

Safety and immunogenicity of immunotherapy with accelerated dosage schedules of polymerized grass and ragweed in patients with dual inhalant sensitivity

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Immunotherapy with individually polymerized grasses (IPG) and immunotherapy with polymerized ragweed (PRW) have been demonstrated to be immunogenic and safe and to result in lowering of symptom-medication scores compared to placebo. We conducted this study to evaluate the immunogenicity and safety of immunotherapy with concomitantly administered accelerated dosage schedules of IPG and PRW in 12 patients with dual inhalant sensitivities. Patients were treated in nine weekly visits with IPG, comprising 71,950 PNU; they were treated in 11 weekly visits with PRW comprising 2955 allergy units. Eleven additional patients who had been previously treated with IPG received only PRW. There were no systemic reactions and no clinically significant changes in routine laboratory parameters, including hepatic and renal functions, with injections. There were significant rises in IgG titers by ELISA to each grass-pollen allergen administered, orchard, timothy, and Bermuda, and in total antibody binding of antigen E. Changes in IgE against orchard, timothy, Bermuda, and antigen E were minor. Thus, IPG and PRW administered concomitantly in accelerated dosage schedules are safe and immunogenic in patients with dual inhalant sensitivities. (J ALLERGY CLIN IMMUNOL 1989;83:750-6.)

Polymerized allergen immunotherapy has been extensively studied in numerous immunotherapy trials.¹⁻⁴ Studies with schedules of 12 weekly injections of IPG and of 13 injections of IPG in 9 weeks² have been performed. It was reported that IPG can be administered safely and that it reduced patient symptom and medication scores. Similar findings were reported after studies with a PRW immunotherapy schedule of 15 weekly injections.^{3,4} Moreover, this reduction in symptom and medication scores persists for 4 years with no further need for immunotherapy.⁵

Polymerized allergens are significantly less allergenic than unmodified allergens on a weight basis,^{6,7} which permits patients to receive a full course

Abbreviations used

AgE:	Ragweed antigen E
AU:	Allergy units
IPG:	Individually polymerized grasses
PRW:	Polymerized ragweed
PTRIA:	Polystyrene tube radioimmunoassay
ALO:	Allergy Laboratories of Ohio
CBC:	Complete blood cell count
U/A:	Urinalysis
ESR:	Erythrocyte sedimentation rate
BUN:	Blood urea nitrogen
LDH:	Lactic dehydrogenase
AST (SGOT):	Aspartate aminotransferase
ALT (SGPT):	Alanine aminotransferase
GGT:	Gamma-glutamyltransferase
FDA:	Food and Drug Administration

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of polymerized allergen immunotherapy in a few months in contrast to a complete course of unmodified allergen immunotherapy that often requires years. This reduced allergenicity is also the explanation for a low rate of systemic reactions, an additional major advantage of polymerized allergens. Although the in-

cidence of systemic reactions with usual immunotherapy has been reported to be 8% to 30% per patient course,⁸ the incidence with polymerized allergens is <1%.³ Very importantly, while allergenicity of polymerized allergens is significantly reduced, activity and immunogenicity are retained.^{3, 9, 10}

The purpose of this study was to evaluate the immunogenicity and safety of concomitantly administered accelerated dosage schedules of IPG and PRW in patients with dual inhalant pollinosis. Patients' symptoms and medication scores were not studied because a significantly larger number of patients and control subjects would be required for these analyses, and reduction in this type of scoring has already been demonstrated with both IPG^{1, 2} and PRW.^{3, 4}

MATERIAL AND METHODS

Patients

Twenty-three patients, aged 24 to 61 years, with characteristic histories of grass pollinosis were recruited by media advertisement. The criteria for characteristic grass pollinosis were significant nasal congestion, rhinorrhea, and pruritus correlating with the grass-pollen season. Each patient had at least a 5 mm wheal and 10 mm flare reaction to prick tests¹¹ of one or more of the following 100,000 AU/ml grass-pollen extracts (Pharmacia Diagnostics, Piscataway, N.J.), Bermuda (lot No. HF25630), orchard (lot No. IB25633), and timothy (lot No. IB25631). An intradermal cutaneous end point titration¹¹ was performed on each patient with appropriate tenfold dilutions of each of the 100,000 AU/ml grass-pollen extracts to which the patient was sensitive by prick tests.

