

Allergy to insect sting

III. Allergenic cross-reactivity among the vespid venoms*

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Recent reports have indicated that venoms may be more beneficial than whole body extracts for the diagnosis and treatment of Hymenoptera sensitive patients. These studies were undertaken to determine the cross-reactivity among the vespid venoms. Eighteen patients who were anaphylactically sensitive to vespid venoms were studied using in vitro leukocyte histamine release. The results (venom concentration for 50% histamine release) were analyzed by linear regression analysis; there was no allergic cross-reactivity between any of the venoms, except for a modest association between yellow hornet and white hornet venom. In spite of this result 13 of the 18 patients studied were sensitive to three or four of the venoms tested. There is no clear explanation for this observation, but it suggests the existence of multiple major allergens in the vespid venoms, some of which are cross-reactive. Since immunotherapy with inappropriate proteins may lead to the development of IgE and the possibility of clinical sensitivity and since the majority of patients were not sensitive to all venom preparations, we suggest that appropriate diagnostic studies be carried out before the institution of therapy.

It has been assumed that allergic reactions to stinging insects are the result of hypersensitivity to an allergen common to most, if not all, of the Hymenoptera species and present throughout the bodies of these insects. This led to the prevailing method of immunotherapy, using mixtures of whole body extracts obtained from honeybee, yellow jacket, hornet, and wasp. Previous work from this laboratory¹ confirming the earlier work of Schwartz² and Bernton and Brown³ has indicated, however, that Hymenoptera-sensitive individuals cannot be diagnosed by skin testing with whole body extracts. This suggested that the allergens to which these patients are sensitive are not represented in the whole bodies but rather, as Loveless and Fackler⁴ have claimed for many years, in the venoms. This has, in fact, been demonstrated by histamine release studies which revealed clear-cut diagnostic differences between Hymenoptera-sensitive patients and controls when studied by challenge with venom, but nondiagnostic results when the challenge was with whole body extracts or venom-free preparations from the head

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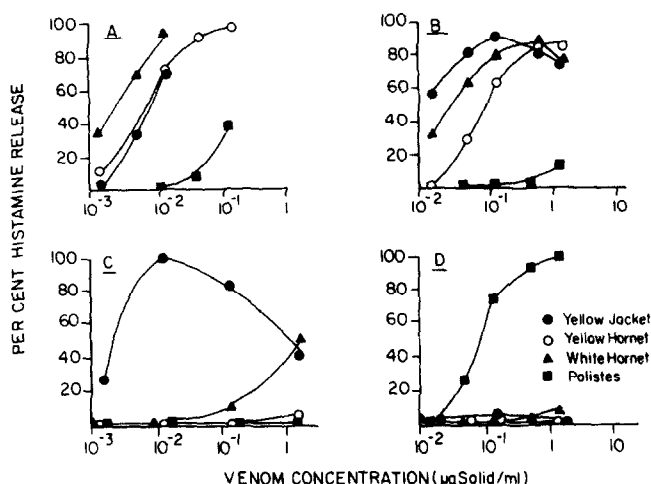


FIG. 1. A-D, Histamine release curves for 4 different patients who are sensitive to 4, 3, 2, and 1 venom, respectively.

and thorax of the insects.¹ More recently, this diagnostic utility of venoms has been confirmed by skin testing with preparations of dialyzed Hymenoptera venoms.⁵

In the course of these studies it became clear that there was no cross-reactivity between honeybee venom and the vespid venoms. This correlates well with the established taxonomy of the Hymenoptera.^{6, 7} Of the vespids (yellow jacket, white hornet, yellow hornet, polistes), three belong to the genus *Vespula* while the fourth, the paper wasp, belongs to the genus *Polistes*. Two species of the *Vespula* (white face hornet and yellow hornet) belong to the subgenus *Dolichovespula*. These are aerial nesters. The ground nesting yellow jackets belong to the subgenus *Vespula*. The taxonomy of this subgenus is extremely complex and its members are indistinctly broken into two species groups, the *Vespula rufa* group and the *Vespula vulgaris* group. The *Vespula rufa* group is normally not a pest of man.

