

Stinging insect allergy: Detection and clinical significance of venom IgE antibodies

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Venom-specific IgE antibodies in 109 sera from patients who had had immediate systemic allergic reactions following insect stings were measured by the radioallergosorbent (RAST) procedure. The majority of sera contained IgE antibodies to either bee, yellow jacket, or hornet venoms. Some sera had positive RAST reactions with 2 or 3 venoms, but others contained single venom-specific IgE antibodies. Of 24 patients who had large local reactions, the sera of 12 contained venom IgE antibodies. The RAST procedure provides an accurate means of documenting IgE-mediated allergic sensitivity to stinging insects.

The immunopathogenesis of anaphylactic type reactions due to stinging insects has not been clarified with the use of presently available whole body insect extracts.¹ Positive immediate skin tests have been found in nonallergic individuals, and negative tests have been noted in a significant number of individuals who had experienced immediate severe reactions. The use of inappropriate testing antigens, whole body extracts, rather than pure venom, has been proposed as one explanation for the failure to document the expected IgE antibody pathogenesis for these reactions. The presence of a tissue-specific antigen in venom has been established, and it would appear that this antigen is present in only minimal quantity in whole body extracts.²

Intradermal skin tests with whole venom may be difficult to interpret due to "nonspecific" reactions. Concentrations of bee venom greater than 10^{-7} are irritative and fail to identify the allergic reactor.* In recent years the radioallergosorbent test (RAST) has been developed as an accurate, reliable in vitro method for the detection of reaginic antibodies.³ We have applied the RAST procedure using pure venom as the testing antigen for the examination of sera of individuals who have had reactions from stinging insects.

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TABLE I. Prevalence and levels of IgE venom antibodies in the sera of 109 patients who had systemic allergic reactions following an insect sting*

RAST % STD	Bee venom	Yellow jacket venom	Bald face hornett venom
0-24	63	52	69
25-49	19	19	16
50-100	5	14	10
101-200	7	10	6
201-500	7	9	2
501-1,000	4	3	2
> 1,000	4	2	1

*RAST levels expressed as per cent of a standard serum. Levels above 25% considered significant.

†106 of the 109 sera examined.

METHODS

Selection of patients

Patients who experienced generalized systemic reactions or large local reactions following insect stings were selected for study. Systemic symptoms included generalized pruritus, urticaria, angioedema, hypotension, shock, unconsciousness, and respiratory problems such as upper airway edema and asthma. Large local reactions were defined as extensive swellings extending from the sting site and usually lasting at least 72 hr.

Whenever possible clinical information was obtained, including details of the reaction, identification of the causative insect (bee, wasp, yellow jacket, hornet), history of previous stings and therapy, and results of routine skin testing with whole body extracts.

Serum was obtained from each patient and stored at 4° C until tested.

Antigens

Commercially available whole body extracts* of honeybee, yellow jacket, wasp, and hornet were used for intradermal tests.

Honeybee venom was obtained by electrical stimulation of bees using the technique described by Benton, Morse, and Stuart.⁴ Venoms from yellow jackets and bald face hornets were obtained by aspiration from venom sacs.

Radioallergosorbent test (RAST)

A modification of the RAST procedure³ was employed using individual venoms in the concentration of 10 mg per 300 mg of cellulose particles as testing allergens.

RESULTS

The RAST proved to be highly reproducible and clearly discriminated sera of insect-sensitive patients from those of controls. Duplicate RAST determinations of patients' sera had a standard deviation of $\pm 16\%$. Thirty control sera obtained from noninsect allergic individuals had low radioactive counts and varied within a 5% range. A pool of such sera was taken as a standard negative control.

RAST results were expressed in relation to a positive control serum that had a radioactive count of $2\frac{1}{2}$ times the normal sera pool. In the case of bee venom, this standard had a passive transfer titer of 100. The RAST results were evaluated by subtracting the count of the negative control from the count of

*Hollister-Stier Laboratories, Spokane, Wash.

TABLE II. Summary of incidence of IgE venom antibodies in sera of systemic reactors

	Bee	Venoms	
		Yellow jacket	Bald face hornet
IgE antibodies			
RAST > 25%	46	57	37
No IgE antibodies			
RAST < 25%	63	52	69
RAST < 25%			
With all 3 venoms		38	
*With rigid criteria		18	

*See text.

TABLE III. Relationship of presence and degree of bee venom and yellow jacket venom IgE antibodies in sera of patients who had systemic reactions*

RAST % STD		Bee venom—RAST %						
		0-24	25-49	50-100	101-200	201-500	501-1,000	> 1,000
Yellow jacket— RAST %	0-24	43	7	2				
	25-49	8	6		2	2	1	
	50-100	6	2	1	3	1	1	
	101-200	1	2	1	2	2	1	1
	201-500	4	2		1	1		1
	501-1,000	1					1	1
	> 1,000					1		1

Numbers in boldface type indicate sera reacting with one venom only.

