291
8+	8	1.00	1,000	5,850	58.5	351
9+	9	1.00	1,000	6,850	68.5	411
10+	10	1.00	1,000	7,850	78.5	471

*PRW 1,000 PNU/ml, 10 μ g AgE/ml, 60 μ g ragweed protein/ml.

†Some patients did not come in for weekly injections and took up to 14 wk to complete 10 injections.

fects of high initial doses of PRW and rapid progression to maintenance levels while monitoring the immunologic response to ragweed antigens. Additionally, the effects of such therapy on symptoms was assessed in 21 patients.

MATERIALS AND METHODS

Subjects

In the first phase of the study 28 subjects were selected (group I). The ages ranged from 22 to 55 yr and included 16 men and 12 nonpregnant women. The subjects were selected on the basis of typical clinical histories of allergic rhinitis and asthma occurring during the ragweed season for the Chicago area.¹¹ All subjects had 3 or 4+ positive prick test responses to commercially prepared aqueous ragweed extract.

After our experience with the first 28 patients, a second group of six allergic individuals aged 25 to 49 yr of age were selected who met the above criteria. They were to receive higher-dose immunotherapy, obtaining the equivalent of 1 yr of standard therapy in 11 injections (group II).

The nature of the study was explained to the subjects, and all gave informed consent. The subjects had no other significant medical problems and each had a normal complete blood count (CBC), urinalysis, and chest radiograph. The subjects took no medications on the day of immunotherapy injections. Two subjects had prior immunotherapy ending approximately 5 to 10 yr prior to this study.

Antigens

Cutaneous testing was performed with commercially prepared aqueous, glycerinated 1:10 (w/v) ragweed extract purchased from Hollister-Stier Labs. Immunotherapy was carried out with a polymerized preparation of ragweed extract prepared as described in detail elsewhere.⁸ Briefly, this material is an aqueous extract of ether-defatted giant and short ragweed pollen, polymerized by glutaraldehyde treatment and collected after molecular fractionation on Sepha-

rose 4B and Sephadex G-200 columns. Sterility was assured by appropriate cultures in the bacteriology laboratories of Northwestern Memorial Hospital. The final preparation of antigen had a molecular weight range of 200,000 to 20,000,000 daltons.

The PRW solution was standardized by optical density analysis at 280 nm and by determining nitrogen by the Kjeldahl method. Optical density analysis tended to overestimate the protein content; therefore, the nitrogen content as determined by Kjeldahl analysis is given in this report. Stock solutions of PRW were adjusted to contain 10,000 PNU/ml (0.6 mg ragweed protein P) and diluted as necessary for immunotherapy. Recent analysis of the ragweed antigen E (AgE) content of monomer ragweed (MRW), which is the starting material for PRW, demonstrated that 10,000 PNU of MRW contains 100 μ g of AgE. Since the AgE in MRW is completely incorporated into the PRW, the AgE content of the PRW is assumed to be 100 μ g AgE/10,000 PNU. For reference, a commercially available short ragweed extract contains 60,000 PNU/ml and 200 μ g AgE/ml. The AgE content of MRW was assayed by Dr. R. Wyrick (Hollister-Stier, Spokane, Wash.) by standard gel diffusion techniques.

Immunologic studies

Serum samples were obtained from each subject at the initiation of this study and at various intervals while receiving immunotherapy. Serum was assayed for IgE antibodies against AgE using the polystyrene tube radioimmunoassay technique.¹² The results are reported as nanograms of IgE specific for AgE. Total serum antibody activity specific for AgE was measured by a modification of the ammonium sulfate precipitation technique of Lidd and Farr.¹³ Most of the binding activity is due to IgG antibody, and the results are expressed as nanograms of AgE bound per milliliter of serum.¹⁴

In the six group II patients, leukocyte histamine release was determined using standard methods¹⁵ and was ex-

