

Brief Communication

Soluble copolymer of wasp venom with human albumin for venom immunotherapy

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Polymerization of allergens decreases allergenicity while retaining immunogenicity, as we have demonstrated for ragweed, grass, and tree pollens. We have also polymerized bee venom with human albumin to form soluble, high-molecular-weight copolymers that are immunogenic in rabbits. We now have prepared a soluble wasp venom-albumin polymer (WVAP), molecular weight $\geq 240,000$ daltons, by glutaraldehyde treatment and Sephacryl S-300 column fractionation. Rabbits immunized with WVAP produced IgG to both WVAP and wasp venom (WV), as measured by ELISA. IgG against WVAP was totally inhibitable by a mixture of WV and albumin, demonstrating both retention of native antigens and absence of new antigenic determinants in WVAP. IgG against WV in serum from patients receiving maintenance doses of WV immunotherapy was inhibited by WVAP. In summary, we have synthesized a soluble, high-molecular-weight copolymer of WV that retains the immunogenicity of native WV, contains no new antigenic determinants, and has potential value in the treatment of patients with WV anaphylaxis. (J ALLERGY CLIN IMMUNOL 77:520-3, 1986.)

Patients at risk of Hymenoptera venom anaphylaxis may require venom immunotherapy indefinitely,¹ and up to 15% or more may experience systemic allergic reactions during treatment with unmodified venom.^{2,3} The use of polymerized venoms for immunotherapy may overcome these drawbacks. Studies of ragweed,^{4,5} grass,⁶ and tree⁷ pollen immunotherapy have demonstrated that polymerization decreases allergenicity,⁸⁻¹⁰ rate of local absorption,⁸ and the risk of iatrogenic anaphylaxis.¹¹ Polymerized allergens are immunogenic,⁸⁻¹³ efficacious,¹¹⁻¹⁴ safe,^{15, 16} and stable.^{12, 17} We have demonstrated that higher doses of polymerized allergen can be administered and a therapeutic response obtained with significantly fewer injections.¹¹

To assess whether these benefits may extend to treatment with venoms, we have polymerized honeybee venom with HSA to form a soluble, high-molec-

Abbreviations used

HSA:	Human serum albumin
WVAP:	Wasp venom-albumin polymer
OD ²⁸⁰ , OD ⁴⁰⁵ :	Optical density 280 nm or 405 nm
WV:	Wasp venom
PBS:	Phosphate-buffered saline

ular-weight venom-A polymer that is immunogenic in rabbits.¹⁸ HSA was used for copolymerization because venoms have a tendency to form insoluble polymers if a solubilizing protein is not added.¹⁹ We have now prepared WVAP. The results of our evaluation suggest WVAP has potential value in the treatment of WV anaphylaxis.

MATERIAL AND METHODS

Preparation of WVAP

WVAP was prepared by the method used to polymerize bee venom with HSA by glutaraldehyde treatment¹⁸ by use of lyophilized sac extract of WV (*Polistes* species; North American mix; Vespa Laboratories, Inc., Spring Mills, Pa.), 1 mg/ml, and chromatographically pure HSA (Cap-pel/Cooperbiomedical, Inc., Malvern, Pa.), 10 mg/ml. Four preparations were made from the same venom and albumin sources. They were pooled, concentrated, and dialyzed against PBS to a final concentration of 4.0 mg/ml. These

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preparations were identical in molecular weight range, and elution profiles were similar. Protein concentrations were standardized by measurement of the optical density at OD₂₈₀. The final preparation of WVAP was centrifuged at $1000 \times g$ for 30 minutes at 4° C, sterilized by ultrafiltration, and stored at 4° C.

Immunization of rabbits with WVAP

Each of two 2.5 to 3 kg rabbits was injected with 2.4 mg of WVAP, subcutaneously, at weekly intervals for 7 weeks. Sera were collected just before inoculation (week 0) and biweekly for 8 weeks thereafter to assess the antibody response. The sera were stored at -20° C.

Measurement of IgG antibody against WV by ELISA

A micro-ELISA for measuring IgG antibodies to WV²⁰ was used with modifications. Immulon polystyrene, flat-bottom microplate (Greiner & Sohne, Nürtingen, W. Germany) wells were coated with 0.2 ml of borate-saline buffer (0.125 mol/L Na borate and 0.15 mol/L NaCl), pH 8.4, containing WV (lyophilized *Polistes* venom *Vespa*), at 5 µg/ml, for 18 hours at 4° C, and then washed with saline-Tween solution 0.15 mol/L NaCl and 0.05% Tween 20). To these were added 0.2 ml dilutions of rabbit sera or sera from WV-allergic or nonallergic human donors in PBS-Tween (PBS and 0.05% Tween 20), and the plates were incubated 30 minutes at 37° C and then washed. In subsequent steps, separated by washes, wells previously reacted with human sera were incubated with rabbit antihuman IgG (γ-chain specific; Calbiochem-Behring Corporation, La Jolla, Calif.), 1:1000 in PBS-Tween, for 30 minutes at 37° C; thereafter, all wells were incubated with 0.2 ml of phosphatase-conjugated, goat antirabbit IgG (Sigma Chemical Co., St. Louis, Mo.), 1:1000 in PBS-Tween, for 1 hour at 37° C. The plates were washed, reacted at 37° C with *p*-nitrophenyl phosphate substrate (Sigma Chemical Co.), 1 mg/ml in diethanolamine buffer,²¹ and the OD₄₀₅ of each well was estimated by an Artek model 210 automated ELISA reader (Artek Systems Corp., Farmingdale, N. Y.). Titers were determined as the inverse of the greatest dilution of test serum with an optical density greater than or equal to twice that of the control serum.