*RAST level expressed as % standard serum.

the positive standard and patient's sample. The net count of the test sample was expressed as percentage of the positive standard serum. (Example: The absolute count of the "standard" sample after subtraction of the count of the negative control is 4,000 cpm, and the count of the test sample after subtracting the count of the negative control is 2,000. The activity of the test serum is expressed as 50%.) RAST levels greater than 25% were considered significant.

Patients with systemic reactions

Incidence of IgE antibodies. Sera from 109 patients who had had systemic reactions following insect stings were analyzed. The results are found in Tables I and II. Significant antibodies (RAST greater than 25%) reacting with bee venom were found in 46 patients, with yellow jacket venom in 57 patients and with bald face hornet venom in 37 patients. Of the total group, there were 71 patients who reacted with at least one of the three venoms and 38 patients who did not react to any of the three venoms.

This latter group of nonreacting sera were analyzed further. Patients were eliminated who had: (1) known wasp stings, (2) sera obtained less than 2 wk following the systemic reaction,* and (3) sera obtained more than 3 yr following the systemic reaction. There still remained 18 patients who failed to have detectable IgE antibodies.

*Unpublished observations indicate that IgE antibodies rise after a sting, reaching a maximum in about 2 wk.

TABLE IV. Relationship of presence and degree of bee venom and bald face hornet venom IgE antibodies in sera of patients who had systemic reactions*

		Bee venom—RAST %						
		0-24	25-49	50-100	101-200	201-500	501-1,000	> 1,000
Bald face hornet — RAST %	0-24	48	13	2	1	3	2	
	25-49	10	2	1	2			1
	50-100	1	3	1	2	1	1	1
	101-200	2		1	1	2		
	201-500				1	1		
	501-1,000						1	1
	> 1,000							1

Numbers in boldface type indicate sera reacting with one venom only.

*RAST level expressed as % standard serum.

TABLE V. Relationship of presence and degree of yellow jacket venom and bald face hornet venom IgE antibodies in sera of patients who had systemic reactions*

		Yellow jacket—RAST %						
		0-24	25-49	50-100	101-200	201-500	501-1,000	> 1,000
Bald face hornet — RAST %	0-24	45	14	8	1			
	25-49	5	2	5	3	2		
	50-100		1	2	3	4		
	101-200				3	2	1	
	201-500					1		1
	501-1,000						2	
	> 1,000							1

Numbers in boldface type indicate sera reacting with one venom only.

*RAST level expressed as % standard serum.

TABLE VI. Prevalence and incidence of IgE venom antibodies in the sera of 24 patients who had large local reactions following an insect sting*

RAST % STD	Bee venom	Yellow jacket venom	Bald face hornet venom
0-24	19	14	17
25-49	0	4	4
50-100	2	4	2
101-200	2	0	0
201-500	1	1	1
501-1,000	0	1	0
> 1,000	0	0	0

*RAST levels expressed as per cent of a standard serum. Levels above 25% considered significant.

Specificity of IgE venom antibodies. Comparisons of the RAST results of individual sera using the three different testing venoms are shown in Tables III, IV and V. In Table III, bee and yellow jacket RASTs are compared. While many of the sera have IgE antibodies reacting to both venoms, quite specific reactions to one of the two venoms were also found. Nine sera reacted to bee venom (RAST greater than 25%) and did not react to yellow jacket. More impressively, 20 sera contained yellow jacket venom IgE antibodies and showed no reaction to bee venom. In Table IV, the relationships of bee venom and bald face hornet venom are shown. While there are parallel reactions to both venoms,

TABLE VII. Relationship of bee venom IgE antibodies and patient identification of bee as causative insect for systemic reaction

	Patient identification	No. of patients
RAST > 25%		46
	Bee	26
	Others	11
RAST > 100%		22
	Bee	17
	Others	3
RAST < 25%		63
	Bee	7

numerous sera contain species-specific IgE antibody only. Thirteen sera reacted only with hornet venom and 21 sera reacted only with bee venom. Table V shows the results of bald face hornet and yellow jacket venom RASTs. These results are somewhat more parallel, yet 23 patients had yellow jacket venom-specific IgE antibodies only and 5 hornet-specific IgE antibodies only.

Patients with large local reactions

Serum samples were studied from 24 patients who experienced large local reactions following insect stings (Table VI). Significant reactions to bee venom were found in 5 patients, to yellow jacket venom in 10 patients, and to bald face hornet venom in 7 patients. Twelve of the 24 patients reacted to at least one of the three venoms while the other 12 did not react to any of the three venoms. Thus a reagenic pathogenesis for these large local reactions might be implicated in half of the patients studied.

