

Immune complex-mediated disease not a factor in patients on maintenance venom immunotherapy

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Although a serum sickness-like presentation has been reported as a sequela of Hymenoptera stings, the possible role of an immune complex-mediated pathologic condition in patients receiving regular venom immunotherapy has never been addressed. To evaluate this problem, 30 adult and 15 pediatric patients receiving regular monthly doses of venom (100 µg of antigen) were studied. All had been receiving immunotherapy for 12 to 29 mo. At the time of venom administration, a questionnaire related to symptoms of immune complex disease was completed. A urinalysis was performed 12 hr later. In addition, blood was drawn to evaluate the presence of immune complexes by C1q and Raji cell assays. Symptom surveys revealed no clinical manifestations suggestive of immune complex pathology. All urinalyses were negative for gross and microscopic hematuria. None of the specimens was elevated on the C1q assay. Only four of the 45 patients had significantly positive Raji cell assays. Prospective reevaluation showed the presence of immune complex before venom administration, with no change in acute-phase reactants or Raji cell titers 12 hr later. Monthly administration of Hymenoptera venom appears to be unassociated with immune complex-mediated disease, by either clinical or immunologic parameters. (J ALLERGY CLIN IMMUNOL 72:199-203, 1983.)

Since the introduction of Hymenoptera venom for commercial use in 1979, concern has been expressed regarding the possible long-term side effects of continued venom administration. Symptoms resembling serum sickness have occasionally been reported after insect stings.^{1, 2} These reports have suggested immune complex-mediated pathologic conditions, but specific immune complex determination studies have not been carried out. The purpose of this paper was to investigate the incidence of immune complexes in Hymenoptera-sensitive patients regularly receiving venom immunotherapy.

MATERIALS AND METHODS

Individuals receiving regular monthly immunotherapy with commercially available honeybee, yellow jacket, yellow hornet, white-faced hornet, and wasp venoms (Hollister-Stier Laboratories, Spokane, Wash.) were invited to participate in the study. Those consenting received their

regular venom administration either in early morning or in late afternoon. At that time a questionnaire related to symptoms surrounding immune complex disease was completed. This included the presence of urticaria, generalized skin rash, arthralgia, fever, hematuria, or cephalalgia 6 to 48 hr after venom administration. The patients returned to the clinic 12 hr after injection, at which time urine was collected for macroscopic and microscopic examination and blood was drawn for immune complex studies. The studies included Raji cell and solid-phase C1q assays performed at the Scripps-Miles Immunology Laboratory.

Those patients who demonstrated the presence of immune complexes were subsequently reevaluated. At this time, blood was drawn before venom administration for sedimentation rate, C3, C4, Raji cell, and C1q assays. Twelve hours later, these studies were repeated and a urinalysis was obtained.

Venom-specific IgE antibodies were measured by means of a RAST technique. Solid-phase allergens were prepared with the use of commercially available honeybee, yellow jacket, and wasp venoms. These were coupled to quarter-inch CNBr-activated filter paper discs. ¹²⁵I-labeled anti-human IgE was purchased from Pharmacia Fine Chemicals, Piscataway, N. J. All serum specimens from one individual were tested with a single RAST assay in triplicate. Venom-specific antibody levels were expressed as a percent of binding produced by a serum from a nonsensitive (negative) individual.

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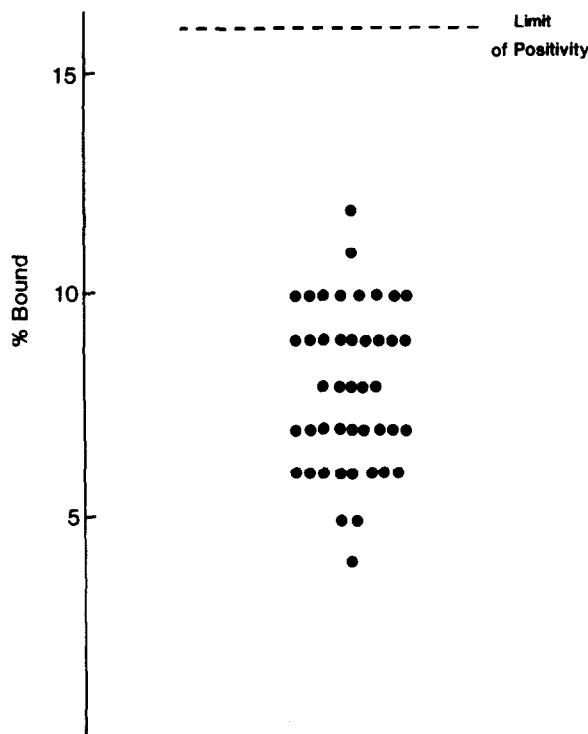


FIG. 1. C1q 12 hr after venom.

