

Diagnosis of Allergy to Stinging Insects by Skin Testing with Hymenoptera Venoms

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Skin testing was done on 30 patients with a history of anaphylactic reactions after a Hymenoptera sting and on 30 control subjects. The patients all had positive basophil-histamine release to one or more venoms on challenge with the specific venoms used for skin testing (honey bee, yellow jacket, white-faced hornet, yellow hornet, and *Polistes*). At 0.1 μ g of venom/ml and at 1.0 μ g of venom/ml, 75% and 100%, respectively, of the sensitive patients had a positive skin test. There was a significant ($P < 0.001$) correlation between skin test and histamine release results. Of the 150 skin tests in control patients, only 1 was positive. Venom skin tests provide, for the first time, a simple, readily available technique to accurately diagnose allergy to stinging insects.

TECHNIQUES for the diagnosis of anaphylactic sensitivity to Hymenoptera stings have been difficult to establish. After the work of Benson and Semenov (1) in 1930, there were a number of uncontrolled reports that skin test reactivity to whole body extracts of Hymenoptera correlated well with the severity of the sting reaction (2). In two controlled studies it was found, however, that skin tests using the only commercially available diagnostic reagents, whole body extracts of Hymenoptera, did not discriminate between hypersensitive patients and control subjects (3, 4). Consequently, the diagnosis of anaphylactic sensitivity to Hymenoptera stings, in the estimated 0.4% to 0.8% of various population samples reporting systemic reactions after a sting, is left to the history alone (5, 6). If the experience with skin testing in penicillin allergy can be used as a comparison, 66% to 85% of patients reporting reactions suggestive of anaphylactic sensitivity fail to show persistent sensitivity when skin tested and challenged (7, 8). This has important therapeutic implications, since many of these people live in fear of another sting or are begun on a lifelong treatment regimen that may not be entirely effective (9-12).

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In an attempt to clarify this diagnostic situation, we used an in-vitro model of human allergic reactions, IgE-mediated histamine release from human basophils. In this system, cell-bound IgE antibodies to venom proteins trigger the release of histamine, which is measured fluorometrically. We showed that, in most patients with a clear history of systemic reactions to Hymenoptera stings, IgE antibodies were present to mediate the release of histamine from basophils, and that sensitive patients could be clearly distinguished from normal subjects (13). This distinction, however, was not possible until the venoms of the various Hymenoptera species were used. Commercially available whole body extracts of Hymenoptera species failed to demonstrate histamine release significantly different from that which occurred in normal control subjects. We also confirmed previous reports that skin tests with the whole body extracts failed to distinguish between allergic patients and normal control subjects.

Since results obtained by histamine release have consistently correlated with skin tests results in pollen-sensitive patients (14), it seemed important to explore the diagnostic usefulness of skin testing with Hymenoptera venoms. Our study shows the correlation of skin tests and histamine release using Hymenoptera venoms. More importantly, it documents the ability of venom skin tests to clearly discriminate between Hymenoptera-allergic patients and control subjects.

Materials and Methods

Thirty patients were selected who, after an insect sting, experienced one or all of the following: [1] generalized urticaria or angioedema, or both; [2] respiratory difficulty due either to laryngeal edema or bronchospasm; and [3] vascular collapse, with or without the loss of consciousness. These reactions developed within minutes of the sting, were severe, and required emergency treatment by a physician. In addition, all patients studied had 50% histamine release to at least one venom. The reaction to the insect sting occurred at least 2 weeks before skin testing or histamine-release studies, and in most cases within the preceding 2 to 6-month period. Thirty volunteers with no history of Hymenoptera sensitivity served as control subjects. Fifteen of these controls were atopic, that is, they had seasonal allergic rhinitis with positive skin tests to ragweed or grass pollen, or both. Informed consent was given by patients and control subjects before study.