Relationship of RAST results to identification of causative agent

It is usually most difficult for patients to identify the specific type of stinging insect responsible for their reaction. Because the honeybee leaves its stinger in place, it is somewhat easier for this identification to be made. Of the 46 patients with elevated bee venom RAST levels, 26 identified the bee as the causative agent of their reactions. The yellow jacket was identified by 8 patients, the wasp by 2, the hornet by 1, and 9 patients could not identify the responsible stinging insect. Of the 22 patients whose RAST levels were greater than 100%, 17 were able to identify the bee as the cause of their reaction. Of the 63 patients who had bee RAST levels below 25%, only 7 believed the bee caused their reactions (Table VII). These results do suggest fairly good agreement between reactions produced by suspected bee stings and the finding of elevated IgE antibodies to bee venom.

Relationship of RAST results to whole body extract skin tests

Skin test data using whole body bee extracts were available in 47 patients, all of whom were studied by the authors. Considering a reaction to a 1:10,000 dilution (0.01%) of this extract as significant, the majority of patients with elevated bee venom RAST levels had positive tests. However, there were also

22 patients with bee venom RAST levels below 25% who had similar positive skin tests. Thus these results confirm the poor reliability of skin tests with whole body bee extract.

Preliminary experiments indicated that whole body extracts were not as useful as venom in the RAST procedure.

DISCUSSION

Several significant conclusions can be reached from results of these studies. The use of pure venom as diagnostic antigens in the RAST procedure has provided an accurate, reproducible test for measuring the presence of reaginic antibodies and confirming the diagnosis of a stinging insect sensitivity. This method also provides a means of analyzing serial serum samples and thus assessing effects of specific factors, such as repeated stings and immunotherapy.

These results indicate that the great majority, but not all, of systemic allergic reactions following insect stings are mediated by IgE antibodies. Such conclusions could not be reached from results of direct skin testing with whole body extracts. Previous studies in several animal models have raised the question of the reliability of whole body extracts as diagnostic agents. Studies in the rabbit model have shown the presence of a "tissue"-specific antigen in pure venom, not present in the sacless whole body extract and present in minimal amounts at best in extracts prepared from the whole body insect.² Similarly, antivenom reaginic-type antibodies raised in mice failed to react to whole body extracts.⁵ In more recent studies using the human leukocyte histamine release assay system, Sobotka and associates⁶ found pure venoms to be appropriate allergens for inducing positive reactions. It can be concluded that pure venom is a more reliable diagnostic allergen than whole body extracts. Removing or neutralizing potent pharmacologically active materials may eventually allow venoms to be suitable skin testing agents as well as reliable agents in the *in vitro* systems that detect IgE antibody.

Of great interest are the small, but still significant, number of sera, that failed to show reactions with any of the three venoms studied. It is possible that the appropriate venom was not used for testing. These patients might have been sensitive to wasp venom or even perhaps to a very specific species of yellow jacket. Possibly IgE antibodies were present only transiently, less than the 3-yr empirical cutoff point. The alternative possibility is that the reaction in these individuals was not mediated by IgE antibodies but had either another type of immunological mediator or a nonimmunological pathogenesis. Such possibilities are presently under study.

Differing patterns of specific insect allergenicity also emerge from the study. Some patients appear sensitive to several insects, having IgE antibodies reacting to 2 or 3 of the venoms studied. Other patients manifest unique sensitivity to only one stinging insect. For example, 20 patients showed moderate to marked sensitivity to yellow jacket but no reaction to bee venom. Recognition of this factor is most important in any attempt to evaluate or analyze current problems of stinging insect sensitivity. For example, numerous attempts have been made to evaluate the role of immunotherapy in this problem, but without considering differences in insects responsible for repeat stings.

The problem with patients with large local reactions following insect stings has also been controversial. There have been suggestions based on clinical observation that such reactions very often precede subsequent systemic reactions. The results of the studies presented here suggest that about half of these patients do indeed have some reagins and might well be at risk with further stings. The question then of appropriate therapy for these individuals is still not clarified except by specific analysis of the IgE antibody.

The obvious benefits of pure venom in diagnostic tests certainly suggest a potential role in therapy. Two well-documented cases have been reported of successful hyposensitization treatment with pure bee venom following treatment failure with conventional whole body insect extracts.^{7, 8} Furthermore, venom has been shown to induce blocking antibodies capable of affording protection both in allergic individuals and in beekeepers, while whole body extracts lack this antigenicity.⁹ These immunological studies have cast serious doubt upon the therapeutic efficiency of the whole body extracts. On the other hand, clinical studies, collected particularly by the Insect Committee of the American Academy of Allergy,¹⁰ have suggested that patients receiving this conventional type of therapy do indeed fair better after subsequent stings. Whether this apparent improvement is a function of time or specific treatment is yet to be verified.

Sequential studies measuring venom IgE antibodies in the sera of allergic individuals are now in progress and should lead to further definitive conclusions.

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