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Insect venom allergy: Diagnosis and treatment

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In the early part of this century, it was recognized that a substance in the part of the body of a honeybee worker where the venom sac is located was capable of eliciting a violent cutaneous and even a systemic reaction in a patient with a previous history of a generalized reaction to a honeybee sting when the substance was applied to an area of scarified skin.¹ Inoculation of the patient with a filtered extract that was made from the posterior part of the worker bees resulted in a gradual lessening of the reactions that occurred when the patient was stung. This appears to have been the first instance of the successful immunization of a sting-allergic patient with a venom-rich solution. It was confirmed several years later that a sting-allergic beekeeper would have a positive skin test to honeybee venom, and it was also demonstrated that in an individual who developed respiratory symptoms from inhaling the bee body detritus around bee hives, an extract of bee body could also elicit a positive test.² The correct interpretation of this finding appears to be that respiratory exposure to bee body "fluff" from the hives leads to the development of IgE antibodies to bee body proteins.

Since to obtain clinically significant quantities of venom seemed a formidable task, attempts were made to determine whether allergy to insect stings also might be due to a protein or proteins shared by the bodies of all of the common Hymenoptera.^{3, 4} Although animals immunized with various Hymenoptera body preparations formed antibodies that could be used in the double diffusion in agar analysis of antigenic components, the results never clearly indicated the relevance of the antigens thus identified to human allergy to insect stings. For a number of years, most allergists were content to use commercially prepared extracts of whole insect bodies for diagnosis and for treatment of this condition. One or two voices were raised to point out either the inefficacy of whole-body extracts as diagnostic materials^{5, 6} or the apparent superiority of hand-collected venoms as therapeutic agents.^{7, 8} Comment was made regarding the possibility that reactions to stings might be toxic rather than allergic,⁹ whereas fatal reactions from stings were noted to occur in patients who were immunized with whole-body extracts.¹⁰ It was with this background that two groups of investigators nearly simultaneously reported the immunologic consequences of immunizing humans with honeybee venom and the use of venoms in vivo and in vitro to establish the immunologic nature of reactions to insect stings.^{11, 12}

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Abbreviation used

RAST: Radioallergosorbent test

The scope of the problem

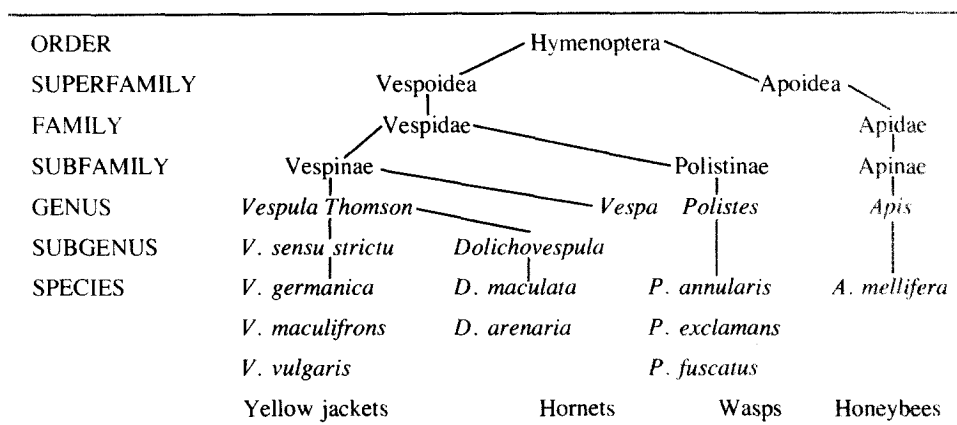
No one is certain how many deaths occur from stinging insect anaphylaxis. Although fewer than 50 fatalities a year in the United States have been reported, this number might be larger if accurate causes of sudden, unexplained death occurring in persons where stinging insects abound could be determined. Observations of large groups of individuals with natural exposure to stinging insects have led to the belief that 0.4% to 0.8% of the population may be at risk of an allergic reaction to a sting.^{13, 14} These figures represent the incidence of sting reactions, however, and do not tell us what the prevalence of the sensitized state may be or what proportion of those sensitized may be at risk of an allergic reaction if they are stung. A study that was begun in the suburbs of Baltimore by use of a stratified, random sample of the employees at a manufacturing plant has revealed some startling figures: 4% reported a history of systemic-sting reactions, and slightly more than 20% had either RAST or skin-test evidence of sensitization to at least one Hymenoptera venom.¹⁵ It is known from studies that involve deliberate sting challenges of adults with previous histories of systemic reactions that the repeat reaction rate in unimmunized persons is about 60%.¹⁶ We do not have a figure for the reaction rate of individuals with positive skin tests *without* previous reactions, although from studies of patients with positive skin tests and histories of large local but no systemic reactions, we know that about 5% of such persons will subsequently have systemic reactions to stings. Whatever the risk of a systemic reaction may be in the individual with a positive skin test to venom but without a previous reaction, it seems that the figure will be sufficiently low to make the routine use of venom skin testing as a screening procedure unwarranted.