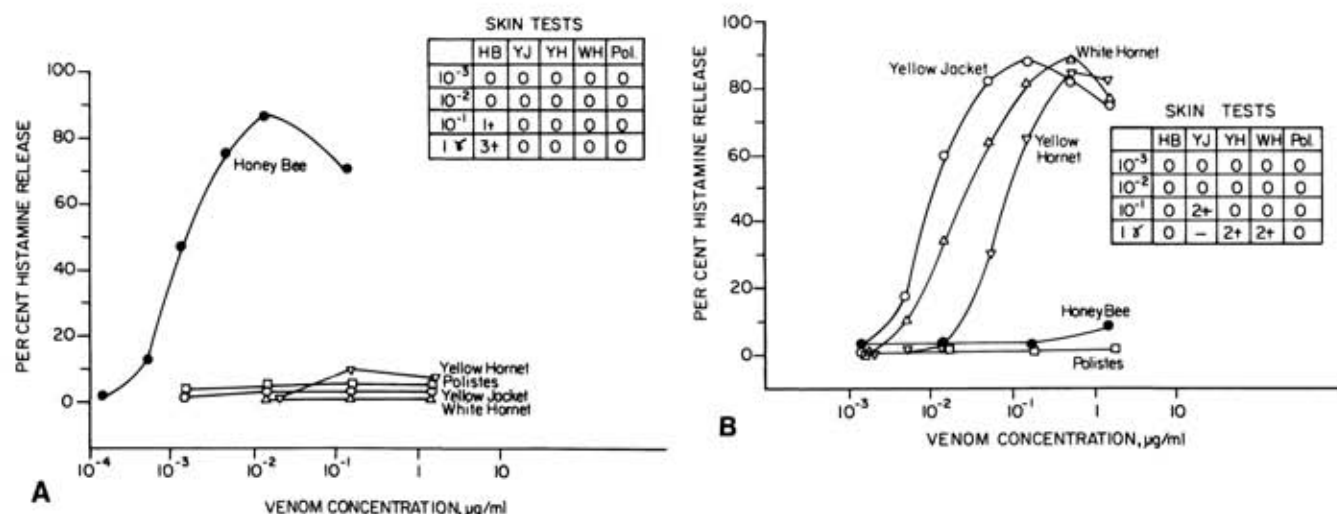


Figure 1. Histamine release-dose response curves compared with the skin test results in two patients. **A.** The patient is sensitive only to honey bee venom. **B.** The patient is sensitive to the three vespid venoms.

The antigens used for skin testing and histamine release included the individual venoms of honey bee, yellow jacket, yellow hornet, white-faced hornet, and a species of the *Polistes* paper wasp. These venoms appear to be responsible for more than 95% of reported reactions to Hymenoptera stings (15). Honey bee venom was collected by electrical stimulation (16) and the other venoms by extraction from crushed venom sacs. Materials so obtained behaved identically in histamine-release studies to those obtained by micropipet from the venom sac. The venoms were lyophilized after collections, weighed, diluted in Tris-buffered saline at pH 7.6, and stored as 10 mg/ml solutions at -20°C until used. Each month venoms were thawed, and serial dilutions using Tris-buffered saline with 0.03% human serum albumin were made for skin testing.

Determination of hypersensitivity to venoms by histamine release was carried out as previously reported (13). After preliminary scratch tests were found to be negative, direct skin testing of patients and control subjects was carried out by the intradermal injection of 0.05 ml of test solution. The starting concentration of the venoms was 1 ng solid/ml and was increased in tenfold increments to 1 $\mu\text{g}/\text{ml}$ or until a positive skin test was obtained. Skin reactions were evaluated at 15 minutes, and a positive skin test was defined as any reaction graded 1+ or more, that is, wheal, 5 to 10 mm, and erythema, 10 to 20 mm.

Results

Figure 1 shows two representative dose response curves of a patient's leukocytes challenged with increasing concentrations of the five Hymenoptera venoms. Shown in the inset are the corresponding skin test results. The first patient (Figure 1A) was sensitive only to honey bee venom; 50% histamine release was obtained at 10^{-3} $\mu\text{g}/\text{ml}$ and a 1+ positive skin test at 10^{-1} $\mu\text{g}/\text{ml}$. The second patient (Figure 1B) showed sensitivity to three of the five venoms tested—yellow jacket, yellow hornet, and white-faced hornet. Again, the histamine-release data were paralleled by positive and negative skin responses. The correlation between the results of histamine release and skin tests with the five venoms in 30 hypersensitive patients was satisfactory ($r_s = 0.6$, $P < 0.001$), as indicated in Figure 2. This correlation is exclusive of data obtained when a patient did not have 50% histamine release to a particular venom, remembering a patient may be sensitive to only one venom.

If the double negative data were included, the correlation coefficient would increase to 0.92, $P < 0.001$. Control subjects are not shown since no control tested released 50% of his cellular histamine at any concentration of venom used.

There were four patients, each sensitive to more than one venom, who had a positive skin test to a particular venom but did not have a corresponding 50% histamine release. By history, these patients did not differ from the others, and, while we have no exact explanation for this finding at present, this phenomenon has been previously described with grass and ragweed extracts (14, 17). In no instance was histamine release obtained when skin tests were negative. The data obtained from skin testing 30 patients who were Hymenoptera hypersensitive and 30

