

Reduced allergenicity of high molecular weight ragweed polymers

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Polymerized ragweed antigens of different molecular weight ranges were studied to determine if the degree of allergenicity was dependent on molecular size of the polymers. In this study, allergenicity is defined as the ability to elicit IgE-mediated skin reactivity. Ragweed antigen treated with glutaraldehyde was fractionated into one preparation with a molecular weight range of 200,000 to 20,000,000 and another with molecular weights less than 200,000. Except for the difference in molecular sizes of the two preparations, all ragweed polymers had been treated in an identical fashion. The low molecular weight fraction had cutaneous endpoint reactivity greater than 10^5 to 10^8 times that of the high molecular weight materials. In these and other studies, immunogenicity defined as the ability to induce an IgG antibody response was retained by the high molecular weight material. The results are consistent with the hypothesis that allergenicity decreases as the molecular weight of the polymerized allergen increases.

In a series of experiments it was demonstrated that polymerization of allergens resulted in reduced allergenicity but retained immunogenicity. Allergenicity refers to elicitation of IgE-mediated reactions and immunogenicity refers to induction of IgG antibodies. The initial experiments used ragweed (RW) antigen E (AgE) as a model system so that the biologic activity of a single RW allergen of major importance¹ could be characterized in the monomeric and polymeric states. Polymerized AgE had markedly reduced ability to elicit IgE-mediated reactions when compared with monomeric AgE² but retained immunogenicity when compared with monomeric AgE.³ A clinical comparison of the effect of RW immunotherapy with the use of polymerized AgE and monomeric AgE demonstrated that the polymerized AgE was at least as efficacious as monomeric AgE.⁴ In order to determine whether the polymerization process was applicable to other pollen allergens, we demonstrated that mixed grass pollen allergens could be polymerized in a manner similar to RW AgE.⁵ To develop the RW polymerized material to the status at which it could be used for commercial and general clinical application,

it appeared necessary to include a wider range of known RW allergens and to use a process less complex than that required to produce RW AgE.¹ For these reasons, an ammonium sulfate-precipitated Sephadex G15-purified fraction of RW was polymerized and evaluated by comparison with the non-polymerized RW fraction. The results showed that the polymerized RW had markedly reduced allergenicity but was more immunogenic in rabbits as determined by IgG antibody responses against RW AgE.⁶

We have suggested that one explanation for the reduced allergenicity of the polymerized allergens could be that the higher molecular weight materials have less opportunity to diffuse through tissues and interact with IgE antibody on the surface of mast cells. Slower diffusion rates of radiolabeled polymers have been shown.^{2, 3} Further, the reduced allergenicity may result from antigenic determinants that are concealed, perhaps by steric hindrance, in the polymers.⁷ The molecular weight of the RW polymers studied varied from 200,000 to 20,000,000 and the immunologic analysis used detection of antibodies against RW AgE. Polymers prepared from AgE (molecular weight about 40,000) could contain from 5 to 500 molecules of RW AgE. With the use of the mixture of RW allergens from pollen extracts it was shown that all RW antigenic determinants of AgE were present in the polymers but that a higher weight ratio of the polymer was required to neutralize anti-AgE antibody when compared with nonpolymerized materials.⁶ This is consistent with the concept of con-