Trials of venom therapy

The first patient immunized with venom by the Baltimore investigators was a beekeeper's son whose sister had previously succumbed to a honeybee-sting reaction.¹¹ The initial attempts to immunize him with whole-body extract met with failure, and his father agreed to collect bee venom manually for use in the son's treatment. Each droplet of venom, fresh from a bee, dried in moments; the remaining solids weighed about 50 μg per droplet (or "sting"). On this basis it was planned to aim for an eventual immunizing dose of 100 μg . It was believed that if a patient could

tolerate an injection containing twice the venom in an average sting, then a satisfactory immune state would have been achieved. The patient's father was stung from 50 to 100 times a week without reaction while at work, and a high titer of "blocking" antibody was found in his serum, when the antibody was measured by the capacity of dilutions of his serum to inhibit *in vitro* bee-venom induced histamine release from the leukocytes of a bee-venom sensitive patient. The child was immunized with a sterile solution of the venom collected by his father, and when he, too, had a high titer of blocking antibody to bee venom, he was challenged with a bee sting as an inpatient. He had a mild local reaction to the sting but no systemic reaction. The success of this case led to the treatment of a small additional series of patients with venom who also were challenged successfully with stings from live insects.¹⁷ About the same time, the presumption that insect allergy was IgE mediated was being confirmed by experiments in the laboratory. As mentioned above the leukocytes of venom-allergic patients released histamine on being challenged *in vitro* with venoms, and normal control cells did not release histamine unless exposed to toxic concentrations of venom (10 μg and above). It was found that the serum of many insect-allergic patients had the ability to sensitize the basophils of nonallergic individuals for venom-induced histamine release and that removal of the IgE from such sera with an appropriate immunosorbent removed this ability.¹⁸

Soon after leukocyte (basophil) histamine-release experiments established the useful range of venom concentrations, skin testing with venoms was cautiously attempted and found to be a usable technique for the confirmation of the venom-sensitive state.¹⁹ The maximum concentration of venom that could be used without increasing the possibility of false positive response to an unacceptable level was 1.0 $\mu\text{g}/\text{ml}$, although more recently the 3.0 $\mu\text{g}/\text{ml}$ concentration has been used without problems. A troublesome question is whether a positive response to a high venom test concentration means as much as a positive response to a low concentration. Hunt²⁰ found that nearly 25% of patients with systemic reactions to stings would have been missed if the patients had not been tested with a range of concentrations that included 1.0 $\mu\text{g}/\text{ml}$. One might wonder if the severity of the sting reaction can be correlated with the sensitivity of the patient on skin testing. Although in general these variables may be correlated, there are such large numbers of significant exceptions (e.g., severe reactions with positive skin tests only at 1.0 $\mu\text{g}/\text{ml}$), it is not safe to assume that less skin test reactivity means less risk of a severe reaction to a sting.



Graph I. Taxonomy of honeybees, hornets, wasps, and yellow jackets.

