

Safety and immunogenicity of immunotherapy with polymerized tree, grass, and ragweed in patients with multiple inhalant sensitivities

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Immunotherapy with polymerized ragweed (PRW) and individually polymerized grasses (IPG) previously has been demonstrated to be safe and effective. We conducted this trial in order to assess safety and immunogenicity of individually polymerized trees (IPT) and to assess safety of concomitantly administering IPT, IPG, and PRW in patients with multiple inhalant sensitivities. Sixteen patients with typical tree pollinosis were recruited; all but one had grass pollinosis, and all had ragweed pollinosis. Patients were treated in 12 weekly visits with allergens to which they were sensitive, 48,850 PNU of IPT; 2668 AU (50,000 PNU) of PRW; and 48,850 PNU of IPG. There were no systemic reactions and no changes in laboratory parameters, including renal and hepatic functions with injections. Blood was drawn for immunologic studies before and after the injection series. There were significant rises in IgG titers by ELISA to each tree allergen administered, oak, elm, and box elder. There were significant rises in total antibody binding of ragweed AgE and rye grass group I in patients treated with PRW and IPG, respectively. Changes in IgE to oak, elm, box elder, AgE, and rye grass group I were minimal. In summary, IPT is safe, immunogenic, and can be administered concomitantly with PRW and IPG in patients with multiple inhalant sensitivities. (J ALLERGY CLIN IMMUNOL 77:53-8, 1986.)

Polymerized allergens have been extensively studied in trials of human immunotherapy. A regimen of 12 weekly injections of IPG has been demonstrated to be immunogenic, safe, and efficacious in the treatment of grass pollinosis.¹ Similarly, in several trials²⁻⁴ a 15 weekly injection regimen of PRW has been demonstrated to be safe, immunogenic, and effective treatment for ragweed hay fever. Furthermore, this efficacy persists for at least 4 years without need for further immunotherapy.⁵

Polymerized allergens are significantly less allergenic than unmodified allergens,⁶⁻⁸ and that is the reason that patients can be administered a course of

Abbreviations used

PRW: Polymerized ragweed
IPG: Individually polymerized grass
IPT: Individually polymerized tree
RGGI: Rye grass group I
AU: Allergy units
PTRIA: Polystyrene tube radioimmunoassay

polymerized allergen immunotherapy in a few months, whereas a course involving a similar amount of unmodified allergen would require years. This reduced allergenicity also accounts for an additional advantage of polymerized allergens, the lower rate of systemic reactions. Although the incidence of systemic reactions has been reported to be 8% to 30% per patient course with usual immunotherapy,⁹ the incidence with polymerized allergens is <1%.² Significantly, although allergenicity of polymerized allergens is reduced by several logs, immunogenicity and efficacy are retained.^{10, 11}

Just as pollen extracts of ragweed and of grasses

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TABLE I. Immunotherapy schedules for IPT, PRW, and IPG

Injection No.	IPT (PNU per injection)	PRW (AU per injection*)	IPG (PNU per injection)
1	100	2.5	100
2	250	5	250
3	500	10	500
4	1000	25	1000
5	1500	50	1500
6	3000	125	3000
7	5000	250	5000
8	7500	300	7500
9	7500	400	7500
10 to 12	7500	500	7500

*Based on RAST inhibition analyses compared to ragweed reference standard.

have been polymerized, the same process was applied to tree pollens. Initially, a mixture of tree pollen extracts was polymerized.¹² Subsequently, individual extracts of tree pollens were polymerized so that each patient's immunotherapy vial could be individualized to include only those extracts to which the patient demonstrated immediate cutaneous reactivity.⁸

The objectives of this study were two. First, we wished to study the safety and immunogenicity of IPT in patients with tree pollinosis. Second, we wished to study the safety of administering PRW, IPG, and IPT simultaneously, since many patients with tree pollinosis have multiple other inhalant allergies. Efficacy was not studied in this trial because tree pollinosis in the midwest is not nearly as severe as ragweed or grass pollinosis, and therefore it is not an optimal locale in which to perform efficacy trials of tree pollen immunotherapy.