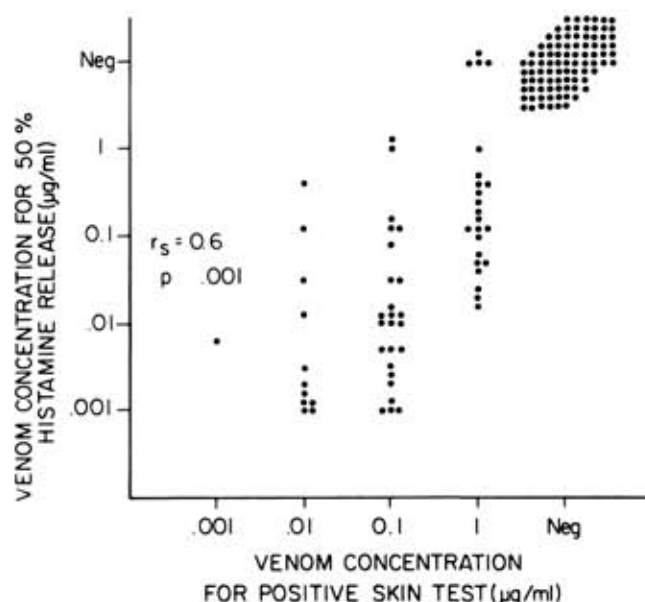


Figure 2. Correlation of 50% histamine release with positive skin test in sensitive patients using Hymenoptera venoms.

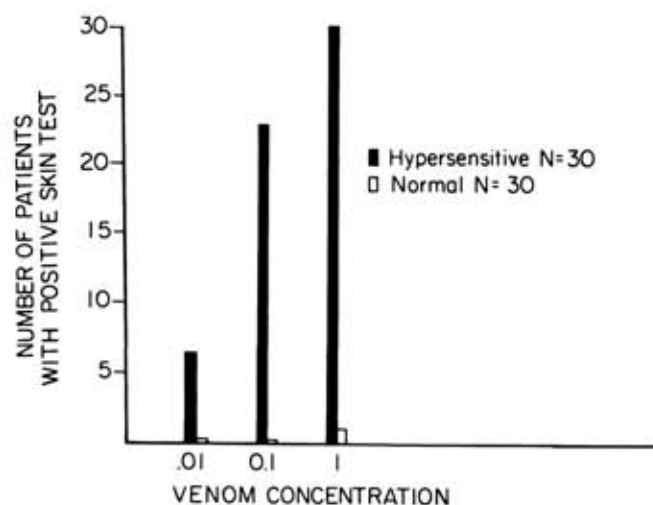


Figure 3. A comparison of skin test results with Hymenoptera venoms in hypersensitive patients and control subjects.

control subjects with the five venoms are presented graphically in Figure 3. Twenty-three of the hypersensitive patients had a positive skin test to one or more of the venoms at a concentration of 0.1 µg/ml, and all 30 patients had a positive skin test to at least one venom at the 1 µg/ml concentration. Only 1 control subject in 30, an atopic person, had a single positive skin test.

Discussion

The inability to diagnose patients anaphylactically sensitive to Hymenoptera stings by skin testing with whole body extracts represents a major deficit in our ability to cope with the potential 1 000 000 Americans at risk. It also led us to question the pathogenesis of this entity, that is, were these reactions truly allergic in the sense of being mediated by IgE antibody? In the case of penicillin allergy, it has been shown that the reliability of skin testing is critically dependent on using the correct antigens for testing (18). Once these were identified, the presence of IgE antibodies to penicillin and its metabolites could be regularly identified by skin test (7). In the case of Hymenoptera sensitivity, once it was established that sensitive patients had IgE antibody against venom antigens and not to the whole body extract proteins, it seemed highly probable that venom antigens would prove to be diagnostically useful.

Because of the novelty of these reagents, they were first evaluated in vitro using histamine-release techniques. Since this methodology is complex, expensive, and not generally available, the next step was to carry out skin testing studies. Our report indicates unequivocally that the venom skin test results correlate with the histamine-release data, and that these reagents are highly useful for diagnostic purposes.

During the course of this study, it was found that dialysis of the venoms did not alter histamine release or skin test results. To rule out the possibility of excluding an appropriate antigen, nondialyzed venoms were used. When venom concentrations of 10 µg/ml and 100 µg/ml were used for skin testing, the difference between hypersensitive

patients and control subjects became less marked. Both groups showed a wheal and flare response to all venoms at 100 µg/ml. We believed that this represented a toxic response to the venoms at concentrations that approach those of a natural sting—about 50 µg.

It is difficult to overstate the clinical importance of these observations. The inadequacy of present skin test materials has limited the physician's ability to gauge correctly the presence of anaphylactic sensitivity and to recommend appropriate therapy. In this situation, allergists have relied on years of clinical experience as their basis for recommending desensitization with mixed Hymenoptera whole body extracts for any patient having a history of a systemic reaction to an insect sting. This, combined with the use of emergency kits containing epinephrine for injection, has kept mortalities to a minimum and will need to be used as necessary until venom materials become available.

Hymenoptera venoms prepared under a contract supported by the National Institute of Allergy and Infectious Diseases will be available to some of that institute's Allergic Disease Centers for further uses as a diagnostic material in 1976. Once the patients at risk are better defined, then a more rational therapeutic plan can be devised. Unnecessary fear and treatment regimens hopefully can be obviated. Development of venom preparations for immunotherapy is presently underway and should be of additional assistance in the management of Hymenoptera hypersensitivity (9, 11).

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