TABLE II. Immunotherapy protocol for polymerized ragweed—group II

Week	Concentration (PNU/ml)	Dose (ml)	Dose (PNU)	Cumulative dose (PNU)	Cumulative dose AgE (μ g)	Cumulative dose PRW protein (μ g)
1	1,000	0.10	100	100	1.0	6
2	1,000	0.25	250	350	3.5	21
3	1,000	0.50	500	850	8.5	51
4	1,000	1.00	1,000	1,850	18.5	111
5	10,000	0.15	1,500	3,350	33.5	201
6	10,000	0.20	2,000	5,350	53.5	321
7	10,000	0.30	3,000	8,350	83.5	501
8	10,000	0.40	4,000	12,350	123.5	741
9	10,000	0.50	5,000	17,350	173.5	1,041
10	10,000	0.60	6,000	23,350	233.5	1,401
11	10,000	0.70	7,000	30,350	303.5	1,821

TABLE III. Local reactions as related to dose of antigen—group I

Dose (PNU)	Local reaction				Total
	None	Small	Medium	Large	
A. Immediate type					
100	12	15	0	1	28
250	12	15	0	1	28
500	10	17	0	0	27
1,000	11	14	1	1	27
Total	45	61	1	3	110
B. Late type					
100	22	1	1	4	28
250	24	1	1	2	28
500	22	3	1	1	27
1,000	23	2	0	2	27
Total	91	7	3	9	110

pressed as the in vitro concentration of antigen E required to cause 50% release of histamine.

Clinical protocols

The protocol for immunotherapy and cumulative dose of antigen given to group I is shown in Table I. The incidences of local and systemic reactions were recorded; IgE and IgG anti-AgE antibodies were monitored prior to therapy and serially after the subjects received PRW by subcutaneous injection.

Reactions were recorded during a 30-min observation period after each injection and by telephone interview 24 hr after each injection. Subjects were questioned about adverse systemic reactions prior to the next injection. This protocol was to be broken only if large reactions or systemic reactions occurred. If a clinically significant local reaction occurred, the same dose was repeated at the time of the next injection. Patients with systemic reactions were to be removed from the study.

All patients in group I were asked to complete symptom score sheets which included nine symptom categories. They

were asked to grade each symptom category from 0 to 10 with 10 being the most severe. This symptom evaluation was their global impression of the severity of each symptom category for the entire ragweed season. The maximum score for an entire ragweed season was 90. This symptom evaluation was carried out at the end of the 1977 ragweed season prior to immunotherapy and after the 1978 ragweed season following PRW therapy. After the 1978 ragweed season, they completed an identical symptom score sheet without reference to their 1977 symptom score analysis.

Six patients (group II) received 11 injections of PRW according to the protocol outlined in Table II. The initial dose contained 100 PNU PRW (6 μ g P), and the final dose contained 7,000 PNU PRW (420 μ g P). In this study, approximately 30,000 PNU were given over the 11 wk period. This was four times the dose given to group I patients. Both local and systemic reactions were recorded in order to determine clinical safety. Reactions were recorded during a 30 min observation period after each injection. Subjects were questioned about adverse systemic reactions prior to the next injection. No attempt was made to determine clinical

TABLE IV. Antibody response during immunotherapy with polymerized ragweed—group I

	Total dose ragweed (PNU)			
	0	1,850	4,850	7,850
IgG*				
Mean	264	2,083	2,844	3,182
SD	443	2,496	2,698	2,661
Range	0-1,960	0-9,600	0-8,400	168-10,800
No.	25	25	24	19
IgE†				
Mean	103	165	183	159
SD	106	189	255	201
Range	6-340	0-828	0-1,158	4-821
No.	27	25	22	19
Injections	0	4	7	10
Time (wk)	0	5	8	10-14

*Specific anti-AgE IgG expressed as nanograms AgE bound per milliliter serum.

†Specific anti-AgE IgE expressed as nanograms of specific IgE.

efficacy by symptom score indices in the six patients. Blood was obtained for the immunologic studies before and after the immunotherapy program.

RESULTS

Twenty-eight subjects (group I) were started on polymerized ragweed protein at an initial dose of 100 PNU ($6 \mu\text{g P}$) PRW. Twenty-seven subjects reached the maintenance dose of 1,000 PNU ($60 \mu\text{g P}$) in four weekly injections and subsequently completed the 10-injection protocol. They then received five additional injections of 1,000 PNU at monthly intervals up to the 1978 ragweed season. One subject dropped out of the project after two injections due to scheduling difficulties.