MATERIAL AND METHODS

Patients

Sixteen patients with typical histories of tree pollinosis were recruited. All patients had at least 5 mm wheal and 10 mm flare reaction to prick tests of one or more of the following 1:10 tree extracts: white oak (lot No. A2061201), elm (lot No. A2060201), or box elder (lot No. A3013101) (Allergy Laboratories of Ohio [ALO], Columbus, Ohio). Patients had intradermal cutaneous titrations to weight/volume dilutions of each tree pollen extract to which the patient had a positive prick reaction.

All patients also had histories of ragweed pollinosis, grass pollinosis, or both. Patients were prick tested with the following 1:10 extracts: giant ragweed (lot No. A3012102), short ragweed (lot No. A2091304), Bermuda (lot No. A3010601), timothy (lot No. A2110801), and orchard (lot No. A2052501) grass (ALO). The tests were considered positive if the patient had at least 5 mm wheal and 10 mm flare. Each patient's immunotherapy vials were individu-

alized to include only those allergens to which the patient had a positive prick reaction.

Patients were otherwise normal as determined by history and physical examination. They also had values that were within normal limits for laboratory tests. Patients had not received immunotherapy for at least 5 years before the study.

Polymerized allergens

All polymerized allergens were prepared at ALO. Details of preparation of PRW, IPG, and IPT can be found elsewhere.^{3,7,8} Determination of protein nitrogen unit per milliliter and general safety and sterility requirements of the Food and Drug Administration, Bureau of Biologics, were conducted by ALO. Allergy units on the basis of RAST inhibition were determined by Roche Biomedical Laboratories (Burlington, N. C.).

Allergenicity of each polymerized allergen was determined by intradermal end point titration on at least three sensitive subjects.¹³

Immunotherapy schedules

During the spring and summer of 1983, the patient's immunotherapy was administered in 12 weekly visits. In order to individually assess reactions associated with each polymerized allergen, a separate injection was administered for each extract. At each visit all but one patient received three injections, IPT, IPG, and PRW. One patient did not have grass pollinosis and thus received two injections, IPT and PRW. The schedules for IPT, PRW, and IPG are illustrated in Table I. IPT and IPG were administered on the basis of protein nitrogen units, whereas PRW was administered on the basis of allergy units as determined by RAST-inhibition analyses compared against aqueous short ragweed reference standard of the Office of Biologics.

Quantitation of allergic reactions to immunotherapy

Immediate local reactions were recorded as centimeters of induration and erythema. If doses were repeated because

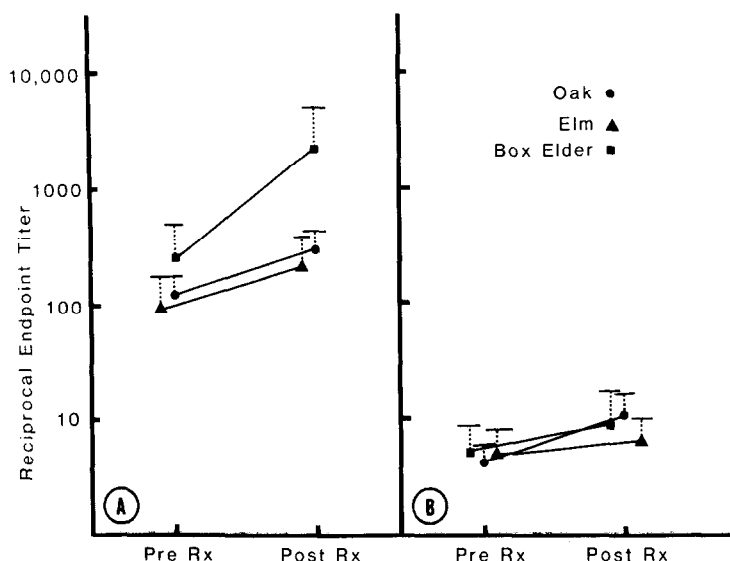


FIG. 1. A, Change in IgG titers by ELISA to box elder (■—■), elm (▲—▲), and oak (●—●) for patients receiving IPT. The vertical bars represent SEM. The titer rises are significant for box elder ($p < 0.02$), elm ($p < 0.05$), and oak ($p < 0.01$). **B,** Change in IgE titers by ELISA to box elder (■—■), elm (▲—▲), and oak (●—●). The vertical bars represent SEM. The changes are not significant for elm or box elder, but there is a significant rise in oak titer ($p < 0.02$).

of a large local reaction, additional injections were administered to reach the total doses illustrated in Table I.

