

Comparison of the allergenic properties of bee venom and whole bee body extract

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Summary

The allergenic properties of bee venom and whole bee body extract were compared by *in vivo* and *in vitro* tests. The majority of patients with known bee sting sensitivity had positive intracutaneous skin test reactions with bee venom and had bee venom specific IgE in their sera. Of seventeen patients with positive bee venom skin tests, nine had positive tests with whole bee body extract. Of thirty sera containing elevated levels of bee venom specific IgE obtained from untreated patients, fourteen sera contained whole body specific IgE but in much lower titres.

In RAST inhibition experiments using both bee venom and whole bee body extract as coupling antigens, bee venom was a more potent inhibiting antigen than whole body extract.

From these experiments we conclude that bee venom is a more potent allergen than whole bee body extract.

Introduction

Stinging insect allergy is a clinical problem of significant morbidity and mortality (Parrish, 1962; Barnard, 1973). The traditional treatment for insect sensitive patients has been the prophylactic injection of the appropriate whole insect body extract. This therapeutic approach originated from the concept that the allergen responsible for the reactions is found throughout the body tissues as well as in the venom (Benson & Semonov, 1930; Benson, 1939). Subsequently, Loveless & Fackler in 1956 suggested the use of pure venom for optimal treatment. Recently two individuals have been described who were successfully immunized with venom after treatment failure with whole body extract (Busse *et al.*, 1975; Lichtenstein, Valentine & Sobotka, 1974). Our own studies have failed to document a significant immunological response following treatment with whole body extracts (Reisman *et al.*, in press).

This study was supported in part by United States Public Health Service Allergic Diseases Clinical Centers Grant 2-P15-AI-10397 and in part by United States Public Health Service Research Grant 5-RO1-AI-01303 of the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland.

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The purpose of this study was to re-examine the relationship between whole bee body extract and bee venom, utilizing *in vivo* and *in vitro* techniques.

Materials and methods

(A) Materials

Bee venom was obtained from the Sigma Chemical Company (St Louis, Missouri) or by electrical stimulation (Benton, Morse & Stuart, 1963). This latter source is indicated by an asterisk*.

Whole bee body extract was prepared in our laboratory (Shulman *et al.*, 1966). Briefly, 15 g of whole bee bodies were added to 100 ml of buffered saline in a Waring Blender and mixed for 3 min. This was clarified further by centrifugation in an ultracentrifuge at 35,000 r.p.m. for 1 hr. Whole bee body extracts for skin testing were obtained from a commercial source (Hollister-Stier Co., Spokane, Washington).

(B) Skin tests

Intradermal skin tests were performed in forty-six patients with histories of stinging insect hypersensitivity. Bee venom* was used in dilutions varying from 0.01 to 1 µg/ml. Concentrations greater than 1 µg/ml gave false positive skin tests in non-allergic volunteers. Skin tests with whole bee body extracts were performed using dilutions of a 1-10 extract varying from 10^{-3} to 10^{-5} . Dilutions less than 10^{-3} gave non-specific reactions in many non-allergic subjects.

(C) In vitro tests

Sera and in some instances cells were collected from (1) patients who had had immediate allergic reactions following bee stings, (2) beekeepers who had little local reaction following bee stings and who represented an 'immune' population and (3) normal, non-allergic individuals.

(1) *Radioallergosorbent test (RAST)*. Bee venom specific IgE antibodies and whole bee body specific IgE antibodies were measured by the RAST using a modification of the technique described by Ceska, Eriksson & Varga, 1972. Twenty milligrams of bee venom or 2 ml of 15% whole bee body extract in a total volume of 20 ml were coupled to 200 paper discs and allowed to incubate at room temperature for 3 hr. After washing four times with normal saline, 0.05 ml of radioactive anti-human IgE (Hunter & Greenwood, 1962) was added to each tube and the samples were incubated overnight at room temperature. The following day, after further washing, the supernatant was removed and the tubes were counted in a Packard autospectrogamma counter. Each serum was run in duplicate and each positive and negative control in quadruplicate. Assays for bee venom and whole bee body antibodies were run simultaneously.