All the patients also had histories of typical ragweed-allergic rhinitis. Criteria for ragweed pollinosis were significant nasal congestion, rhinorrhea, and pruritus correlating with the ragweed-pollen season. Patients were tested by prick method with each of the following 100,000 AU/ml of ragweed-pollen extracts (Pharmacia): giant ragweed (lot No. HF25628) and short ragweed (lot No. GF22674). Tests were considered positive if the reaction resulted in at least a 5 mm wheal and 10 mm flare. Patients originally participated in a grass study as placebo-control subjects and thus were treated in an open fashion in this study.

The immunotherapy vials for each patient were individualized to include only those allergens to which the patient had a positive prick test reaction. No patient had received immunotherapy for at least 5 years before the study, except for the 11 patients previously treated with IPG. The patients were otherwise healthy, as determined by history, physical examination, and laboratory values: CBC with differential, U/A, ESR, serum creatinine, BUN, LDH, AST (SGOT), ALT (SGPT), GGT, alkaline phosphatase, and direct and indirect bilirubin.

Polymerized allergens

All polymerized allergens were manufactured at ALO, Columbus, Ohio. Details of the preparation of IPG and PRW

can be found in previous articles.^{2, 3} RAST-inhibition analyses to determine allergy units were conducted by Roche Biomedical Laboratories (Burlington, N.C.). The micro-Kjeldahl method was used to determine the protein nitrogen units per milliliter of each polymerized allergen (ALO). Sterility and general safety requirements of the FDA were also conducted by ALO.

Immunotherapy schedule

During the spring of 1984, 11 patients received IPG as part of a previously reported trial.² During the fall of 1984 and winter of 1985, the other 12 patients received IPG immunotherapy in 10 injections administered in nine weekly visits. All 23 patients received PRW immunotherapy with 13 injections in 11 weekly visits. A separate injection was administered for each extract (IPG and PRW) so that reactions associated with each polymerized allergen could be individually assessed. The immunotherapy schedules for IPG and PRW are listed in Table I. IPG was administered on the basis of protein nitrogen units, whereas PRW was administered on the basis of AUs, as determined by RAST-inhibition analyses compared with an aqueous short ragweed reference standard of the Office of Biologics, FDA.

Evaluation of allergic reactions to immunotherapy

Millimeters of induration and erythema were recorded for each local reaction 30 minutes after the injection(s). Patients were also queried at that time whether they had had any additional symptoms, in particular, symptoms that might be compatible with a systemic allergic reaction.

Delayed local reactions and any other symptoms the patients experienced after 30 minutes were recorded by patients on reporting forms.

Clinical laboratory tests

Before initiation of this study and after the final injection, a U/A, CBC with differential, ESR, serum creatinine, BUN, LDH, AST (SGOT), ALT (SGPT), GGT, alkaline phosphatase, and direct and indirect bilirubin were performed on each patient.

ELISA

For each of the immunologic assays, blood samples were drawn from each patient before the first injection and immediately after the final injection. Sera were separated from cells and stored at -20° C until immunologic assays were performed.

The ELISA was used for determination of IgG and IgE against each of the grass pollens: Bermuda, orchard, and timothy. For the detection of IgG, reagents were added in the following order to Immulon micro-ELISA plates (Greiner & Sons, Nürtingen, West Germany) with appropriate washes and incubation periods, as previously reported.^{2, 12, 13} Bermuda-pollen (lot No. HF25630), orchard-pollen (lot No. IB25633), or timothy-pollen (lot No. IB26531) extracts (Pharmacia Diagnostics) at 75 µg of protein per milliliter, patient-serum dilutions, rabbit antihuman