No systemic, immediate-type reactions occurred in this group of subjects despite the high initial dose or rapid progression in dose. No other generalized adverse reactions were reported by the subjects.

Local reactions at the injection site were observed and reported by the subjects. The reactions were divided into two types: immediate type, characterized by mild pruritis, erythema, and edema, occurring within 30 min of injection; and late type, characterized by pain, edema, and occasional erythema, occurring usually 4 to 6 hr after injection. The number of reactions and relationship to dose of antigen are shown in Table III. The reactions were classified by size: mild, if erythema and/or edema < 3 cm; moderate, if erythema and/or edema 3 to 6 cm; and large, if erythema and/or edema > 6 cm.

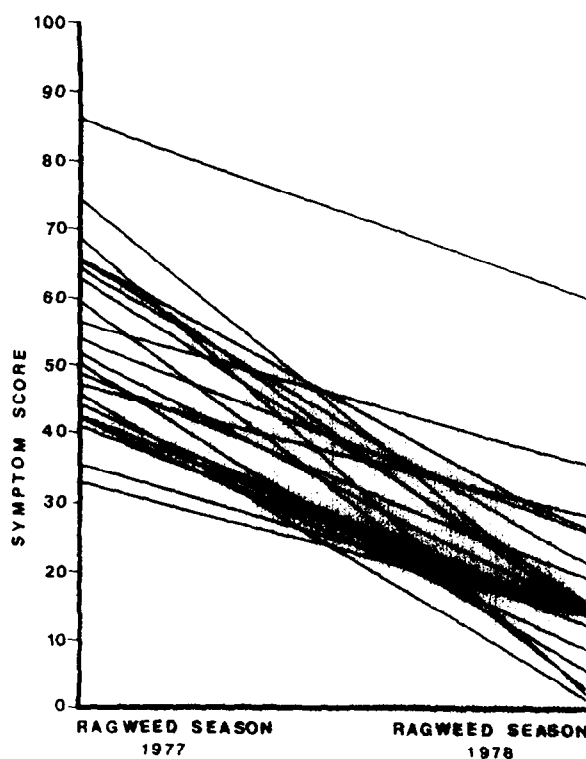


FIG. 1. The symptomatic improvement of 21 patients (group I) treated with PRW. The mean symptom score during the 1977 ragweed season was 54, falling to 16 during the 1978 ragweed season.

Immediate-type local reactions were not clinically significant except in one subject. In 106 of the 110 injections given between the initial dose and progression to maintenance, no reaction or a small reaction occurred. Occasional small reactions did occur during maintenance therapy. One subject developed a large reaction on the initial dose and the subsequent dose. This subject dropped out of the study due to scheduling difficulties but later was placed on a modified program starting at 50 PNU ($3 \mu\text{g P}$) with slow progression without difficulty.

Late-type local reactions were noted in 19 of 110 injections. These reactions did not correlate with the presence or absence of immediate-type reactions. All 19 reactions occurred in six subjects, and seven of the nine large reactions occurred in three subjects. None of the six subjects with late local reactions demonstrated progression in reaction size with increasing doses of antigen.

The immune response of these subjects to immunotherapy is shown in Table IV. Wide variation was noted in both IgG and IgE antibodies at the start of and during immunotherapy. The mean IgG antibody level rose almost eightfold after 4 wk of therapy and thereafter demonstrated only small incremental

TABLE V. Leukocyte histamine release and serum antibody to AgE in six subjects before and after progressive dose, short-term immunotherapy with polymerized ragweed

Subject	50% Leukocyte histamine release (μ AgE/ml)*		IgE anti-AgE (ng IgE/ml)		IgG Anti-AgE (ng AgE bound/ml)	
	Before	After	Before	After	Before	After
1	NR	NR	11	12	54	740
2	0.75	0.34	21	20	50	1,200
3	0.87	2.80	172	163	144	4,000
4	NR	NR	7	5	24	2,000
5	0.40	0.25	287	389	108	3,200
6	0.35	0.44	25	17	21	1,600
Mean	0.59	0.96	87	100	66	2,123

*Concentration of AgE in vitro required to produce 50% histamine release. NR = No release.

changes. All subjects showed a significant IgG response, except for one subject who did not show a response until he had received 7,850 PNU PRW. The mean IgE anti-AgE antibody also increased slightly during initial therapy but started to decline with continued therapy. No apparent correlation was observed between local reactions and antibody levels or the ratio of specific IgG to IgE.