RAST results were expressed in relationship to a known ragweed pooled sera standard which was assayed in each experiment. This standard was obtained by pooling serum from several ragweed sensitive individuals. The ragweed IgE antibody concentration of this pool was measured by a radioimmunoassay (Fugita *et al.*, 1975) and was approximately 133 ng of IgE per ml. The ragweed sera standard was incubated with cellulose discs prepared by coupling 10 ml of 15% ragweed extract in 10 ml of 0.1 M sodium bicarbonate per 200 discs by the same method described for bee venom. The amount of a fixed quantity of IgE antibody in the standard could be compared to the

unknown quantity of specific IgE antibody in the test sample by comparison of the c.p.m. This is a similar approach to that described by Johansson, Bennich & Berg, 1971. Calculations were performed as follows:

$$\frac{\text{Net c.p.m. bee venom}}{\text{Net c.p.m. ragweed standard}} = \frac{X}{133\%}$$

Thus, if the net c.p.m. for bee venom was 8,000 and the net c.p.m. for ragweed standard was 10,000 then $X = 106\%$. The disc RAST had an average standard deviation equal to 7% of the mean c.p.m.

(2) *RAST inhibition studies.* A standard reference sera pool was obtained by mixing serum from six bee sensitive individuals. Serial ten-fold dilutions of bee venom or whole bee body extract were added to each tube in duplicate. A 0.05 ml portion of pooled sera was incubated overnight with 0.15 ml of either antigen or 2% HSA. The next day the appropriate disc was added and the RAST procedure continued as described for the direct RAST. The calculations were performed as follows:

$$\% \text{ Inhibition} = 1 - \frac{\text{net c.p.m. sample}}{\text{net c.p.m. without antigen}} \times 100$$

(3) *Histamine release from leucocytes.* The May modification (May *et al.*, 1970) of the Lichtenstein & Osler method (1964) was used for this assay. Cells from nine bee sensitive patients were challenged with 1 $\mu\text{g/ml}$ or less bee venom and histamine was assayed fluorometrically.

Results

(1) Skin testing

Positive skin tests to bee venom were found in seventeen of forty-six patients tested. Nine of these patients with positive bee venom skin tests also had positive skin tests to whole bee body extract. Of twenty-nine patients with negative bee venom skin tests, seven had positive whole bee body extract skin tests. All patients who could definitely identify the bee as the causative insect, had positive bee venom skin tests.

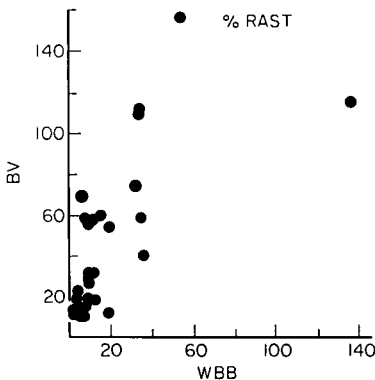


Fig. 1. Levels of bee venom (BV) and whole bee body (WBB) specific IgE in the sera of thirty untreated allergic patients. Rank correlation between these two antibodies was significant ($r = 0.72$, $P = 0.001$).

Table 1. IgE antibodies reacting with bee venom (BV) and whole body extract (WBE) in the sera of untreated allergic patients, 'immune' and allergic beekeepers and non-allergic control population. Antibody levels are expressed as a percentage of a positive standard (see text). Levels over 10% are considered significant

	No.	IgE antibodies			
		BV		WBE	
		Mean %	Range	Mean %	Range
Untreated patients	30	45	(10-157)	18	(0-136)
Beekeepers with reactions	10	53	(0-148)	12	(0-44)
Beekeepers, active	26	2	(0-29)	5	(0-30)
Controls	60	1	(0-8)		
	20			2	(0-7)

(2) Venom and WBE specific IgE serum antibodies

Elevated levels of bee venom specific IgE were found in the serum of thirty untreated bee sensitive individuals. Fourteen of these patients also had elevated amounts of IgE antibody reacting with whole bee body extract, generally in proportion to those of bee venom but at lower levels (Fig. 1). There was a significant rank correlation between these antibodies ($r = 0.72$, $P = 0.001$). Relative amounts of bee venom specific IgE and whole bee body specific IgE in the serum of these patients and in groups of twenty-six active non-allergic beekeepers and ten beekeepers who had had systemic reactions are found in Table 1. Protected beekeepers generally had negligible levels of bee venom and whole bee body specific IgE.