Laboratory tests

Each patient had the following laboratory tests before receiving injections in order to determine that there were no abnormalities: complete blood count with differential leukocyte count, erythrocyte sedimentation rate, urinalysis, blood urea nitrogen, creatinine, lactic dehydrogenase, serum glutamyl oxytransferase, serum glutamyl phosphotransferase, gamma glutamyl transferase, alkaline phosphatase, and direct and indirect bilirubin. Each of these tests was repeated after the final injection.

ELISA

For each of the immunologic assays, blood samples were drawn from each patient before the first injection and after the final injection. Serum was separated and stored frozen until immunologic studies were performed.

The ELISA assay was used for detection of IgE and IgG against each of the tree pollens, oak, elm, and box elder.

In order to detect IgE to each of the tree pollens, the following reagents were added in sequence to Immulon micro-ELISA plates (Greiner and Son, Nürtingen, West Germany) with appropriate washes and incubation periods according to the methods of Voller et al.¹⁴ and Sepulveda et al.¹⁵: oak (lot No. A3022201), elm (lot No. A3022102), or box elder tree pollen (lot No. A3013101) extract (ALO) diluted to 150 µg of protein per milliliter, dilutions of patient's serum, rabbit antihuman IgE, goat antirabbit IgG alkaline phosphatase, and *p*-nitrophenyl phosphate. The en-

zyme reaction was stopped by the addition of 3 mol/L of sodium hydroxide, and the optical density of each well was then read at 410 nm on a Dynatech micro-ELISA minireader MR 590 (Dynatech, Santa Monica, Calif.).

The sequence of addition of reagents to wells to detect IgG was identical except that rabbit antihuman IgG was added in place of rabbit antihuman IgE. Sera from four patients receiving maintenance doses of conventional extracts were also assayed with this system.

Modified Farr assay

Total serum binding capacity of RGGI (National Institutes of Health) was determined by a modification¹⁶ of the ammonium sulfate method of Lidd and Farr.¹⁷ Briefly, 100 µl samples were incubated with 10, 100, and 1000 ng of ¹²⁵I-labeled RGGI. RGGI bound to antibody was precipitated with 40% saturated ammonium sulfate, and the amount of RGGI bound by 1 ml of serum in antigen excess was calculated. This is a quantitative estimate of blocking antibody and is expressed as nanograms of RGGI bound per milliliter of serum.

Total serum binding capacity of AgE (National Institutes of Health) was determined in an analogous method with ¹²⁵I-labeled AgE being substituted for ¹²⁵I-RGGI.

PTRIA

RGGI binding by IgE was measured by a previously described polystyrene tube technique.¹⁸ Polystyrene tubes were coated with myeloma IgE. Rabbit antihuman IgE was then added to form an IgE immunosorbent. After a 48-hour incubation with 100 µl of patient serum, the tubes were washed, and ¹²⁵I-RGGI was added in excess. After a 48-