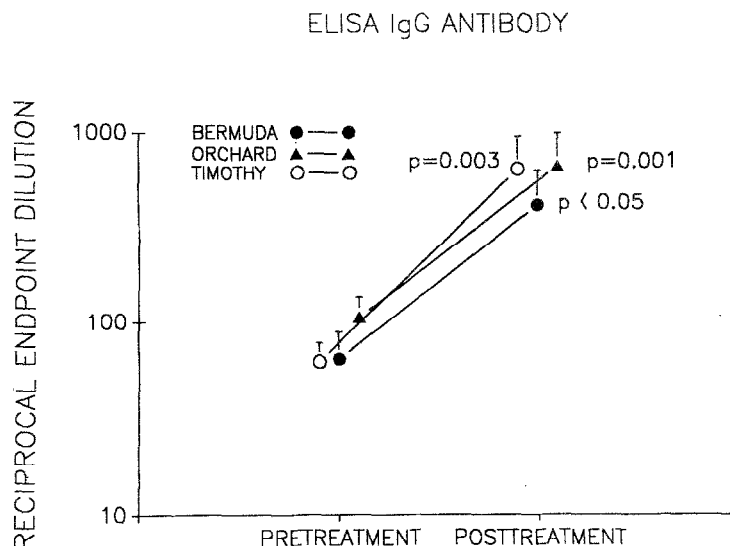


FIG. 2. Change in IgG titers by ELISA to Bermuda (●—●), orchard (▲—▲), and timothy (○—○) grass in patients receiving IPG. The vertical bars are SEM. The titer rises are significant for Bermuda ($p < 0.05$), orchard ($p = 0.001$), and timothy ($p = 0.003$) grass.

of serum was calculated and expressed as nanograms of AgE bound per milliliter of serum.

RESULTS

Patients

Of the 23 patients, 17 were prick positive to Bermuda, 21 to orchard, and 22 patients to timothy grass. The median end point of patients sensitive to Bermuda was 1 AU/ml; to orchard, 0.1 AU/ml; and to timothy, 0.1 AU/ml. A scattergram of individual patient results is illustrated in Fig. 1.

All 23 patients had positive prick tests to both giant and short ragweed. The patients ranged in age from 24 to 61 years with a mean of 37.5 ± 9 and median of 37 years. There were 18 male and five female subjects of whom three were black and 20 were white.

Evaluation of allergic reactions to immunotherapy

Of the 12 patients receiving IPG, one patient had at least one immediate local reaction consisting of erythema and wheal, whereas nine patients had flare alone. Reactions averaged 3.0 mm of erythema and 0.1 mm of induration per injection. No patient receiving IPG had a large local reaction that warranted an additional injection.

Of the 23 patients receiving PRW, eight patients had at least one immediate local reaction consisting of both wheal and flare, whereas 12 patients had at least one immediate local reaction consisting of flare alone. They averaged 5.0 mm of erythema and 0.2

mm of induration per injection. No patients receiving PRW required an additional injection because of a large local reaction. These local reactions occurred throughout the injection schedule, were always less than 48 hours in duration, and did not produce significant distress for the patient.

There were no systemic reactions.

Clinical laboratory tests

There were no clinically significant differences in results of the following laboratory tests when preimmunotherapy results were compared to postimmunotherapy results: U/A, CBC with differential, ESR, serum creatinine, BUN, LDH, AST (SGOT), ALT (SGPT), GGT, alkaline phosphatase, and direct and indirect bilirubin.

ELISA

Changes in IgG titer after IPG are illustrated in Fig. 2 for each pollen: Bermuda, orchard, and timothy. Statistical analyses of the changes with treatment were performed with paired Student's *t* test on log transformations of the data. One-tailed levels of significance were used because IgG antibody has been demonstrated to increase with treatment. There were statistically significant increases in IgG titer to each grass pollen, Bermuda (sevenfold; $p < 0.05$), orchard (sixfold; $p = 0.001$), and timothy (tenfold; $p = 0.003$). There was no change in IgE to Bermuda, a 1.3-fold rise ($p < 0.05$) in IgE to orchard and a 1.2-fold rise in IgE to timothy, which was not statistically significant.