Venom-specific IgG antibody levels against yellow jacket and wasp venoms were measured by the solid-phase radioimmunoassay technique of Hamilton and Adkinson.³ Purified protein A from *Staphylococcus aureus* was purchased from Pharmacia. Ten micrograms of protein A and 100 μ g of commercially available venom (Hollister-Stier) were each iodinated with 0.5 mCi of ¹²⁵I-labeled anti-human IgE by means of a modification of the chloramine-T method of Greenwood et al.⁴

RESULTS

During the summer of 1981, 45 individuals undergoing regular monthly venom immunotherapy were investigated. Thirty were adults (mean age 43 yr) and 15 were children (mean age of 13 yr). All patients had been advanced to regular monthly administration of 100 μ g of each administered venom, on a modified rush immunotherapy schedule.⁵ Duration of therapy ranged from 12 to 29 mo (mean 22 mo). Four patients were receiving five venoms, 16 received four, four received three, six received two, and 15 patients received only one antigen. None of the patients evaluated had a history of clinical symptoms connected with venom administration that suggested immune complex-mediated disease. Gross and microscopic studies showed no evidence of hematuria in urine specimens received approximately 12 hr after the administration of venom.

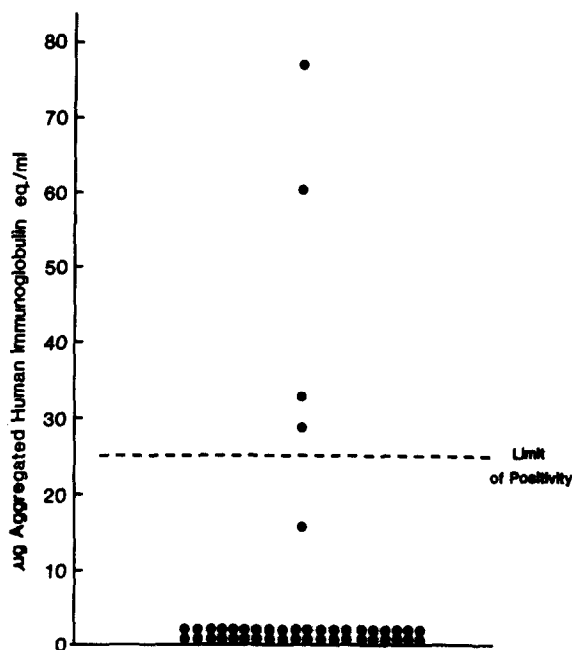


FIG. 2. Raji cell 12 hr after venom.

The results of immune complex assays utilizing a C1q and Raji cell technique are given in Figs. 1 and 2, respectively. Four of the 45 patients had significant elevations of immune complexes by the Raji cell assay, whereas none of the specimens was elevated by the C1q assay.

Three of the four patients with elevated Raji cell assays were again studied, this time prospectively. Results are detailed in Table I. Three of the four patients were over the age of 55. All demonstrated positive Raji cell assays before the administration of venom, with no significant changes in acute-phase reactants (C3, C4, sedimentation rate) 12 hr later. One patient with scleroderma was initially lost to follow-up. She returned to the clinic 6 months later, with no venom administered during the interval, but her elevated Raji cell assays were unaffected. Increases in venom-specific IgE titers after institution of regular venom immunotherapy, though varying individually, fell within 1 S.D. of the norm for patients evaluated at this center (see Figs. 3 and 4). Venom-specific IgG antibody titers, like IgE titers, demonstrated individual variation (see Figs. 5 and 6). All, however, fell within 1 S.D. of the mean.