One study comparing the efficacy of venom therapy with placebo or whole-body extract therapy has been performed.¹⁶ In that study the test of efficacy was the response to a deliberate sting challenge, and under the conditions of the study, venom was clearly the immunizing agent of choice. The sting-reaction rate in both placebo and whole-body extract treated patients was about 60%, but only 5% sting-reaction rate in venom-treated patients. Subsequent studies have demonstrated that the IgG response and treatment efficacy are venom-dose dependent²¹ and have prompted critics of the comparative trial to suggest that had a larger dose of whole-body extract been used, a therapeutic effect would have been observed. This seems unlikely, since most whole-body extract preparations are found to have little or no venom content.¹⁸

Other trials of venom therapy have been conducted but without controls. Although in all trials a small failure rate has been observed, it was at first not clear what patients were at risk of reactions in spite of venom therapy. Although venom therapy was associated with an increase in serum-IgG antibodies to venom, the number of treatment failures has been so low as to make it difficult to collect a sufficient number for evaluation. As the number of such patients has accumulated and with the development of a more dependable assay for IgG antibodies on the basis of the affinity of *Staphylococcus* protein A for three of the four human-IgG subclasses,²² a pattern has emerged that helps clarify the quantitative relationship of IgG antibodies to protection from insect-sting reactions. After 4 mo of venom therapy, about 5% of adult patients had an IgG-antibody level below 3 $\mu\text{g/ml}$.²³ Coupled with the observation that treatment failures are more frequent when IgG-antibody levels are below 3 $\mu\text{g/ml}$, it now appears possible to detect that fraction of the venom-immunized population that is at risk of a sting reaction in spite of venom immunotherapy. Increasing the immunizing dose by 50% to

100% is usually associated with the development of adequate serum levels of IgG antibody.²¹

Venom immunotherapy also leads to a rise in IgE antibodies to venom within a few weeks after starting treatment. This does not persist, however, and the fear that venom immunotherapy might iatrogenically prolong the venom-sensitized state appears unfounded. Additional evidence for the protective role of IgG antibodies to venom was provided by experiments in which honeybee-allergic patients were injected with a gamma globulin fraction of the plasma of hyperimmune beekeepers.²⁴ In this study, it was first established at what dose of venom each patient would demonstrate signs of a systemic allergic reaction. Administration of venom was followed by a 2 to 3 wk period during which the patients were refractory to venom administration; sensitivity to venom injections returned after the immune globulin dissipated.

Venom constituents

The teleologic explanation for the variety of substances found in Hymenopteran venoms is that in general these insects use stinging either as a means of defense or of capturing prey. A large vespid found in North America, the Cicada Killer, paralyzes a cicada with a sting before hauling it back to its burrow for lunch for its offspring. The honeybee is apparently vegetarian, however, and will sting only in defense. In all Hymenoptera the stinging apparatus is a modified ovipositor; thus males do not sting.

The pain of a sting is due to the irritant effects of the venom constituents, which include vasoactive amines, small polypeptides, and larger protein molecules.

The taxonomy of the honeybee and the vespids is presented in Graph I.²⁵ This is shown in order to clarify the relationship of cross-reacting antigens that are found in the venoms of certain of these insects. In Table I are listed the known proteins and peptides of

TABLE I. Known protein and peptide components in the venoms of honeybee and vespids

	Honeybee venom		Vespid venoms	
	Molecular weight	% of venom	Molecular weight	% of venom
<i>Proteins</i>				
Phospholipase A ₂	15,800	12	—	0
Phospholipase A ₁	—	0	37,000	6 to 14
Hyaluronidase	45,000	2	43,000	1 to 3
Antigen 5	—	0	23,000	3 to 10
Acid phosphatase	49,000	na	49,000	na
<i>Peptides</i>				
Melittin	2,840	50	—	0
Apamin	2,038	2	—	0
Mast cell degranulating peptide	2,593	2	1,600	na
Kinin	—	0	1,200	na

na = not available.

honeybee and vespid venoms.²⁵ Venom peptides have a variety of physiologic activities, but none is known to be antigenic except melittin in honeybee venom. All of the venom proteins have enzyme activities except vespid antigen 5. This antigen resembles a protein that is found in the venom of an Asian hornet for which neurotoxicity has been found in a crustacean.