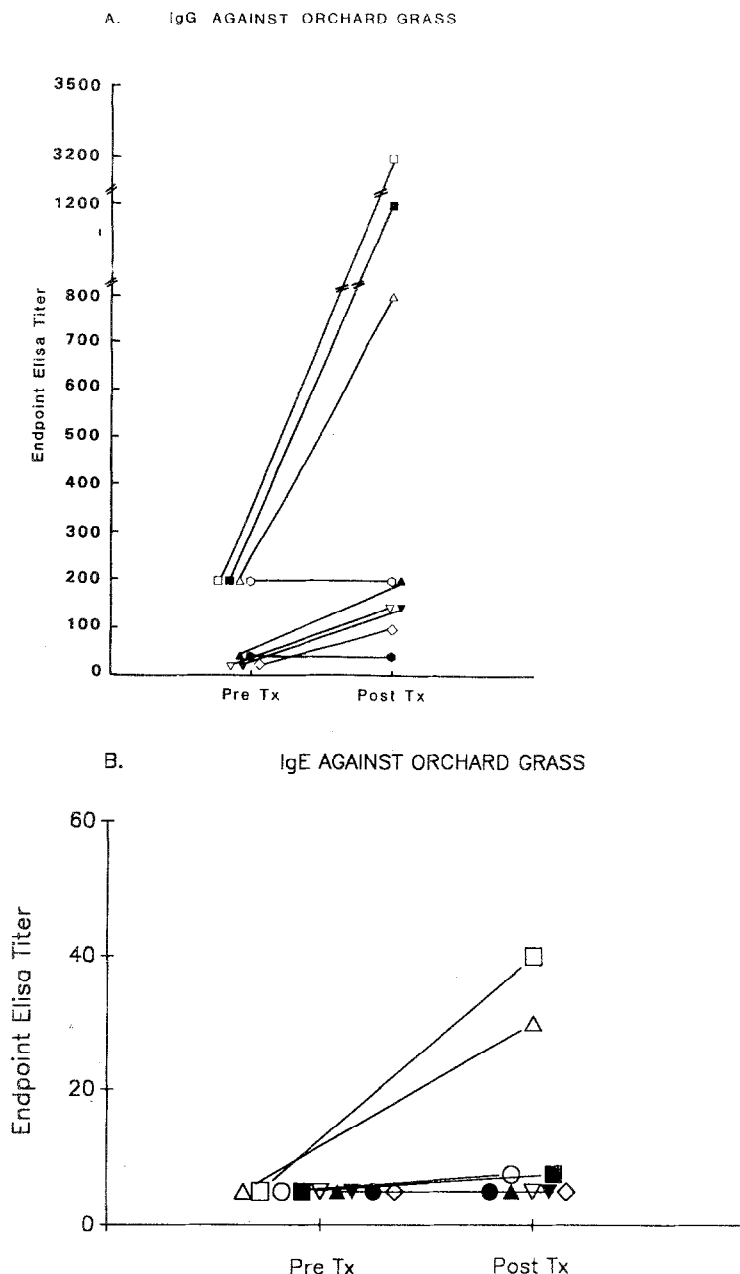


FIG. 3. **A.** Individual patient ELISA IgG titers are illustrated for orchard grass before (Pre Tx) and after (Post Tx) treatment. These are representative of the individual changes in titers that occurred with administration of the other antigens. **B.** Individual patient's ELISA IgE titers are illustrated for orchard grass before (Pre Tx) and after (Post Tx) treatment.

A representative scattergram for individual orchard grass antibody results is illustrated in Fig. 3.

Modified Farr assay

The statistical analysis of the change in total serum binding capacities for AgE with treatment was performed by use of a one-tailed paired Student's *t* test on log-transformed data. There was a statistically significant twelvefold rise in total serum binding capacity for AgE ($p < 0.0001$).

PTRIA

The statistical analysis of changes in IgE against AgE with PRW injections by one-tailed paired Student's *t* test was performed on log-transformed data. There was a 1.3-fold rise in IgE against AgE that was statistically significant ($p = 0.008$).