To measure IgG against WVAP, a similar assay was performed in plates coated with WVAP at 100 µg/ml.

Inhibition analyses of rabbit and human anti-WV or anti-WVAP

Sera were preincubated with increasing amounts of inhibitor (WVAP or both WV and HSA) for 30 minutes at 37° C. Then, assays for IgG antibodies against WV or WVAP were performed as described above.

RESULTS

Elution profiles of WV, HSA, and WVAP

Fig. 1 illustrates the chromatograms of WV and HSA with the use of dextran blue (2×10^6 daltons) and catalase as markers. After polymerization,

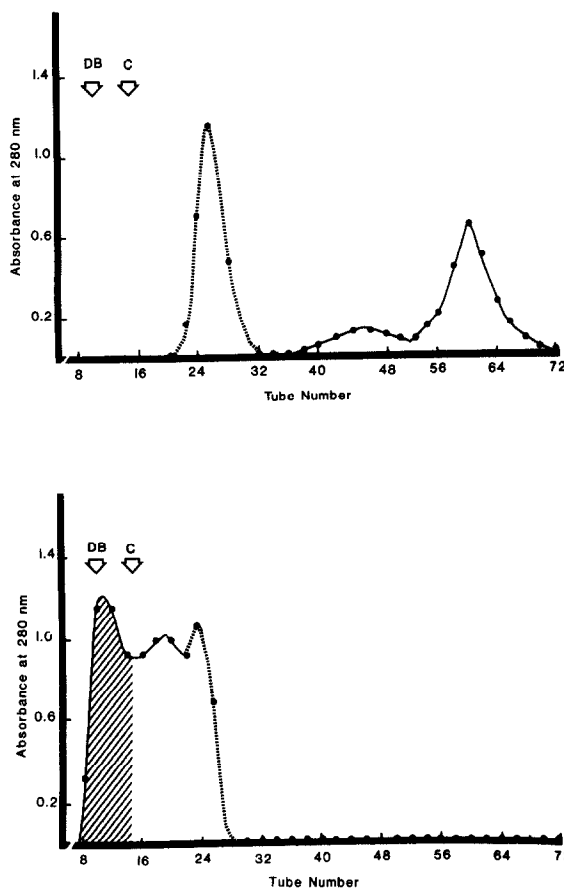


FIG. 1. Sephacryl S-300 chromatography profiles of (upper panel) WV (●—●) and HSA (●---●), molecular weights 60,000 daltons before polymerization, and (lower panel) WVAP (shaded area) after polymerization. Column markers are dextran blue 2000 (DB), molecular weight 2×10^6 daltons, and catalase (C), molecular weight 240,000 daltons, illustrated by arrows. The eluant was PBS.

WVAP elutes before catalase in a new high-molecular-weight peak extending into the void volume. Elution profiles of multiple preparations were similar. Fractions with molecular weight exceeding that of catalase, i.e., $\geq 240,000$ daltons, were pooled and concentrated.

Immune response of rabbits immunized with WVAP

Rabbits immunized with WVAP produced IgG to both WV and WVAP, as determined by ELISA in plates coated with WV or WVAP, respectively (Fig. 2). Titers of IgG against WV reached peak levels after the sixth injection and declined after the last injection. (The titer decline is not presented in Fig. 2) The magnitude and duration of the rabbit IgG response to WVAP immunization paralleled the antibody response in rabbits immunized with bee VAP.¹⁸ The titer of antibody against WVAP is higher than that against

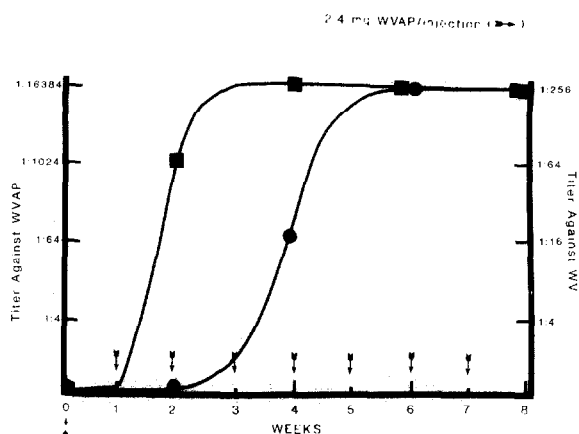


FIG. 2. Rabbit IgG antibody response to immunization with WVAP, 2.4 mg per injection ($\uparrow$) Left ordinate, titer against WVAP ($\blacksquare$); right ordinate, titer against WV ($\bullet$).