DISCUSSION

Recently, assays have been developed that indirectly measure circulating immune complexes and have led to the detection of these complexes in a large number of diseases.⁶⁻¹¹ In some conditions such as nephritis and vasculitis, the findings support the evi-

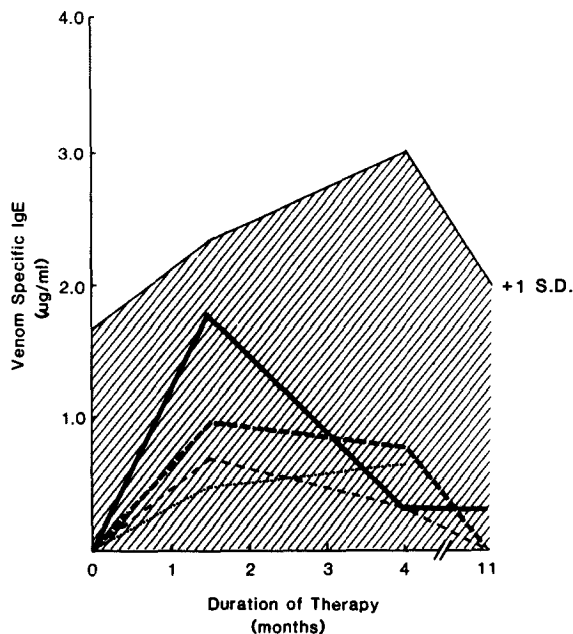


FIG. 3. Yellow jacket-specific IgE response to immunotherapy.

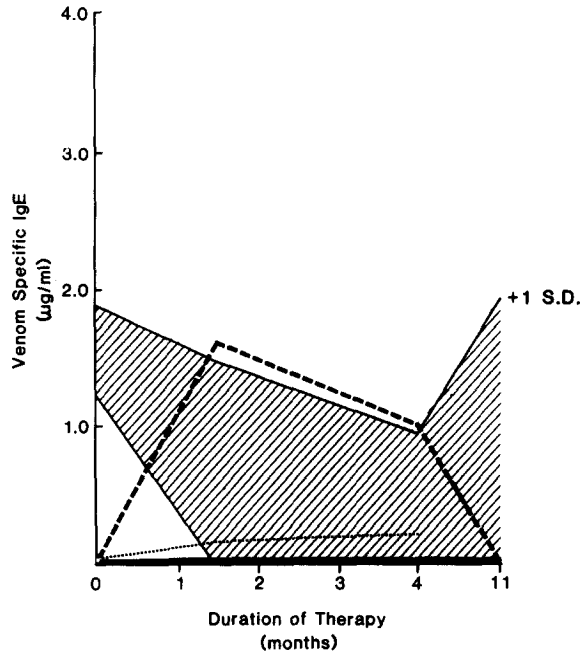


FIG. 4. Wasp-specific IgE response to immunotherapy.

TABLE I. Patients with elevations of Raji cell assays

Patient	Age (yr)	Studies before venom injection					Studies 12 hr after venom injection					Urinalysis
		ESR (mm/hr)	C ₃ ^A (mg/dl)	C ₄ ^B (mg/dl)	C1q ^C (%)	Raji ^D (μg/ml)	ESR (mm/hr)	C ₃ ^A (mg/dl)	C ₄ ^B (mg/dl)	C1q ^C (%)	Raji ^D (μg/ml)	
J. H.	59	11	109	14	4	164	12	112	13	4	113	0 RBCs
B. M.	34	44	128	18	11	61	42	134	20	11	87	<2 RBCs
S. D.	57	44	127	35	10	129	27	128	36	6	42	0 RBCs

ESR = erythrocyte sedimentation rate.
Normal range: ^A80-120 mg/dl; ^B15-45 mg/dl; ^C<16%; ^DRaji cell <2 μg/ml.

dence that circulating immune complexes play a pathogenic role. In some forms of cancer, nonspecific measurement of immune complexes seems to be a useful tool in monitoring treatment methods. Complexes have also been found in such disorders as Crohn's disease^{12, 13} and diabetes mellitus,¹⁴ where there is little evidence that they are of pathogenic importance.

The sequence of immune complex formation is a major factor in the pathogenicity of immune complexes. Complexes are usually rapidly sequestered by the host's phagocytic system. If antigen exposure is protracted, continued immune complex formation may lead to chronic disease. The nature of the antigen and antibody strongly influences the deposition of the resulting immune complex. Potent antigens such as those found in venom evoke a strong antibody response, and the antigen is rapidly eliminated.