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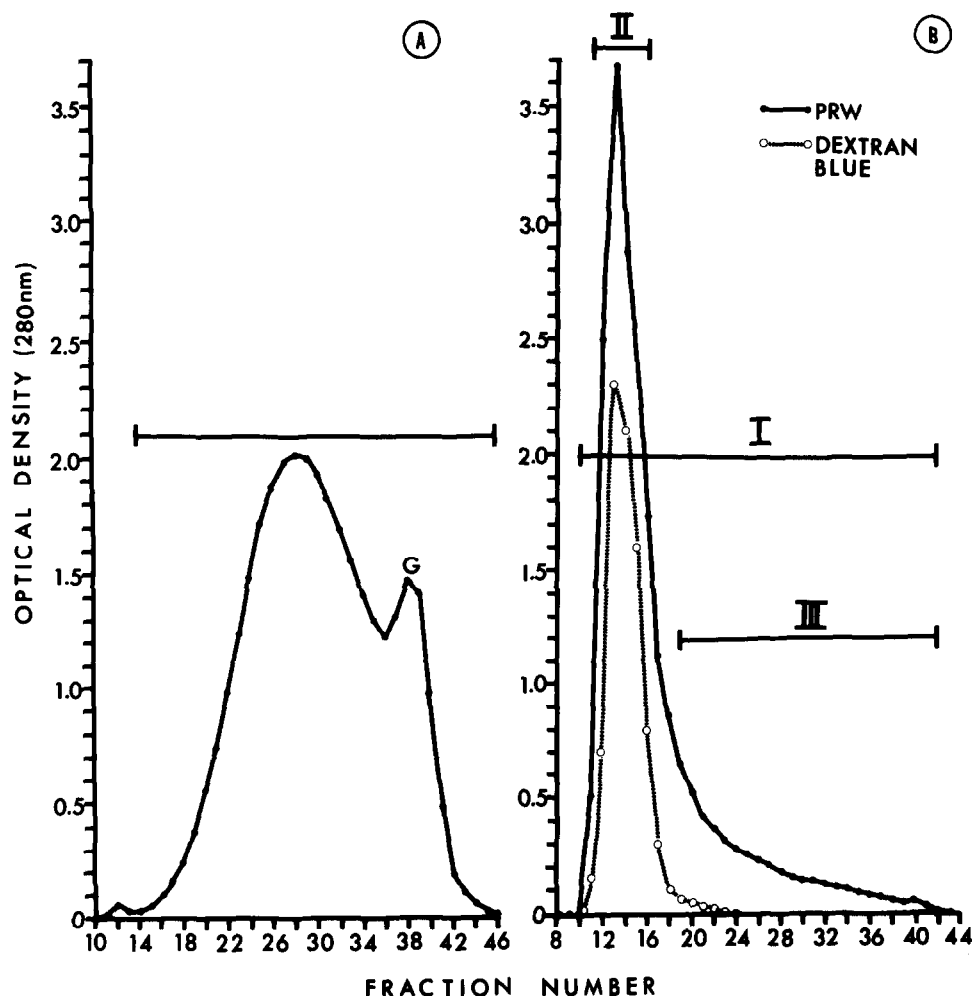


FIG. 1. A, Sephadex 4B fractionation of polymerized RW. G, Excess glutaraldehyde. B, Sephadex G-200 fractionation showing the 3 preparations used for skin testing.

cealed antigenic determinants. The immunogenicity of the RW polymers which exceeded that of the monomers⁶ was suggested as resulting from the retention of antigenic determinants which were available for macrophage processing and antibody induction.⁷

The current study was designed to test this hypothesis further. Multiple experiments in this laboratory have shown that the polymerization procedures utilized for pollen antigens result in a range of molecular weights.^{2, 3, 5, 6} The low molecular weight materials were excluded from experimental animal or human studies based on our concept of the desirability of only polymers of higher molecular weight. This study compares the allergenicity of the high molecular weight materials (200,000 to 20,000,000 daltons) with the low molecular weight materials (less than 200,000 daltons). All of these materials were treated identically during the polymerization procedure and vary only in their molecular weights.

MATERIALS AND METHODS

Preparation of polymerized ragweed (PRW) from monomeric ragweed antigen (MRW)

Procedure of preparation of PRW and MRW was the same as previously described in detail.⁶ Briefly, pollens were obtained from Hollister-Stier Laboratories (Spokane, Wash.). Giant and short ragweed pollens were used. These pollens were defatted with ethyl ether several times. The defatted pollen grains were extracted with isotonic phosphate-buffered saline (PBS), pH 7.2, at 4° C for 24 hr. The preparation was centrifuged at 17,000 × G for 1 hr and the precipitate was discarded. Proteins in the crude extract were precipitated with ammonium sulfate at 90% saturation level. Resulting precipitate was dissolved in PBS and dialyzed at 4° C against PBS until free of ammonium sulfate. Further purification of extract was done by chromatography on a Sephadex G-15 column. The excluded portion of Sephadex G-15 was the monomer ragweed.