Twenty-one patients returned completed symptom score evaluations after the 1977 and 1978 ragweed seasons. The results of the patients assessment of their improvement are shown in Fig. 1. All patients reported a dramatic decrease in symptoms, with a mean fall in symptom index from 54 to 16.

The six subjects in group II completed the experimental protocol. Each subject received at least 300 μ g AgE and 30,000 PNU. No significant local reactions occurred. Four of the six subjects had mild immediate reactions characterized by erythema less than 3 cm in diameter within 30 min of injection. Mild immediate local reactions occurred in 35 of 66 injections during this study. One subject (Subject 5) had late local reactions characterized by erythema and edema less than 3 cm diameter, with onset approximately 8 hr post injection. This reaction was not painful and did not concern the subject. It gradually resolved over a 12-hr period and did not progressively increase in size with increasing doses of antigen.

Five of six subjects had no systemic adverse reactions. Subject 5 had a mild, evanescent, erythematous, nonpruritic skin rash several days after starting immunotherapy. The rash was not urticarial in nature, did not bother the subject, and disappeared spontaneously. The rash did not become progressively more severe as immunotherapy was continued, but injection of 1,500 PNU was given twice to this patient because of the remote possibility of a systemic reac-

tion. There was no rash during the week following the extra injection, and the remainder of the protocol was completed without difficulty. It was not clear to the subject or to us whether the rash was related to the immunotherapy injections.

The results of the histamine release studies and the antibody responses are shown in Table V. Two of the subjects showed a slight increase in histamine release before and after therapy, whereas two of the subjects showed a slight decrease. There were two non-responders in the group. The changes in histamine release noted did not appear to be significant.

The IgE anti-AgE did not change significantly during the course of this study. One subject (Subject 5) did have a slight increase from 287 to 389 ng IgE/ml before and after therapy. This is the same subject that had late local reactions and the evanescent erythematous rash. Overall, there was no clear demonstration of enhanced IgE production.

All subjects showed a significant increase in the IgG anti-AgE levels before and after therapy. There was a mean 32-fold increase in IgG antibody levels. The mean pretreatment level was 66 ng AgE bound/ml, and the mean posttreatment value was 2,123 ng AgE bound/ml.

DISCUSSION

This study was designed to evaluate the safety, immunologic response, and therapeutic impact of polymerized ragweed. In terms of safety, the most significant finding in this study was the absence of immediate-type systemic or anaphylactic reactions in spite of rapid progression to larger doses. Anaphylaxis is widely recognized as the major risk factor for patients undergoing immunotherapy, and this risk has properly been responsible for most of the time-consuming and elaborate dilution and injection pro-

protocols used in the clinical practice of allergy. Considering this report and the efficacy study of Bacal et al⁹, a total of 44 highly sensitive subjects with ragweed pollinosis have now received therapeutic doses of PRW without systemic reactions. In the control group of nine atopic subjects reported by Bacal, seven systemic reactions were recorded using standard monomeric extracts for therapy. When the absence of anaphylaxis is combined with the rapid dose schedules of this study, the risk of anaphylaxis appears to be greatly decreased. However, the report of Metzger et al⁵ indicates two systemic reactions to polymerized ragweed AgE and suggests that caution and physician supervision will continue to be required in providing ragweed immunotherapy to patients.

Local reactions occurred in this study. An immediate local reaction was important in only one of 28 patients and was easily managed by a reduction in dose. The late local reactions were a more significant problem. Pain from large reactions was common and a complaint from the subjects in nine of 110 injections given. Whether these reactions are related to injection technique, the polymerization of the antigens, the immunologic status of the subjects, or the dose of the antigen is not certain. Prior studies have indicated late local reactions in skin tests with ragweed extracts,^{15, 16} and the late asthmatic responses have been seen in some subjects undergoing bronchial challenge with ragweed.¹⁷ Injection technique probably contributed to some of the large reactions and is suggested by changes in nursing personnel during the course of the study and by the absence of any pattern or relationship to dose in 12 of the late local reactions. However, seven of the nine large late local reactions did occur in three subjects; this implies that the immunologic status of the subject or individual sensitivity to the PRW does play a role. Prophylactic antihistamine therapy was not a part of the experimental protocol so that the occurrence of immediate or late reactions would not be masked. When the therapy has had more evaluation, pretreatment with a long-acting antihistamine may prevent or modify the late local reactions.

