

Venom skin tests in insect-allergic and insect-nonallergic populations

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Intradermal skin tests with varying concentrations of honeybee, yellow jacket, white-faced hornet, yellow hornet, and Polistes venoms were done on 85 patients with histories of insect-stinging anaphylaxis and on 56 insect-nonallergic subjects. Positive skin tests (wheal ≥ 5 to 10 mm and flare ≥ 11 to 20 mm) were present in 67 insect-allergic patients at venom concentrations ranging from 0.001 $\mu\text{g/ml}$ to 0.1 $\mu\text{g/ml}$. Seven additional allergic patients had positive skin tests with the 1.0 $\mu\text{g/ml}$ venom concentration. Twenty-six nonallergic subjects had positive skin tests at the venom concentration of 1.0 $\mu\text{g/ml}$, and two patients had positive skin tests at the lower venom concentrations (0.001 to 0.1 $\mu\text{g/ml}$). These results confirm venom skin tests as a highly sensitive method of detecting venom-specific IgE in the evaluation of patients with stinging-insect hypersensitivity. Since a large percentage of insect-nonallergic subjects reacted to the 1.0 $\mu\text{g/ml}$ concentration, clinical judgment and further in vitro testing should be considered in the evaluation of patients who react only at this venom concentration. (J ALLERGY CLIN IMMUNOL 76:803-7, 1985.)

Commercially available insect venoms are very reliable in detecting venom-specific IgE in patients with suspected stinging-insect sensitivity.¹⁻³ Prior studies have suggested that a $\geq 1+$ skin test reaction elicited by venom concentrations of ≤ 1 $\mu\text{g/ml}$ are immunologically mediated, thus identifying potentially allergic individuals.⁴ Higher concentrations of venom cause nonspecific reactions, probably as the result of pharmacologically active agents contained in these venoms.^{5, 6}

In assessing these skin test criteria, we have been concerned about patients who react only to the 1.0 $\mu\text{g/ml}$ venom concentration. This led to a reassessment of the skin test reactions in individuals with histories of stinging-insect allergy and in insect-nonallergic subjects. The results confirm the reliability of skin tests with venoms for the detection of specific

IgE antibody and also indicate that the 1.0 $\mu\text{g/ml}$ dose may elicit an irritative reaction in some individuals.

MATERIAL AND METHODS

Venom preparations

Honeybee, yellow jacket, white-faced hornet, yellow hornet, and *Polistes* venoms were kindly supplied by Pharmacia Laboratories, Piscataway, N. J. Venoms were stored as a lyophilized powder at 4° C until they were used. The freeze-dried powder was reconstituted with a special diluent (0.9% NaCl, 0.4% phenol, and 0.03% normal human serum albumin) to a concentration of 100 $\mu\text{g/ml}$. Serial tenfold dilutions were prepared with the same diluent and stored at 4° C. The concentrate was prepared every 2 weeks, and subsequent dilutions were reconstituted daily before testing.

Patient selection

Patients observed routinely for evaluation of stinging-insect anaphylaxis were selected. Anaphylaxis was defined as the occurrence of any of the following symptoms shortly after an insect sting: urticaria and/or angioedema, wheezing, upper airway swelling, hypotension, loss of consciousness, nausea, vomiting, or diarrhea. None of the subjects had received venom immunotherapy or whole body extract immunotherapy. Each allergic patient was evaluated within 3 years of the anaphylactic sting episode.

The insect-nonallergic subjects were recruited from the private practice of one of the authors (R. E. R.) and evaluated in the same manner. These control subjects had a high incidence of asthma and allergic rhinitis. None had experienced any type of adverse reaction after an insect sting.