This study was directed to ascertaining the allergenic cross-reactivity of the vespid venoms. Since it is possible to sensitize individuals by immunization with irrelevant proteins⁸ and inasmuch as we believe that venom immunotherapy will prove to be more efficacious than therapy with whole body extracts, it seemed clinically important to address this question.

MATERIALS AND METHODS

Patients

Eighteen patients who had experienced the symptoms of anaphylaxis following a sting by an insect of the *Vespula* or *Polistes* genus of the order Hymenoptera were selected for study. These patients were all the history-positive, histamine release-positive individuals who had been referred to us at the time the study began. Seven of these patients had undergone previous immunotherapy with mixed Hymenoptera whole body extracts. Patients shown to be sensitive only to honeybee venom using in vitro histamine release, as well as a group of laboratory employees with no history of Hymenoptera sensitivity, served as controls.

TABLE I. Venom concentration (μg solid/ml) required for 50% histamine release

Patient	Yellow jacket	Yellow hornet	White hornet	Polistes
1	0.066	0.040	0.037	0.50
2	0.167	0.189	0.055	*
3	0.0028	*	*	*
4	*	*	0.68	0.05
5	0.120	*	*	*
6	0.160	1.6	1.1	6.5
7†	0.010	1.04	0.11	*
8	0.0048	0.37	0.60	0.045
9†	0.051	0.396	0.11	*
10†	0.102	1.08	0.14	0.05
11	0.06	*	*	*
12†	0.13	0.18	0.026	1.3
13	1.8	1.8	*	0.38
14	0.276	*	1.6	*
15†	0.029	0.116	0.057	3.5
16†	0.023	0.119	0.119	0.032
17	0.18	0.18	0.16	1.0
18†	0.026	0.015	0.079	0.76
AV	0.18(17)‡	0.54(13)	0.34(14)	1.28(11)

*Greater than 10.0.

†Patient received whole body extract therapy prior to study.

‡Number of patients.

Antigens

The venoms were obtained by an entomologist (A. W. B.) by dissection of the venom sac and removal of the venom with micropipettes. Venoms from the specific Hymenoptera species were pooled and lyophilized. They were subsequently weighed and suspended in a Tris-buffered solution at 100 $\mu\text{g}/\text{ml}$ and frozen at -20°C . A freshly thawed aliquot was used for each experiment.

Histamine release techniques

Histamine release from the washed leukocytes of allergic and control patients was carried out as has been described previously.¹ The cell preparations were challenged with each venom at concentrations ranging from 10^{-3} to 10 $\mu\text{g}/\text{ml}$ in threefold increments. The concentration of any venom producing 50% histamine release was determined by interpolation from the dose-response curves. In some instances, a patient's cells were completely insensitive to one or more of the venoms, and in others less than 50% histamine release was obtained at the highest concentration used. In these instances, results are plotted on the graphs (Fig. 2) as showing a 50% histamine release at 100 $\mu\text{g}/\text{ml}$. These points were not, however, used in the calculations described below.

Calculations

The concentrations required for 50% histamine release for all possible venom pairs were evaluated by linear regression analysis. The correlation coefficients and their respective significance levels are provided in Fig. 2. As noted, for these calculations only venoms which caused 50% histamine release at a concentration less than 10 $\mu\text{g}/\text{ml}$ were utilized.