For each 10 mg MRW, 0.625 ml 1:50 glutaraldehyde (25% grade II, Sigma Chemical Company) was added. The polymerization of MRW was carried out as previously de-

scribed⁶ for 4 hr at which time glycine was added to stop further polymerization. The glutaraldehyde-treated mixture was fractionated on a Sepharose 4B (Pharmacia) column and the fractions included by the Sepharose 4B column were combined. The polymerized ragweed (PRW) was placed in a dialysis bag and concentrated by evaporation to 4 cc and dialyzed free of excess glutaraldehyde and glycine. The PRW was then applied to a Sephadex G-200 (Pharmacia) column. The fractions excluded or included by the Sephadex G-200 were combined and stored at -20° C until use. Before human use, they were dialyzed for 48 hr against phosphate-buffered 0.15 M NaCl, pH 7.35, containing 0.5% phenol.

RW preparations were sterilized by filtration by means of a filter unit (0.20μ plain membrane, Nalge, Sybron Corp., N.Y.). They were cultured for sterility by the microbiology laboratories of Northwestern Memorial Hospital at 48 hr and following 2 wk of culture. Preparations were proved to be sterile before use in the human studies reported below.

Preparation III was concentrated and adjusted to an optical density (OD)₂₈₀ of 0.50. Preparations I and II were also brought to the same reading. All dilutions refer to these stock solutions which can be estimated to contain approximately 0.5 mg protein/ml. Preparation II can be estimated to have a geometric mean molecular weight of 2×10^6 daltons which would make the mean molar concentration about 1/50 that of monomer.

Cutaneous titration (human). Three ragweed-sensitive subjects were tested by intracutaneous injection of 0.02 ml of serial 10-fold dilutions of each preparation. Reactions were graded 1+ to 4+ positive and the endpoint titration was the highest dilution giving a wheal and erythema reaction.

Measurement of total IgG antibody specific for AgE

As previously described⁴ total serum antigen E-specific binding capacity was measured with a modification of the technique described by Lidd and Farr.⁸ Antigen E was labeled with ¹²⁵I, and the antibody free or complexed with antigen was precipitated by 40% ammonium sulfate.

RESULTS

Polymerization of RW and selection of preparations for skin testing

Fig. 1, A shows the Sepharose 4B fractionation of the polymerized RW resulting from treatment with glutaraldehyde. The fractions with a molecular weight less than 20,000,000 daltons which are in the included fractions of Sepharose 4B were pooled and further fractionated through Sephadex G-200. The Sephadex G-200 fractionation profile is shown in Fig. 1, B and compared with the profile of dextran blue from the same column. Three preparations from this fractionation were utilized for study. Preparation I was obtained by recombining aliquots of all fractions from the Sephadex G-200 and thus contained high molecular weight-excluded proteins and low molecu-

TABLE I. Cutaneous reactivity of high molecular weight ragweed polymers (200,000 to 20,000,000 daltons) (preparation II), low molecular weight materials (preparation III treated in an identical fashion but less than 200,000 daltons, and a mixture of both materials (preparation I)

Preparation	Subject A	Subject B	Subject C
I*	10 ⁻³ †	10 ⁻⁵	10 ⁻¹
II	10 ⁻²	Negative	Negative
III	10 ⁻⁸	10 ⁻⁸	10 ⁻⁵

*See Fig. 1, B for composition of ragweed preparations I to III.

†Expressed as end point dilution titers. The number recorded is the highest serial 10-fold dilution giving a positive wheal-and-erythema reaction.