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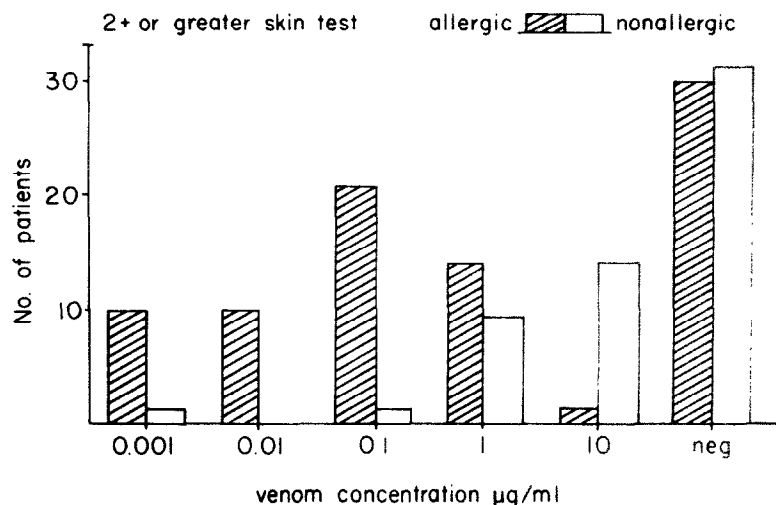


FIG. 1. Comparison of $\geq 2+$ skin test end point reactions with Hymenoptera venoms in insect-allergic and insect-nonallergic subjects. Patients were tested with increasing venom concentrations until a 2+ reaction was elicited or until a maximum concentration of 1.0 $\mu\text{g/ml}$ was administered to allergic subjects and 10 $\mu\text{g/ml}$ to nonallergic subjects. Nine allergic subjects also were tested with the 10 $\mu\text{g/ml}$ dose.

TABLE I. Demographic data

	Insect-allergic patients	Insect-nonallergic patients (control)
No. of patients	85	56
Mean age (yr)	27	32
Range (yr)	5 to 80	11 to 62
History of sting	85 (100%)	19 (34%)
Sting reaction		
Anaphylaxis	85 (100%)	0
Large local	40 (47%)	0
Atopic history	22 (27%)	40 (71%)

Skin testing procedure

Serial end point intradermal titration tests were performed with the five individual venom preparations. A volume of 0.02 ml was injected intradermally starting at the 0.001 $\mu\text{g/ml}$ venom concentration. Increasing concentrations were applied until either a 2+ reaction developed or a maximum concentration of 1.0 $\mu\text{g/ml}$ was administered to the allergic patients and 10 $\mu\text{g/ml}$ to the nonallergic subjects. Testing also was done with the 10 $\mu\text{g/ml}$ concentration in nine allergic subjects. A negative control (diluent) and positive control (histamine, 0.1 mg/ml) were placed concurrently with each set of venom skin tests. Skin tests were read after 15 minutes with the following scale: 1+ = wheal 5 to 10 mm, flare 11 to 20 mm; 2+ = wheal 5 to 10 mm, flare 21 to 30 mm; 3+ = wheal 10 to 15 mm, flare 31 to 40 mm; and 4+ = wheal 15 mm, flare >40 mm.⁷

Statistical analysis

Chi-square analysis was performed on the overall skin test data for both allergic and nonallergic groups.⁸ Because

of the small sample size and small cell frequencies for each venom concentration, the skin test data for individual venoms were considered as independent samples. A two-tailed Fisher exact probability test was used to analyze for differences in the percentages at each venom dilution.⁹ Significance was $p < 0.05$.

RESULTS

Demographic data

Eighty-five insect-allergic and 56 insect-nonallergic subjects participated in this study (Table I). The mean age for the allergic group was 27 years with a range of 5 to 80 years; the mean age for the nonallergic group was 32 years with a range of 11 to 62 years. All 85 insect-allergic patients had experienced anaphylaxis after an insect sting; 40 subjects reported concomitant large local reactions. Of the 56 nonallergic patients, 19 reported a history of an insect sting, none of which resulted in anaphylaxis or large local reactions. The frequency of atopic disorders, such as asthma, allergic rhinitis, or atopic dermatitis was 27% in the insect-allergic group and 71% in the insect-nonallergic group.

Skin test results

The distribution of $\geq 2+$ skin test end point reactivity is presented in Fig. 1 and detailed for the individual venoms in Table II. At the lower venom concentrations (0.001 to 0.1 $\mu\text{g/ml}$) positive skin tests were found principally in the insect-allergic group. At 1.0 $\mu\text{g/ml}$ the number of positive skin tests was still primarily in the insect-allergic group (13 patients), but there were nine nonallergic subjects with positive