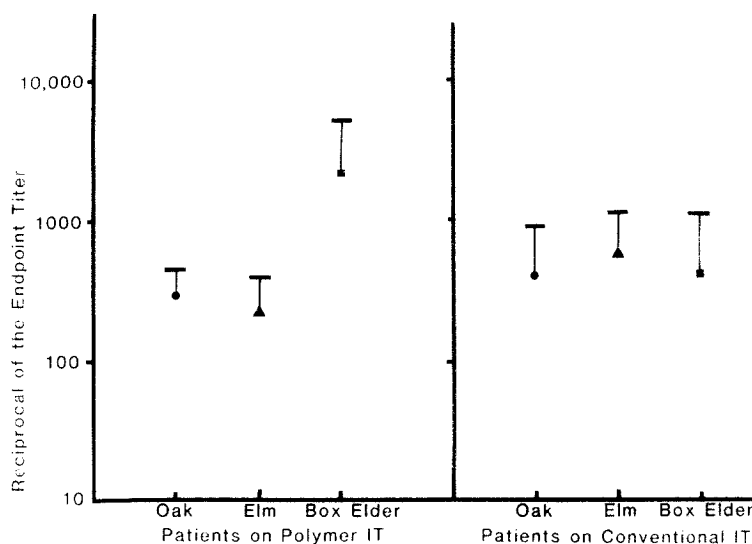


FIG. 2. IgG titers by ELISA to box elder (■), elm (▲), and oak (●) for patients treated with polymerized tree extracts (*left*) and with conventional tree extracts (*right*). The vertical bars are SEM. There are no significant differences in titers between the two groups.

hour incubation, the tubes were washed and counted. The amount of RGGI in 1 ml of serum was calculated.

AgE binding by IgE was measured in an analogous assay by use of ^{125}I -AgE in place of ^{125}I -RGGI.

RESULTS

Patients

Of the 16 recruited patients, 10 were prick positive to box elder, 13 to elm, and 14 to oak. The median end point of the patients sensitive to box elder was 10^{-6} ; to elm, 10^{-6} ; and to oak, 10^{-7} .

All 16 patients had histories compatible with ragweed pollinosis and had positive prick reactions to giant and short ragweed. Fifteen of 16 patients had histories of grass pollinosis and positive prick tests to one or more grasses.

Quantitation of allergic reactions

One patient receiving IPT had two immediate local reactions characterized by erythema alone. Totals of 3.2 cm erythema and 0 cm induration were recorded. This averaged 0.2 mm erythema per injection. No patient required any additional injections for large local reactions caused by IPT.

One patient receiving IPG had one immediate local reaction characterized by erythema alone. Totals of 1.5 cm erythema and 0 cm induration were recorded. This averaged 0.1 mm erythema per injection. No patient required additional injections for large local reactions caused by IPG.

Four patients receiving PRW had immediate local reactions consisting of erythema with or without induration. Totals of 5.5 cm erythema and 0.3 cm in-

duration were recorded. This averages 0.3 mm erythema and 0.02 mm induration per injection. One patient required a repeat injection for a large late local reaction. Her injection schedule was then altered so that she would receive 15 injections instead of 12. There were no systemic reactions in any patients.

Laboratory tests

All patients entered into the study had values that were within normal limits for the following laboratory tests: complete blood count with differential leukocyte count, erythrocyte sedimentation rate, urinalysis, blood urea nitrogen, creatinine, serum glutamyl oxyltransferase, serum glutamyl phosphotransferase, gamma glutamyl transferase, alkaline phosphatase, and direct and indirect bilirubin. There were no significant changes after treatment.

ELISA

The changes in titer to IgG and IgE against each of the tree pollens are presented in Fig. 1. Statistical analyses of the before and after changes were performed by use of one-tailed paired t test on log transformations of the data. There were statistically significant increases in IgG titer to each tree, box elder (ninefold; $p < 0.02$), elm (threefold; $p < 0.05$), and oak (threefold; $p < 0.01$) (Fig. 1, A). The change in IgE was not significant for box elder and elm. There was a 1.5-fold rise in IgE to oak that was significant ($p < 0.02$; Fig. 1, B). The titers of the four patients receiving maintenance dose of conventional extracts were comparable to the posttreatment titers of the

patients receiving polymerized extracts as presented in Fig. 2.

Modified Farr assay

Changes in total serum binding capacities for RGGI with IPG injections and for AgE with PRW injections are presented in Fig. 3, A. Statistical analyses of the before and after changes were done by one-tailed paired *t* test on log transformed data. There was a statistically significant 13-fold rise in total antibody binding of RGGI ($p < 0.01$). There was also a statistically significant rise in total serum binding capacity for AgE ($p < 0.0001$).

PTRIA

Changes of IgE against RGGI and AgE with injections are illustrated in Fig. 3, B. Statistical analyses by one tailed paired *t* test were done on log transformed before and after data. Although the mean fold increase in IgE against RGGI (2.2-fold) and AgE (1.8-fold) was comparable, only the rise in IgE against AgE was statistically significant ($p < 0.01$).