(3) Relationship of skin test results and presence of serum IgE antibodies

Table 2 shows the distribution of positive skin test and serum IgE antibody results in forty-six patients selected for testing on the basis of a history of insect sensitivity,

Table 2. Summary of skin test and serum IgE antibody results in forty-six insect sensitive patients

Bee venom		Whole bee body		No. of patients
Skin test	Serum IgE	Skin test	Serum IgE	
+	+	+	+	7
+	+	+	-	2
+	+	-	-	6
+	+	-	+	1
+	-	-	-	1
-	+	-	-	2
-	+	+	+	1
-	+	+	-	1
-	-	+	-	4
-	-	+	+	1
-	-	-	-	20
Total				46

Table 3. Comparison of the results of bee venom skin testing, histamine release and serum IgE antibody in nine bee sensitive patients. Skin tests were graded from +1 (indicating 3 mm weal) to +4 (indicating weal with pseudo-pods). The concentration of bee venom required for this result in $\mu\text{g/ml}$ is shown in the column to the right. The maximum histamine release and the concentration of bee venom required for this release are indicated in the next two columns. Specific IgE antibody to bee venom measured by RAST and expressed as a percentage of a positive standard is shown in the last column. Levels over 10% are considered significant

Patient	BV skin test result		BV maximum Histamine release		BV RAST (%)
		Conc. ($\mu\text{g/ml}$)	(%)	Conc. ($\mu\text{g/ml}$)	
DT	+2	0.01	66	0.0001	132
TB	+2	0.1	66	0.00001	130
LC	+4	0.1	48	0.0001	93
MM	+3	0.1	64	0.01	91
DC	+2	0.1	48	0.0001	53
JG	+2	1.0	71	0.01	61
RK	+3	1.0	33	0.1	26
JM	+2	1.0	47	0.1	10
WB	$\pm$	1.0	26	1.0	9

but not necessarily bee sting sensitivity. Sixteen of seventeen patients with positive bee venom skin tests had elevated specific IgE serum antibodies to bee venom. Four patients had detectable bee venom specific IgE antibodies and negative bee venom skin tests.

Positive whole bee body skin tests were accompanied by whole bee body specific IgE in nine of sixteen instances. Five patients with whole bee body extract skin test reactions had no evidence of sensitivity to bee venom either by skin test or by RAST.

(4) Relationship of histamine release, serum IgE antibodies and skin test results

Nine patients with known bee venom sensitivity were tested by measuring histamine

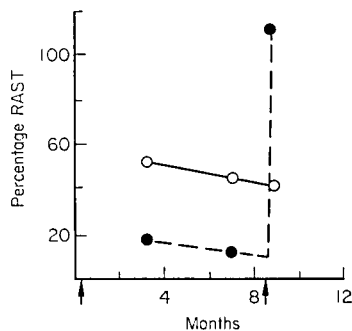


Fig. 2. Bee venom (BV, ●—●) and whole bee body extract (WBB ○—○) specific IgE in the serum of a beekeeper who experienced a generalized allergic reaction following a sting (†). BV specific IgE increased while WBB specific IgE continued to decline.

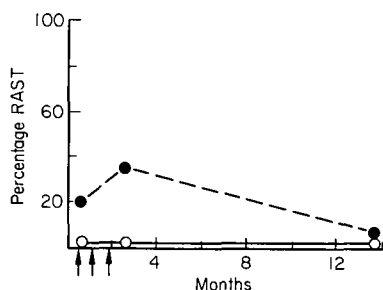


Fig. 3. Bee venom (BV ●—●) and whole bee body extract (WBB, ○—○) specific IgE in the serum of a beekeeper who experienced large local reactions following bee stings (↑↑↑). BV increased while WBB remained consistently low.

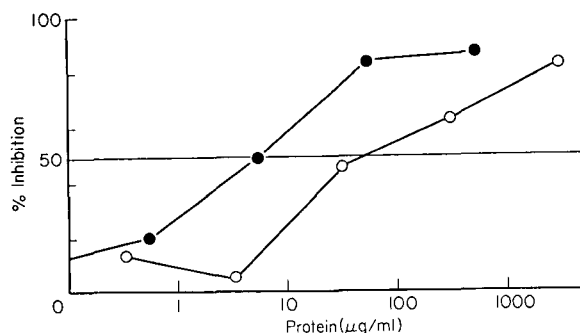


Fig. 4. Typical experiment of inhibition of the bee venom RAST. Bee venom protein (6 μg/ml (●—●) caused 50% inhibition of the RAST as compared to 80 μg/ml protein of whole bee body extract (○—○).

release from leucocytes after challenge with ten-fold dilutions of bee venom in concentrations ranging from 0.00001 μg/ml to 1.0 μg/ml. The maximum histamine release and the concentration of bee venom used to achieve this release are shown in Table 3. The results of skin test titrations with bee venom and the bee venom serum IgE values are also listed. Correlation measured by the Spearman rank test was very good between histamine release and RAST results ($r = 0.92$, $P = 0.001$), and between bee venom skin tests and RAST values ($r = 0.9$, $P = 0.001$). Good correlation was found between venom induced histamine release and skin tests ($r = 0.78$, $P = 0.02$).