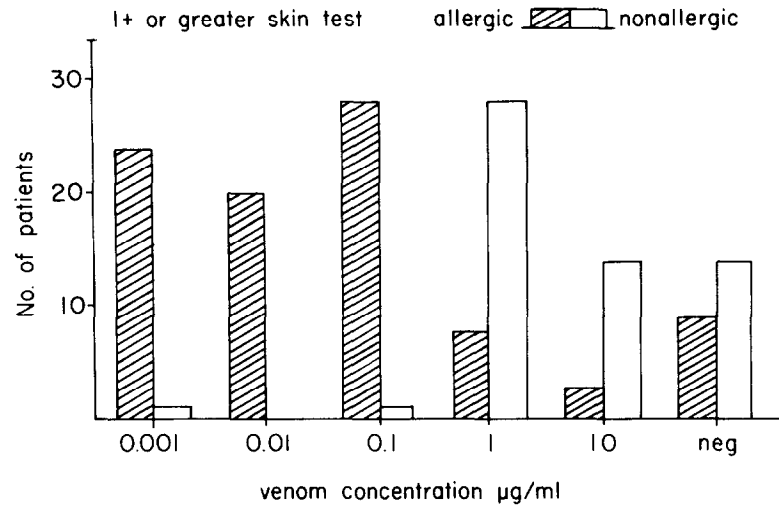


FIG. 2. Comparison of $\geq 1+$ skin test reactions with Hymenoptera venoms in insect-allergic and nonallergic subjects. Data indicate the lowest venom concentration eliciting a 1+ reaction.

TABLE II. Distribution of positive skin test end points ($\geq 2+$) with individual Hymenoptera venoms in 85 allergic and 56 nonallergic individuals

Venom	Group	Venom concentrations (µg/ml)					
		0.001	0.01	0.1	1.0	10*	Neg
Honeybee	Allergic	3	2	3	10	0	67
	Nonallergic	1	0	1	2	9	43
Yellow jacket	Allergic	2	5	14	14	0	50
	Nonallergic	0	0	0	5	10	41
White-faced hornet	Allergic	2	7	12	8	1	55
	Nonallergic	1	0	1	3	5	46
Yellow hornet	Allergic	0	6	8	10	1	60
	Nonallergic	0	0	0	4	4	48
<i>Polistes</i>	Allergic	3	4	11	6	3	58
	Nonallergic	1	0	1	3	3	48

*Only nine allergic patients were tested with 10 µg/ml.

skin tests. The pattern of reactivity was the same with the individual venoms. By use of the $\geq 2+$ skin test criterion, there were 31 patients with completely negative skin tests in the insect-allergic group.

The distribution of $\geq 1+$ reactivity is presented in Fig. 2 and Table III. At the lower venom concentrations (0.001 to 0.1 µg/ml), positive skin tests again were principally in the allergic group. At the 1.0 µg/ml dilution, positive skin tests were found only in an additional seven insect-allergic patients. By use of a 1+ positive skin test, the number of completely negative skin tests was much lower in the insect-allergic group (nine patients). However, a high number of nonallergic subjects (26 patients) had a 1+ positive skin test at the 1.0 µg/ml venom concentration.

Frequency analysis of 1+ positive skin tests demonstrates that at the lower venom concentrations

(0.001 to 0.1 µg/ml) there is a statistically significant difference between the allergic and nonallergic groups ($p < 0.05$). At the 1.0 µg/ml venom concentration, the frequency of positive skin tests in the allergic and nonallergic groups was not statistically significant for any individual venom tested (honeybee, $p > 0.38$; yellow jacket, $p > 0.67$; white-faced hornet, $p = 1.00$; yellow hornet, $p > 0.81$; and *Polistes*, $p < 0.50$).

DISCUSSION

Skin tests constitute the major method of demonstrating the presence of IgE antibodies in allergic individuals. For subjects with insect-sting anaphylaxis, venom skin testing helps to identify the culprit insect.¹⁻⁴ The individual venoms contain pharmacologically active compounds, such as histamine, kinins,

TABLE III. Distribution of positive skin test end points ($\geq 1+$) with individual Hymenoptera venoms in 85 allergic and 56 nonallergic individuals