DISCUSSION

This clinical study was designed to evaluate the safety and immunogenicity of an accelerated dosage

schedule of PRW therapy and of concomitantly administering accelerated dosage schedules of PRW and IPG to patients with dual inhalant sensitivities. We have previously successfully treated patients with PRW totaling about 1350 AU administered in 12 to 15 weekly injections.^{18, 19} In one of these previous articles¹⁹ we reported the administration of 2668 AU; however, since that time, the Office of Biologics of the FDA has redefined the potency of its short ragweed reference standard so that we actually administered 1334 AU in that study, as defined by the potency of the current short ragweed reference standard. In this study, we administered more than twice that amount of material, 2955 AU in 13 injections delivered at 11 weekly visits.

As for IPG, we have previously reported successful clinical results with 12 weekly injections totaling 48,000 PNU, and with 72,000 PNU in 13 injections in nine weekly visits. In this trial we delivered 71,950 PNU in 10 injections at nine weekly visits.

In terms of safety considerations, there were no systemic reactions in spite of large doses of both polymerized pollen allergens administered concomitantly in accelerated schedules. There were no repeat injections required for large local reactions from any of the 283 PRW injections or the 113 IPG injections. In addition, when postimmunotherapy general laboratory tests were compared to preimmunotherapy tests, there were no clinically significant changes in any of the clinical laboratory parameters measured.

In several of our past studies of IPG, we have used grass Group I (Rye I) as an allergenic marker for northern grass pollens. With that purified protein, we used a modified Farr assay and PTRIA as the immunoassays to measure immunologic changes with immunotherapy. We have used the ELISA to measure antibodies to various other antigen mixtures and have modified that assay to measure antibodies to partially purified grass pollens.²⁰ In a previous study we found that the IgG rises after IPG were similar to the rises after conventional grass-pollen immunotherapy.²⁰ In the current study there were statistically significant rises in IgG titer to each grass of comparable magnitude to that in our previous studies. There was also a significant rise in total antibody binding of AgE similar to those antibody responses in our previous studies.

In summary, this clinical study demonstrates the safety and immunogenicity of an accelerated dosage schedule of PRW and of concomitantly administering accelerated dosage schedules of PRW and IPG to patients with dual inhalant sensitivities. Patients developed increases in total antibody binding of AgE and in IgG titer to grass pollens, had no systemic reactions, had no clinically significant changes in laboratory pa-

rameters, and received significantly fewer injections than would have been required to administer similar weights of conventional allergens used in immunotherapy.

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Therapy of chronic idiopathic urticaria with nifedipine: Demonstration of beneficial effect in a double-blinded, placebo-controlled, crossover trial

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*The efficacy of nifedipine, a calcium channel antagonist, in the treatment of chronic idiopathic urticaria, was evaluated in a double-blinded, placebo-controlled, crossover trial. Ten patients with chronic urticaria refractory to maximally tolerated doses of H₁ and H₂ antihistamines and in whom extensive evaluation failed to identify a cause for their urticaria were entered into the study. Patients continued with their prestudy antihistamine regimens. A study drug dosage was titrated in each patient and maintained throughout the trial. Patients were treated with placebo or nifedipine for 4 weeks and then crossed over to the other medication for 4 weeks. One patient withdrew because of unrelated medical illness, two patients withdrew after crossover to placebo because of intolerable urticaria, and seven patients completed the study. A beneficial effect of nifedipine was clearly demonstrated. Hive count, hive index, and itch index were all significantly improved at the end of 4 weeks of nifedipine treatment ($p = 0.023$, 0.003 , and 0.003 , respectively) but not placebo treatment ($p = 0.194$, 0.664 , and 0.944 , respectively). Additionally, end point hive index and itch index scores with nifedipine compared to corresponding placebo scores were significantly improved ($p = 0.010$ and 0.008 , respectively). Nifedipine was well tolerated. Thus, when nifedipine is used as an adjunct to antihistamines, it appears to be a safe, efficacious drug for the treatment of chronic idiopathic urticaria. (*J ALLERGY CLIN IMMUNOL* 1989;83:756-63.)*

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Chronic idiopathic urticaria is defined as urticaria persisting for at least 6 weeks and in which a specific etiologic factor cannot be determined. The treatment of chronic idiopathic urticaria is mostly symptomatic. Histamine, released from dermal mast cells, has been implicated as an important contributory factor in the clinical manifestations of chronic idiopathic urticaria.¹⁻⁶ Evidence for such a role includes increased