

The immune response of patients with ragweed hay fever treated with polymerized ragweed antigens

C. R. Zeiss, M.D., Charles J. Unga, B.S., Doris Levitz, B.S.,
Irena M. Suszko, B.S., and Roy Patterson, M.D. Chicago, Ill.

This report describes the immune response of patients with ragweed hay fever treated with polymerized ragweed antigens (PRW). Their IgG antibody responses to crude ragweed extract, antigen E, antigen K, and antigen Ra3 were determined by a solid-phase radioimmunoassay. The results indicate that PRW contains an array of clinically important antigens that are available for immunologic processing and result in an immune response in patients treated with this new form of immunotherapy for ragweed hay fever.

Previous studies showed that polymerization of ragweed antigen E (AgE), the major allergen of ragweed pollen, had reduced allergenicity but retained immunogenicity.¹ When the polymerized ragweed AgE was compared with monomeric AgE, the polymeric antigen was at least as immunogenic as assessed by antibody responses in rabbits. Polymerized AgE was shown to be less allergenic in man as demonstrated by decreased ability to elicit immediate-type skin reactivity and leukocyte histamine release.² A clinical trial of polymerized AgE immunotherapy for ragweed hay fever showed that the polymer produced as great an immune response with fewer immediate-type reactions and equal clinical efficacy as compared with the monomer antigen.³

Limitations of polymerized AgE as a therapeutic agent included technical difficulties of obtaining large amounts of antigen for general clinical use and the fact that AgE is not the only clinically important allergen in ragweed extract.⁴ Therefore, a more complex fraction of ragweed extract containing an array of antigens was polymerized with a molecular weight range of 200,000 to 20,000,000 daltons.⁵

The polymerized ragweed antigens (PRW) were found to be more immunogenic in rabbits than the unpolymerized, monomer preparation (MRW). MRW

and PRW resulted in equal peak reaginic responses in rabbits, but the duration of reagin production was longer after immunization with MRW than after PRW. PRW had 100- to 1000-fold less cutaneous reactivity in man than MRW.⁵

In a recent clinical trial in man, PRW was compared with MRW as an immunotherapeutic agent for the treatment of ragweed hay fever.⁶ PRW was found to have markedly reduced allergenicity as compared with MRW and resulted in comparable antibody responses to the major antigen of ragweed, AgE.

This report compares the IgG antibody responses of these patients after immunization with PRW and MRW, not only to AgE but also to the other ragweed antigens available to us, which were antigen K (AgK) and Ra3. The results indicate that PRW contains not only AgE but also AgK and Ra3 and that these antigens in PRW induce a specific immune response in patients treated with PRW.

MATERIALS AND METHODS

Patients studied

Patients with typical ragweed pollenosis were immunized with either PRW or MRW according to a standard protocol as previously described.⁶ The antibody responses of each subject after 15,000 PNU of antigen (900 μ g ragweed protein) had been given are reported herein. Five subjects from each group were chosen after preliminary investigation revealed an equal distribution of antibody response to AgE in both groups.

Antigens

AgE, AgK, and Ra3 were obtained from the Research Resources Branch, NIAID, National Institutes of Health. A crude ragweed extract was made by extracting giant and short ragweed pollen obtained from Hollister-Stier Labora-

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

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Reprint requests to: C. R. Zeiss, M.D., 303 East Chicago Ave., Chicago, IL 60611.