The immunologic responses to PRW were comparable to those previously reported in which similar cumulative doses of monomeric antigen were used.^{5, 9} Of interest is that only four injections of PRW (1,850 PNU, 18.5 μ g AgE) were able to stimulate this response before a plateau effect was seen in the IgG response. Giving higher doses in the group II patients did not result in a substantial increase in blocking antibody levels. The levels of IgG antibody activity against AgE measured in this study were as high or higher than in our previous studies. This indicates that

TABLE VI. Advantages of polymerized ragweed protein in immunotherapy

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1. Initial dose is 40 times higher than standard therapy.
 2. Maintenance dose is reached in 4 wk with four injections as compared to 5 to 6 mo with standard therapy.
 3. Rise to significant blocking antibody levels is 4 wk as compared to 4 to 5 mo.
 4. Systemic reactions are markedly reduced.
 5. Convenience to patient is increased.
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a significant immune response to AgE can be achieved in just 4 wk of therapy. The increase in specific IgE observed in our subjects after therapy has also been noted in previous reports of immunotherapy with pollen antigens.¹⁸ However, the increases reported here are limited and started to decline with continued therapy. Only antibody activity against AgE was measured in this study, and this probably accounts for the low IgG responses observed in some subjects (Table IV). Zeiss et al.¹⁰ have reported significant IgG responses to AgE, antigen K, and antigen Ra3 in subjects treated with PRW, but they found that the responses to each antigen vary with the individual subject. In this study, AgE was only used as one index of response to therapy with PRW and hence does not include all individual responses. With rapid dose progression in PRW antigen given to group II, we looked for acute changes in histamine release.²⁰ No significant changes were noted in this short-term study.

Although this study was primarily one of the effect of rapid progression to high doses on the patients' immune response, patients in group I recorded a marked reduction in symptom scores as seen in Fig. 1. This dramatic reduction is encouraging, and multicenter study of PRW is now underway which should give a complete answer to the question of the efficacy of PRW.

The protocol used in this study has many advantages over the standard allergen injection program¹⁹ which is in use at the Northwestern University Allergy Clinics and which is representative of many clinical protocols. These programs are briefly compared in Table VI. The initial dose is 40 times greater than that with standard aqueous therapy (100 PNU ragweed protein as opposed to 2.5 PNU). The maintenance dose can be reached in only 4 wk with four injections in the majority of patients as compared to the standard program of 26 to 30 injections on a weekly basis. Additionally, as demonstrated by the group II patients, 30,000 PNU can be given in 11 wk, which is equivalent to 1 yr of standard therapy.

Significant blocking antibody is produced within 4 wk instead of the usual 16 to 20 wk period observed with standard aqueous therapy.⁵ The incidence of systemic reactions is markedly reduced, and local reactions are easily managed by slight reductions in the dose of antigen. In addition, the PRW offers a significant potential for making immunotherapy more convenient and practical for patients. Cost has not been specifically evaluated to date, but the preparation of polymerized antigens is not complex, and commercial preparation may minimize any increase in preparation costs. A slight increase in preparation cost may well be offset by reductions in the number of injections required. If expense and inconvenience are reduced while maintaining efficacy, immunotherapy with polymerized antigens may be utilized for a wider population who would not accept it currently because of expense and inconvenience.

The most appropriate utilization of polymerized antigens for immunotherapy has not yet been established and warrants further study. Combining the results of the group I and II study patients, a maintenance dose of 4,000 PNU could be reached in 8 wk in most patients. Monthly injection of 4,000 PNU from this point on would result in approximately 60,000 PNU (600 μ g AgE) being given over a 1-yr period. With the increased safety and retained immunogenicity of PRW, a number of different schedules could be tailored to meet different patients' needs.

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