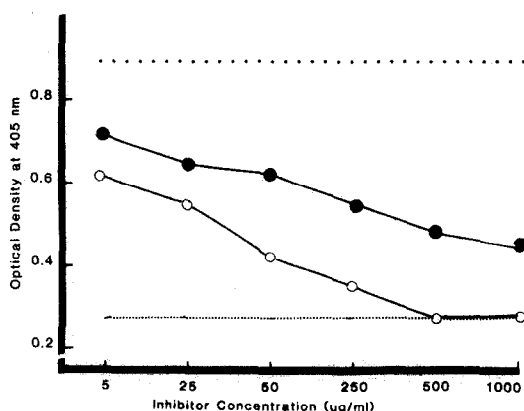
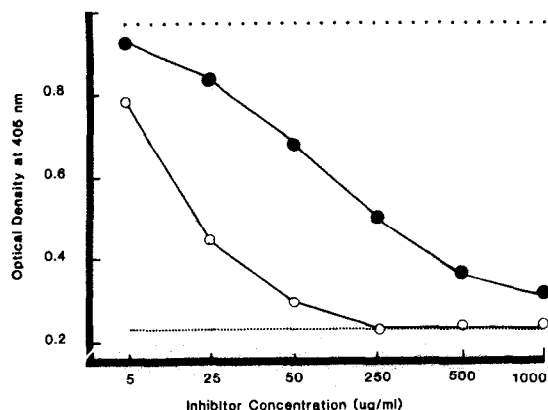


FIG. 3. Antigenic completeness of WVAP, as illustrated by (upper panel) inhibition of rabbit IgG against WV after preincubation with WVAP ($\bullet$) or with WV ($\circ$), and by (lower panel) inhibition of human IgG against WV after preincubation with WVAP ($\bullet$) or with WV ($\circ$). In each panel, test serum with no inhibitor added ($\cdots$) and control serum ($---$) are presented for comparison.

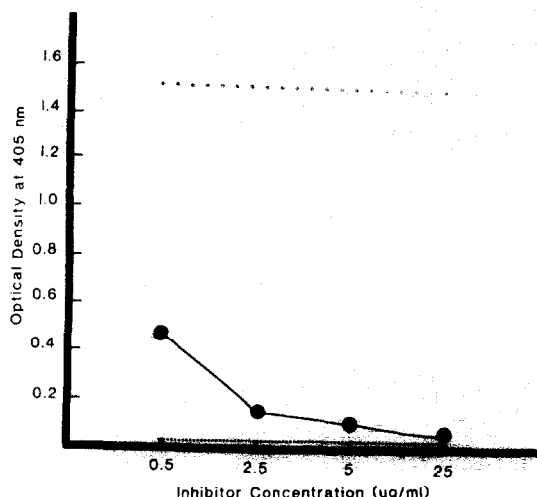


FIG. 4. Absence of new antigenic determinants in WVAP, as illustrated by inhibition of rabbit IgG against WVAP by a mixture of WV plus HSA ($\bullet$), each at the quantities presented. Test serum with no inhibitor added ($\cdots$) and control serum ($---$) are presented for comparison.

WV as would be expected, since WVAP also contains HSA that is a heterologous protein in rabbits.

Inhibition analyses demonstrating antigenic completeness of WVAP

This study was done to determine if WVAP would inhibit IgG binding to WV with rabbit serum containing IgG against WV or sera obtained from patients receiving maintenance immunotherapy with WV. The results demonstrate rabbit or human IgG to WV is inhibited by WVAP, indicating the antigenic completeness of WVAP (Fig. 3). The results obtained with a representative human serum are presented. Inhibition experiments were performed in duplicate on at least two different days and were reproducible. Higher concentrations of WVAP, compared to WV, are required to effect comparable degrees of inhibition. Similar results were observed in our studies comparing bee venom and bee VAP.¹⁸

Inhibition analyses demonstrating absence of new antigenic determinants in WVAP

Studies were also done to determine whether new antigenic determinants were introduced by glutaraldehyde treatment of WV and HSA. Fig. 4 demonstrates WV plus HSA inhibited all rabbit IgG to WVAP, thus providing no indication that copolymerization produced new antigenic determinants.

DISCUSSION

The clinical benefits of immunotherapy can be achieved more rapidly, safely, and with fewer injec-

tions by use of polymerized pollen allergens instead of conventional allergens. We would expect comparable clinical benefits from polymerized venom immunotherapy. In our initial experiments we determined that venoms tended to form insoluble polymers. Thus, we chose HSA as a copolymerizing agent because it is a constituent of the venom diluents in current use. HSA has proved safe as an immunotherapy diluent.²²

In this article we describe the preparation of WVAP and demonstrate its immunogenicity in rabbits, its antigenic completeness, and the absence of new antigenic determinants. We therefore conclude WVAP has potential value in the treatment of WV anaphylaxis.

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