Venom	Group	Venom concentrations ($\mu\text{g/ml}$)					
		0.001	0.01	0.1	1.0	10*	Neg
Honeybee	Allergic	12 [†]	8 [†]	16 [‡]	12	1	36
	Nonallergic	1 [†]	0 [†]	1 [†]	18	12	24
Yellow jacket	Allergic	7	12 [†]	21 [†]	16	1	28
	Nonallergic	1	0 [†]	0 [†]	17	8	30
White-faced hornet	Allergic	8 [†]	12 [†]	22 [†]	11	2	30
	Nonallergic	1 [‡]	0 [†]	1 [†]	13	15	26
Yellow hornet	Allergic	5	12 [†]	13 [†]	11	1	43
	Nonallergic	1	0 [†]	0 [†]	12	11	32
<i>Polistes</i>	Allergic	9 [‡]	10 [†]	12 [†]	11	3	40
	Nonallergic	1 [‡]	0 [†]	1 [†]	15	9	30

*Only nine allergic patients were tested with 10 $\mu\text{g/ml}$.[†] $p < 0.05$.[‡] $p < 0.10$.

melittin, phospholipases, and hyaluronidase.^{5,6} These biologic amines, polypeptide toxins, and enzymes can cause immediate wheal-and-flare reactions. Thus, it is critical to distinguish between immunologically mediated reactions and a reaction caused by the direct pharmacologic action of these agents. Prior recommendations have suggested that venom concentrations of $>1.0 \mu\text{g/ml}$ cause irritative skin reactions. Thus, the $1.0 \mu\text{g/ml}$ has been used as the cutoff points to distinguish potentially allergic patients from nonallergic individuals.

In this study most insect-allergic patients had positive skin tests with venom concentrations of $0.1 \mu\text{g/ml}$ or less. Inclusion of the $1.0 \mu\text{g/ml}$ concentration resulted in positive tests in an additional seven patients whose tests were negative at the lower venom concentrations. Some patients with positive histories of stinging-insect anaphylaxis had negative skin tests. Other investigators have reported similar findings in patients with negative venom skin tests.¹⁰⁻¹⁹ As a point of comparison, Schuberth et al.¹¹ found negative venom skin tests in 27 of 235 children (11%) evaluated for insect allergy. In addition, the multicenter insect study conducted by the American Academy of Allergy and Immunology¹⁹ also noted a similar frequency of patients with negative skin tests. These negative reactions could reflect a loss of sensitivity during time or these patients may be a select minority of individuals without detectable venom-specific IgE antibody, even when examined shortly after sting anaphylaxis. In total, these studies confirm the sensitivity of intradermal skin tests with venoms as an appropriate diagnostic procedure in the detection of potentially allergic individuals.

By contrast, insect-nonallergic patients exhibited a high frequency of positive skin tests at the $1.0 \mu\text{g/ml}$ venom concentration. Venom-specific IgE could not be detected in the sera of those individuals with positive skin tests. Nineteen of the 56 insect-nonallergic patients reported a history of an accidental insect sting without reactions. The incidence of positive skin tests in the "stung" patients was no different from that in the total group. Thus, in these insect-nonallergic individuals, the positive skin tests must be interpreted as irritative or nonspecific, rather than indicating a specific IgE pathogenesis.

There have been multiple other studies of venom skin tests that have included insect-nonallergic subjects. This present study uses the largest number of control subjects. The findings in our studies agree with those of Schwartz et al.¹⁹ and Miyachi et al.²⁰ in which a small but significant percentage (12% and 29%) of normal individuals have positive skin tests at the $1.0 \mu\text{g/ml}$ concentration. Hunt et al.⁴ found one of 30 nonallergic subjects with a positive skin test. In several other studies²¹⁻²³ control subjects have not reacted at this concentration; however, these studies included very few control subjects. Golden et al.²⁴ reported positive venom skin tests in 12% of 147 insect-nonallergic individuals, although specific information regarding the dose eliciting the positive response is not presented. The higher incidence of positive reactions (26 of 56 patients, 46%) to at least one venom in our insect-nonallergic patients may be related to the increased incidence of atopy in this population.

These results suggest that individuals reacting only to a venom concentration of $1.0 \mu\text{g/ml}$, particularly with a mild reaction, require careful clinical evaluation

and in vitro studies (RAST) to define their allergic status. Although the skin test is usually considered more sensitive than the RAST for detection of specific IgE, on occasion patients with borderline skin test reactions have elevated serum IgE antibodies. On the basis of our data, we are currently recommending that the results of skin tests with 0.1 µg/ml venom concentration is a more appropriate cutoff point in the evaluation of the insect-allergic patient.

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