TABLE II. Immunogenicity of high molecular weight ragweed (200,000 to 20,000,000 daltons) as demonstrated by IgG antibody response in 2 subjects shown in Table I

Parameter	Subject A	Subject B
Initial IgG binding activity (pretreatment) (ng AgE P bound/ml serum)	160	140
IgG binding activity after 15,000 protein nitrogen units (PNU) high molecular weight polymer (ng AgEP bound/ml serum)	1,600	1,000

lar weight-included proteins. Preparation II was composed of materials excluded by Sephadex G-200 and thus consisted of polymers of molecular weight 200,000 to 20,000,000 daltons. Preparation III was composed of fractions included in Sephadex G-200 and thus contained proteins with molecular weights less than 200,000.

Cutaneous titrations with the 3 preparations obtained after glutaraldehyde treatment. The skin test titrations of the 3 preparations are shown in Table I. These show striking differences between the endpoint reactions of preparations II and III which were treated identically during preparative procedures but which have different molecular weights. Preparation I shows an intermediate degree of skin reactivity as would be expected since it is composed of both preparations II and III.

Immunogenicity of preparation II. The IgG antibody response of 2 subjects that were skin tested and treated with preparation II is shown in Table II. The mean for 10 additional preparation II-treated patients who were not skin tested was 2,193 with a range of 90 to 11,600 after receiving 15,000 protein nitrogen units (PNU). It is of interest that these antibody re-

sponses are not significantly different from those obtained after treatment of patients with either monomeric or polymeric AgE.⁴ These antibody responses are not dissimilar to those obtained with monomeric RW and demonstrate the immunogenicity of preparation II in humans allergic to RW. Nine monomer-treated patients had a mean response of 3,966 with a range of 1 to 16,000 ng/ml.

DISCUSSION

These results demonstrate that ragweed allergens treated with glutaraldehyde have dramatically reduced allergenicity when they exist as large polymers. Allergenicity is retained in the lower molecular weight materials even though they have been subjected to identical treatment with glutaraldehyde in the preparative procedures. The difference in degree of allergenicity as determined by cutaneous endpoint titration varied from at least 10^5 to 10^8 with the use of different subjects. The retention of immunogenicity has been demonstrated previously in rabbits³ and in the 2 skin test-positive subjects who participated in this study. These subjects were part of a larger clinical trial comparing monomer and polymer.

The reduction in allergenicity of polymerized pollen allergens could be explained as a combination of several factors. Lower molecular weight allergens should have greater diffusibility through tissues, allowing them to reach available mast cells.

The known monomeric allergens of ragweed extract range in molecular weight from about 5,000 to 38,000 daltons. Since the polymer has been selected by Sephadex G-200 chromatography to be at least 200,000 daltons, the minimum molecular concentration of a given weight of monomer would range from 5 to 40 times that of the same weight of polymer. If the average molecular weight of the polymer is 10,000,000 daltons, the molecular concentrations of monomer would range from 500 to 2,000 that of the same weight of polymer.

Lower molecular weight monomeric allergens, in an increased molar ratio, should have increased opportunity to react with specific IgE on the surface of mast cells. Finally, antigenic determinants on the native allergenic molecules are available to react with specific IgE on mast cells. By contrast, a high molecular weight polymer of allergens has been shown to

diffuse more slowly through tissues² and thus have decreased rate of reactivity with mast cells. The low molecular allergens, when combined together as very high molecular weight polymers, should have a markedly diminished opportunity to react with specific IgE on the surface of the same number of mast cells. Finally, when low molecular weight allergens are combined to form what is conceived to be a globular rather than a linear high molecular weight polymeric structure, some antigenic determinants of the allergenic molecule should be concealed within the polymeric structure and be unavailable to react with specific IgE on mast cell surfaces. In a previous study² the possible effect of lower molar concentrations of polymer as contrasted with the molar concentrations of monomer was discussed in relation to the results and conclusions of Levine.⁹

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