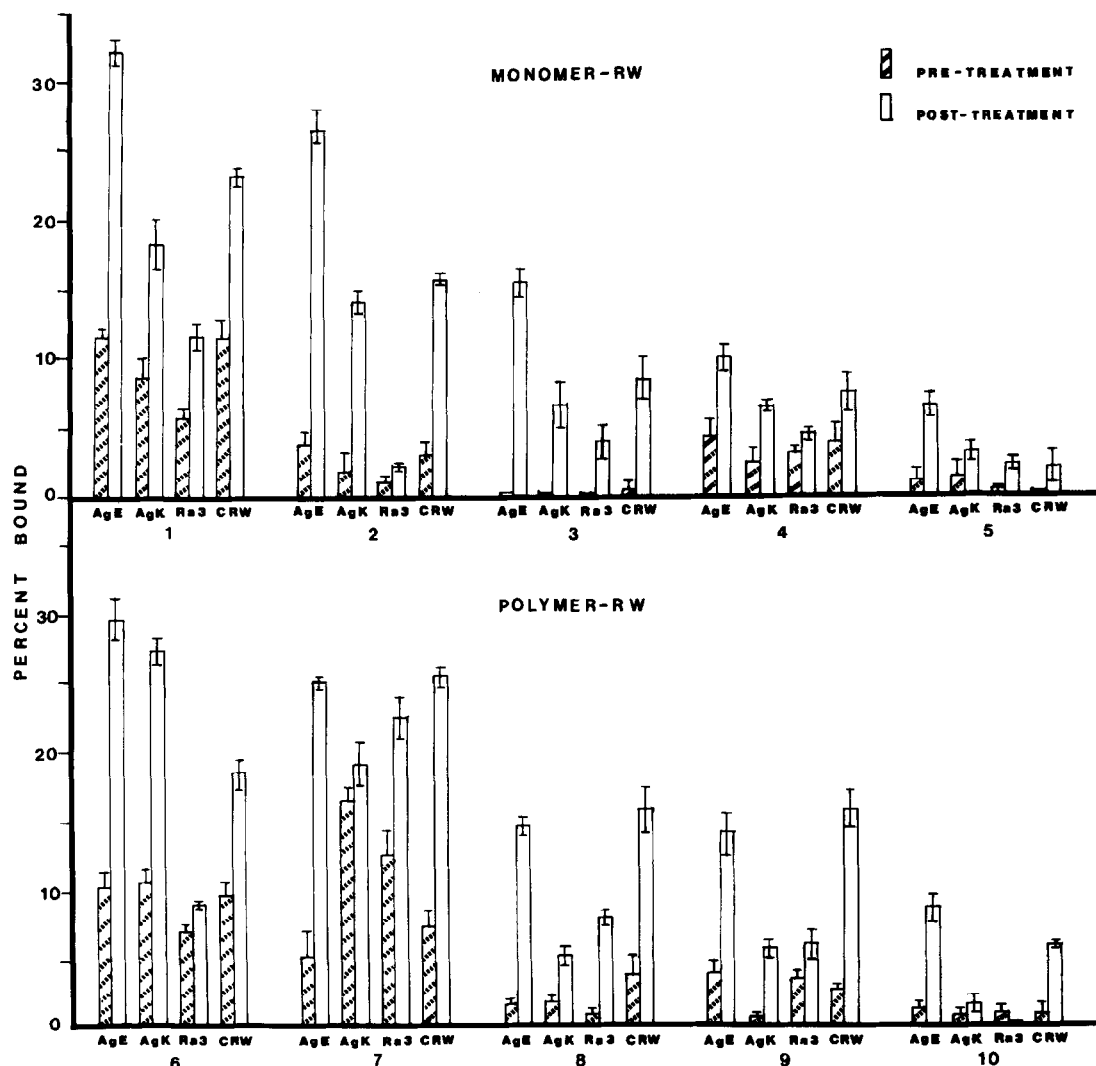


FIG. 1. Patients' IgG antibody activity against CRW, AgE, AgK, and Ra3 before and after therapy with either MRW or PRW. Mean and SEM are shown.

tories, Spokane, Wash. Defatted pollen grains were extracted with isotonic phosphate-buffered saline (PBS, pH 7.2), at 4° C for 24 hr with the use of a magnetic stirrer. The preparation was centrifuged at $17,000 \times g$ for 1 hr and the small amount of precipitate discarded. The supernatant of this preparation was termed crude ragweed extract (CRW). MRW and PRW were prepared as described previously.⁵

Antiserum and control serum

A single control serum from a nonallergic donor was used throughout the study. Rabbit antihuman IgG specific for the Fc portion of human IgG was obtained from Behring Diagnostics, Somerville, N. J.