The three proteins in honeybee venom that are allergenically important are phospholipase A₂, hyaluronidase, and acid phosphatase. In addition, the polypeptide melittin is also antigenic, possibly because it can associate to yield a tetramer the size of a small protein (the monomer contains 26 amino acid residues). Bee-venom phospholipase A₂ contains 128 amino acid residues compared to the 123 amino acid residues in the human enzyme.²⁶ The sequences are known, and there seems to be little or no structural homology between the bee and the human forms.²⁷

The three vespid-venom proteins that have been purified to an extent that is necessary for analysis are antigen 5, hyaluronidase, and phospholipase A₁. Hornet, wasp, and yellow jacket venoms contain these same homologous proteins, although in varying proportions. As noted above no functional activity has been shown for antigen 5. The proteins from the different species of yellow jacket venoms shown in Graph I are very similar chemically and can be considered virtually identical. However, there are some antigenic differences between these and the venom proteins of *Vespula squamosa*, a yellow jacket common in the southeastern United States.²⁸ When the homologous proteins from hornet, wasp, and yellow jacket venoms are compared, small chemical differ-

ences are apparent. However, no structural data are available on any of the vespid-venom proteins.

Although honeybee and vespid venoms both have phospholipase and hyaluronidase activity, the phospholipase A₂ in bee venom is unlike the phospholipase A₁ in vespid venoms. Similarly, the proteins with hyaluronidase activities from the two types of venoms are not the same. Since there is considerable cross-reactivity between the protein antigens of the hornets and yellow jackets, skin testing with these venoms often leads to a picture of multiple positive skin tests. It may be impossible to discriminate on clinical grounds between the case in which sensitization to multiple species has occurred and the case in which sensitization to a single species has resulted in multiple positive skin tests because of cross-reactivity. King and Valentine²⁵ have suggested that skin testing with individual venom proteins may help eliminate confusion because of animal studies that show differing degrees of antibody reactivity depending on whether the authentic or a cross-reacting antigen is used. RAST inhibition is a laboratory technique that may also help solve this problem, albeit at considerable expense.

Criteria for venom immunotherapy

The immunology of insect allergy is somewhat clearer now than two decades ago but many unresolved questions exist. One of the most important questions is how to decide what patient should be started on venom immunotherapy. This question was one of the reasons for beginning a study of insect allergy in children 5 yrs ago.^{29, 30} Children with urticarial, nonlife-threatening reactions were chosen for study because the risk seemed least and because the potential benefit seemed greatest if definite criteria could be established that would help select patients for treatment. Although the long-term intent of the study was to find clinical or immunologic qualities that signaled the need for venom prophylaxis, the short-term findings have been of great interest. The study was designed to compare the results of accidental stings in children with positive skin tests and histories of nonlife-threatening reactions to stings who were randomly assigned either to venom therapy or no venom therapy. Both groups were instructed in sting avoidance and emergency self-treatment of stings. It was anticipated that a significant proportion of the group that did not receive venom treatment would have systemic reactions to stings, and some reactions would be worse than the original reactions. Although the rate of repeat reactions observed in adults had been 60%, it was suspected that the rate would be a little lower. However, the repeat reaction

rate was strikingly lower. Only 10% of stings resulted in reactions, most of these were not as severe, and none were worse than the original sting reaction. Immunologic relevance of the culprit insect was established in about 90% of cases. In the group that received venom treatment, immunotherapy did result in a significant reduction of the reaction rate with subsequent stings. However, one may question whether the small observed benefit in this population of children with urticarial reactions is worth the expense and effort involved, if all such children were to be immunized with venom.

Since not a single child experienced a sting reaction worse than the original, it no longer seems reasonable to frighten patients with the possibility that "the next sting may be your last." By contrast, analysis of sting fatalities reveal that about one half of patients who died from venom allergy had previously experienced a milder reaction. This is not a fair comparison because prospective and retrospective data, which was different population bases, cannot be compared. It does underline the need for better data regarding those patients, rare though they may be, whose reactions become progressively worse or terminate fatally.