DISCUSSION

This study was done as a pilot investigation primarily to assess the safety and immunogenicity of IPT. Additionally, we have studied whether it is safe to administer multiple polymerized inhalant allergens (IPT, IPG, and PRW) concomitantly. In terms of safety, the patients received 48,850 PNU of IPT; 48,850 PNU of IPG; and 2668 AU (approximately 50,000 PNU) of PRW in 12 weekly injections with no changes in clinical laboratory tests, including hepatic and renal function tests. Very importantly, in terms of safety, there were no systemic reactions in this group of patients. This is very significant in that rates of systemic reactions from usual immunotherapy range from 8% to 30% per patient course.⁹ Separate injections were administered of IPT, IPG, and PRW in order to assess rates of local reactions from each. None of the patients required additional injections caused by large local reactions from IPT or IPG. One patient required a repeat injection of PRW for a large local reaction, and then her dosage schedule was modified. Local reactions did not prevent any of the patients from reaching maintenance doses and the total dose as outlined in Table I.

There is no purified tree antigen that can be used as a marker for oak, elm, or box elder pollen in the way that AgE or RGGI can be used as markers for ragweed or northern grass pollens. Thus, the modified Farr assay and PTRIA, both of which are best performed with a purified antigen, are not well suited to performing immunologic studies with tree antigens.

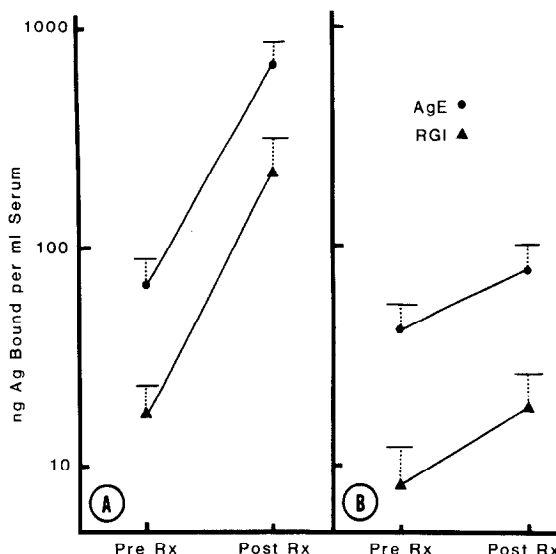


FIG. 3. A, Change in total serum binding activity of AgE (●—●) and RGGI (▲—▲) for patients receiving PRW and IPG. The vertical bars represent SEM. The rises are significant for antibody bound to AgE ($p < 0.0001$) and RGGI ($p < 0.01$). **B,** Change in IgE binding to AgE (●—●) and RGGI (▲—▲). The vertical bars represent SEM. The change in IgE binding of RGGI was not significant, but the change in binding of AgE was significant ($p < 0.01$).

We have used the ELISA method to measure antibodies to other crude extracts¹⁹ and have modified that assay to measure antibodies to partially purified tree extracts. There were significant rises in IgG titer to each of the tree pollens administered. These titers are similar to the average titers of four patients receiving maintenance doses of conventional tree immunotherapy. There were significant rises in total antibody binding capacity to AgE and RGGI similar to our previous studies of PRW and IPG immunotherapy. Changes in IgE by ELISA for trees or by PTRIA for AgE or RGGI were minimal.

In summary, this trial of immunotherapy demonstrates safety and immunogenicity of IPT. Patients developed increases in IgG titer to trees, had no change in laboratory parameters, had no systemic reactions, and received significantly fewer injections than would have been required with conventional immunotherapy. Finally, safety of concomitantly administering multiple polymerized inhalant allergens was demonstrated, and the immunogenicity and safety of PRW and IPG were again demonstrated.

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Erratum

The following correction applies to the article, "Molecular Forms of Tear IgA and Distribution of IgA Subclasses in Human Lacrimal Glands," by Allansmith et al. that appeared on page 569 of the October 1985 issue of *THE JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY*.

The first two lines of the abstract should read, "Tears and sera from 14 subjects were analyzed for IgA levels by radioimmunoassay by use of secretory IgA standards for tear samples and monomeric IgA standards for serum samples."

Correction now appears in reprints.