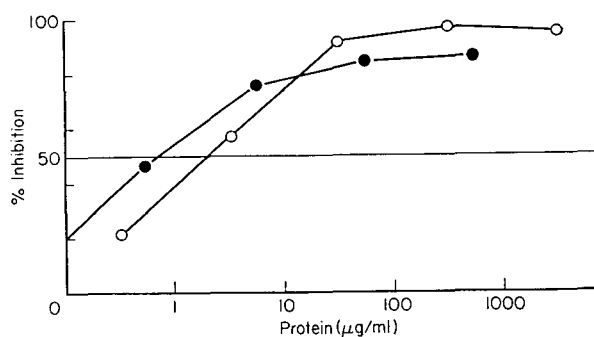


Fig. 5. Typical experiment of inhibition of the whole bee body RAST. Bee venom protein, 0.7 μg/ml (●—●) caused 50% inhibition of the RAST as compared to 1.9 μg/ml protein of whole bee body extract (○—○).

(5) Effect of a sting

Two beekeepers who had had allergic reactions to bee stings received further stings during a time period when sequential sera were available. Beekeeper D.Y. (Fig. 2) kept hives for 6 years during which time she sustained an estimated twenty stings per year without difficulty. In July, 1974 she was stung on the neck and became hypotensive. Epinephrine was given for immediate relief. She was restung in March, 1975 and sustained generalized pruritus and marked local swelling. During the following week she developed arthralgia.

Beekeeper I.C.F. (Fig. 3) had kept bees as a hobby for 5 years sustaining about five stings per year. He reported marked local swelling lasting several days after each sting. He was restung twice between his sera samples.

In these individuals who were stung by their own bees, bee venom specific IgE antibodies were stimulated by the stings while the level of whole bee body antibodies remained relatively constant. During this period neither beekeeper received immunotherapy with whole bee body extract.

(6) RAST inhibition studies

A detailed account of the RAST inhibition methodology as a tool for standardization of allergenic extracts has been reported by Arbesman, Wypych & Reisman (1975). Using bee venom discs, 6 µg/ml protein of bee venom as compared to 80 µg/ml protein of whole bee body extract was required to achieve 50% inhibition (Fig. 4). Using whole bee body discs, 0.7 µg/ml protein of bee venom as compared to 1.9 µg/ml of whole bee body extract was required for 50% inhibition (Fig. 5).

Discussion

Bee venom specific IgE in serum is indicative of the presence or potential presence of bee sting allergy. Venom specific serum IgE correlated well with venom induced histamine release in nine patients studied. The majority of patients with venom specific IgE also had positive skin tests with bee venom. In contrast, only eleven of twenty of these patients had positive skin tests with whole bee body extract.

Skin test reactions to whole bee body occurred in four of forty-six individuals without positive corroborative immunological findings. These studies suggest that bee venom is the allergen of choice for diagnostic testing, and that whole bee body extract testing will miss individuals with bee venom hypersensitivity and falsely suggest sensitivity where there is a lack of supportive immunological data.

In a group of untreated patients with elevated bee venom specific IgE, only one half had elevated WBE specific IgE. Levels of whole bee body IgE antibody were in general proportional to, but lower than, levels of bee venom IgE.

Very rarely IgE antibodies may preferentially be directed against the whole bee body antigen rather than venom. A group of 240 patients with various histories of insect hypersensitivity were screened for both antigens. Only four individuals were found with IgE antibodies to whole bee body antigens without concurrently present IgE antibodies to the major venom specific antigen. Three of these individuals were beekeepers who were exposed to bee dust as well as bee venom. One individual was a patient with a strongly positive skin test to whole bee body but no serological evidence of IgE antibodies to bee venom.

The affinity of bee venom specific IgE antibodies for bee venom and whole bee body antigen was compared by RAST inhibition. Bee venom was by far the most

effective antigen. Interestingly, bee venom was as effective as whole bee body extract in inhibiting specific whole bee body IgE antibodies.

Therapy with whole bee body extract as has been traditionally administered, failed to stimulate production of blocking antibodies or to significantly diminish IgE antibodies above that which could be expected with time alone (Reisman *et al.*, in press). In contrast, venom injection stimulates both IgE and blocking antibodies. As measured by these parameters, whole bee body extract appears to be immunologically ineffective.

The results of these studies suggest that bee venom is the appropriate antigen for diagnosis and treatment of bee sting allergic individuals. Whole bee body extract appears to be a relatively weak allergen.

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