If the criteria for establishing venom immunotherapy were clearly established, then one would know not only when to start it but also how long it should be continued. Many investigators have tried to reduce to a mathematical certainty the quantitative relationship between antivenom IgE and IgG antibodies in the hope of predicting risk of sting reactions. These attempts have failed, which allows us to say only that the presence of IgE antibodies to venom is one of an unknown number of risk factors for reactions to stings. It has thus been of interest to observe that there is, on average, a progressive decline in serum-IgE antibodies to venom in children who have been followed both with and without immunotherapy. There has also been a decline in the skin test reactivity of some but not all children who have been followed. Transient boosts in IgE antibodies have occurred after initiation of venom immunotherapy and after stings. In some treated children whose skin tests have become negative up to 1.0 $\mu\text{g/ml}$ of venom, a positive RAST has been observed.³¹ Although this could be construed to mean that the sensitivity of skin mast cells has declined, it may reflect the occasional inadequacy of even high concentrations of skin-testing venom in detecting low levels of sensitivity. It seems reasonable to believe that a change from a positive to a negative venom skin test signals that venom therapy can be stopped. This hypothesis is being tested, and the positive RAST-negative skin test anomaly is being closely observed.

Treatment of acute reactions

The study that was mentioned earlier¹⁶ provided the investigators with the opportunity to observe the immunologic and physiologic consequences of acute reactions to insect stings.³² In three unimmunized or whole-body extract immunized patients whose reactions included hypotension, hemodynamic effects began within 1 to 3 min of the stings and peaked within 60 sec. Interestingly, in 11 patients with urticaria, even massive (50% to 75% of body surface involvement) urticaria, no cardiopulmonary changes were observed. One of the three hypotensive patients responded rapidly to Trendelenburg position, intravenous infusion of saline, and 0.5 cc of 1:1000 epinephrine administered subcutaneously. In a second patient, similar treatment resulted in no change, and shock persisted for 90 min, which required additional volume replacement, intravenous 1:10,000 epinephrine, and also norepinephrine bitartrate. This patient received 2½ L of fluid and 3.5 mg of epinephrine before recovering. In the third patient, anaphylaxis persisted for 2 hr in spite of aggressive therapy, which eventually included approximately 5 L of fluid, 7 mg of epinephrine, and 1 gm of hydrocortisone acetate. There was a modest increase in plasma histamine in 60% of challenged patients that did not correlate with the presence of skin lesions. In two of the three hypotensive patients, striking increases in plasma histamine were observed. In these patients, clotting factors V and VIII were decreased during anaphylaxis, as were fibrinogen levels. Complement components C3 and C4 showed an acute drop in only one patient. No acute changes in serum-IgE antibodies to venom were seen.

In view of the severity of and the difficulty in treating two of the 14 observed reactions, the physician must temper his enthusiasm for the effectiveness of subcutaneous epinephrine in the management of anaphylaxis. It is without question highly effective in most acute sting reactions but may be insufficient for the very severe reactions. In this regard it should be noted that in all 14 cases, the clinical pattern of reactions resembled the patient's previous reaction. Epinephrine is the drug of first choice for anything but trivial reactions. Mild urticaria may be managed with antihistamines, although large doses parenterally may induce somnolence and hypotension that confuse the clinical course. Corticosteroids cannot be expected to have an effect within any time period relevant to an acute reaction, and it is not clear whether they are of any benefit in acute sting reactions.

Large local reactions to stings are probably IgE mediated but call for a different approach. Antihistamines and nonsteroid anti-inflammatory agents

seem to help, but neither they nor the popular short course of steroids has been compared to placebo therapy.

Summary

Allergy to insect venom is IgE mediated. Untreated, it occasionally terminates fatally and often causes temporary illness. Medical intervention with venom immunotherapy in patients with prior systemic reactions presents reactions to stings by inducing IgG-antibody formation, although in some groups of patients this results in little real benefit. Emergency self-treatment kits, (Epi-Pen and Epi-Pen Jr., Center Laboratories, Port Washington, N.Y.; Ana Kit, Hollister-Stier, Spokane, Wash.) if promptly and intelligently used, may reverse most moderate sting reactions. Some severe reactions require aggressive therapy. Until better treatment criteria are available, the most certain way of reducing the risk of systemic reactions to stings is with venom immunotherapy.

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