Polystyrene tube radioimmunoassay for the detection of IgG antibody activity to ragweed antigens

IgG antibody activity to AgE, AgK, Ra3, and CRW was determined by a solid-phase polystyrene tube radioimmuno-

assay as previously described.⁷ Briefly, 100 μ g of the individual purified antigens, or 1.6 mg of CRW in 1 ml of 0.1 M sodium bicarbonate (NaHCO_3), were added to separate polystyrene tubes for 1 hr at room temperature. The antigen solutions forming the first layer were aspirated and the tubes washed. Human serum albumin (HSA), 1% in PBS, was then added for 1 hr at room temperature to fill all free polystyrene tube adsorption sites. The tubes were washed and the test serum added. One ml of a 1:100 dilution of patient's serum in 1% HSA PBS was added to the antigen-coated tubes and incubated overnight at 4° C. Pre- and posttreatment sera were analyzed simultaneously. The test sera were decanted and the tubes washed. One ml of rabbit antihuman IgG diluted 1:10 in PBS was added for 18 hr at 4° C. After washing, 10 ng of ^{125}I IgG (200,000 cpm) were added and incubated for 18 hr at 4° C. The tubes containing the radiolabeled IgG were counted, washed 10 times with water, and recounted. From the pre-cpm and post-cpm bound, the percent ^{125}I IgG bound in the final layer was

calculated. The percent binding by a control serum was calculated and subtracted from the patient values.

RESULTS

Control binding and analysis of variation in binding

Triplicate determinations of control serum binding for the four antigens ranged from 5.2% to 7.2%. Standard deviations ranged from 0.80% to 1.0% and the standard error of the mean (SEM) ranged from 0.50% to 0.65%. Analysis of variation of triplicate determinations for antibody activity in pre-treatment serum, where antibody activity was low, showed coefficients of variation ranging from 13% to 17%. Analysis of posttreatment antibody activity, when antibody levels were higher, showed coefficients of variation of 4% to 9%.

Analysis of the antibody response in the patients studied

The antibody responses to the antigens studied are shown for 10 patients who were immunized with either MRW or PRW (Fig. 1). The patients varied both in the magnitude of their IgG antibody response to the various antigens and in the specificity of the antibody response. In the MRW- and PRW-treated groups, there was a similar range in the magnitudes of the response to AgE, but there were individual differences in the patients' IgG antibody responses to antigen K and Ra3 in both the PRW- and MRW-treated groups.

DISCUSSION

It has been shown in a previous study⁶ that PRW was an effective and safe immunogen for the treatment of ragweed pollenosis. Although immune responses to AgE had been established in the previous study showing no differences between PRW- and MRW-treated patients, there was the possibility that PRW did not contain other clinically important ragweed antigens such as Ra3, and AgK.⁴ Additionally, because of the complex structure of the PRW, it was theoretically possible that these other important antigens would not be available for immunologic processing.⁵ Using the other important ragweed antigens available to us, Ra3 and AgK, this present study has

established that not only are these antigens present in PRW, but they are available for immunologic processing and result in an IgG antibody response in patients given PRW. In this respect, the group treated with PRW did not differ at all from patients given the unpolymerized preparation.

The IgG antibody response in these patients was greatest for AgE and AgK and of smaller magnitude for Ra3. This was not unexpected since AgE and AgK represent 6% and 3% of pollen proteins, respectively, while Ra3 constitutes only 0.4% of the pollen proteins.⁴

The polystyrene tube radioimmunoassay is reliable, relatively easy to perform, and can detect antibody responses to both purified and highly complex antigens such as CRW. It enabled us to detect individual patient differences in immunologic response to the major antigens of ragweed and should be useful in studying immunotherapy utilizing other aeroallergens.

This study demonstrates that polymerized ragweed contains an array of clinically important antigens that are available for immunologic processing and result in an immune response in patients treated with this material.

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