The fate of circulating immune complexes is also determined by the quantitative relationship between antigen and antibody. Immune complexes formed with a large amount of antibody and a small amount of antigen (antibody excess) are large in size but are not pathologic because they are rapidly phagocytized and removed from circulation before lodging in body filtering structures. To date, there does not appear to be evidence to suggest that situations with antibody excess have manifested clinical disease.

A review of 15 case reports by Light et al.¹ has suggested that at least in an extremely small portion of those patients who have abnormal reactions to Hymenoptera stings, a clinical picture resembling serum sickness or acute renal pathology results. Although specific immunologic and pathologic evidence is lacking, it was suggested that circulating immune complexes were responsible.

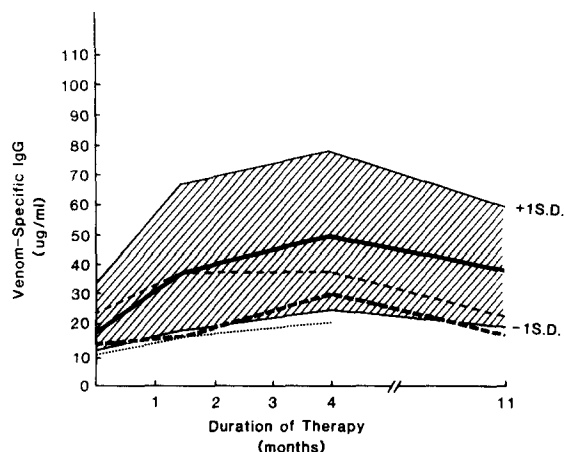


FIG. 5. Yellow jacket-specific IgG response to immunotherapy.

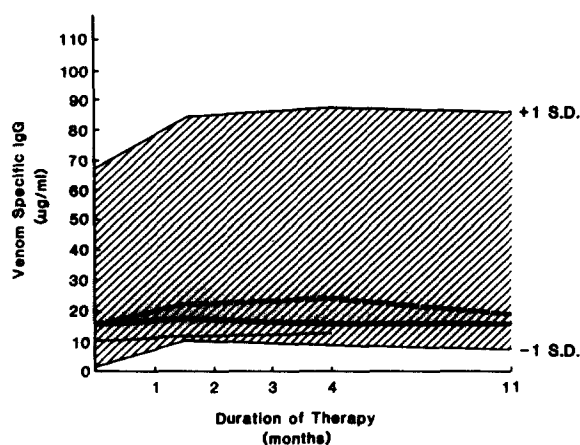


FIG. 6. Wasp-specific IgG response to immunotherapy.

The amount of venom introduced, either by a natural sting or by venom immunotherapy, even if given intravenously, results in a titer well below that which would result in detectable levels of immune complexes by any of the presently available assay systems. However, in light of these case reports and as a result of increased awareness of immunologic mechanisms surrounding the pathogenesis of disease states, concern was raised regarding the safety of patients subjected to long-term venom administration.

The present study of 45 patients revealed no clinical or immunologic evidence of immune complex-mediated pathology secondary to venom administration. Of the four patients who did have immune complexes detected by Raji cell assays, one already suffered from a connective tissue disorder. Those studied prospectively demonstrated significant changes

in neither acute-phase reactants (C3, C4, sedimentation rate) nor absolute immune complex levels. These four patients all demonstrated venom-specific IgG and IgE immunoglobulin levels within 1 S.D. of the mean, compared to other patients evaluated at the Center.

Work by Di Mario and Irvine¹⁵ reported the presence of circulating immune complexes in approximately 10% of the normal population. They used a solid- and liquid-phase C1q assay, a conglutinin binding test, and a bit wider range with the Raji cell assay. There was no difference in immune complex values between males and females, but there was a significant trend for immune complexes to become more prevalent with age was seen when C3-binding (Raji cell) methods were used. This technique commonly measures complexes of larger size. Three of the four patients studied with positive immune complex assays were over the age of 55; the four accounted for 8% of the population studied.

SUMMARY

Evaluation of 45 patients receiving monthly Hymenoptera venom immunotherapy revealed no evidence of venom-related clinical or pathologically mediated immune complex disease. Three of four patients who had elevated levels by one of the two immune complex assays, when studied prospectively, showed the presence of immune complexes before venom administration, with no change in absolute immune complex assay values or acute-phase reactants after venom injection.

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