RESULTS

Most vespid-sensitive individuals responded to multiple venoms. Examples of patients responding to all four, three, two, or only a single venom are provided in Fig. 1. It may be noted that there is no consistent pattern of activity. Even though most patients react to multiple venoms, the complete data presented in Table I show that as much as one hundredfold differences in sensitivity to the

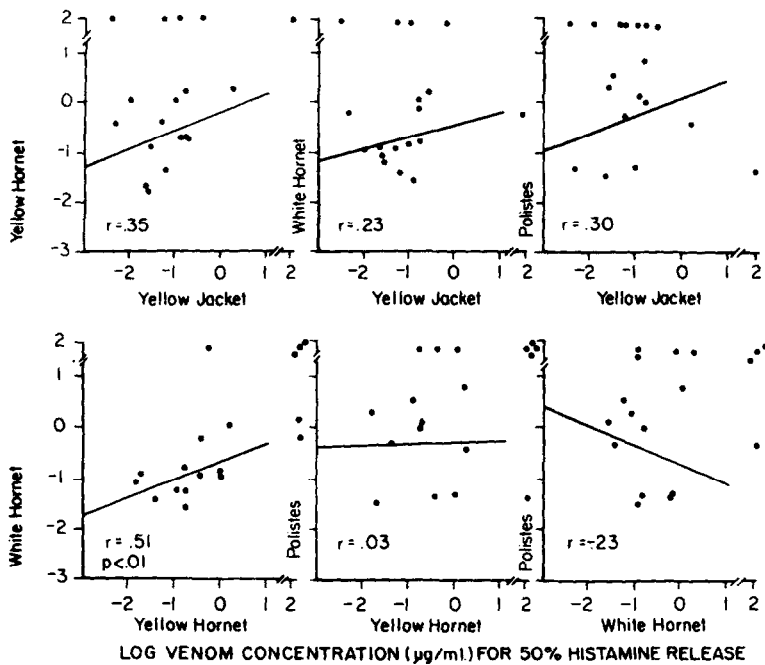


FIG. 2. Logarithm of the venom concentration (μg solid/ml) producing 50% histamine release in each patient is plotted comparing each possible venom pair. A concentration of 100 $\mu\text{g}/\text{ml}$ ($\log = 2$) is assumed in patients not releasing significant histamine to a particular venom. R value and significance level are shown for each venom pair.

different venoms may exist in reactive patients. Only three patients reacted to a single venom, while 13 of the 18 demonstrated significant histamine release to 3, or even 4 venoms. The insect to which most patients were sensitive was the yellow jacket, with 17 out of the total of 18 patients showing $>50\%$ histamine release. The taxonomy of the yellow jacket is complex with some authors claiming that there are multiple species. Without wishing to address this question we have studied the pure venoms of two morphologically distinct "species" of yellow jacket: *V. maculifrons* and *V. squamosa*. In 3 yellow jacket-sensitive patients the reactivity to both venoms was essentially similar.

Yellow hornet and white hornet venoms caused a response in about 70% of the patients, and more than half also responded to the polistes venom. However, only 2 of these 18 patients were found to be sensitive to honeybee venom. Table I also lists the average venom concentrations which caused 50% histamine release in these patients. This ranged from a low of 0.18 $\mu\text{g}/\text{ml}$ for yellow jacket to a high of 1.28 $\mu\text{g}/\text{ml}$ for polistes.

Although most patients were responsive to several of the Hymenoptera venoms, the relative concentration required for a 50% response in different patients showed no consistent pattern. A possible relationship in terms of sensitivity to the several venoms was sought by carrying out linear regression analysis to examine any correlation between the sensitivity of patients to each of the pairs of Hymenoptera venoms. These data are presented in Fig. 2. All of these correla-

tions were insignificant with the exception of a modest association between the activity of yellow hornet and white hornet venoms ($r_s = 0.51$, $p < 0.01$).

DISCUSSION

We have previously utilized techniques similar to those described above to ascertain the relationship between different antigens in pollen extracts. When there were common or cross-reacting antigens (such as antigens E and K in ragweed pollen), there was a highly significant correlation between the concentrations of each antigen required for 50% histamine release from the cells of different patients.⁹ In other instances (such as ragweed antigens E and RA3), while most ragweed-sensitive individuals showed a response to both materials, there was no quantitative relationship between the concentration of each required for a similar response. We concluded, in this latter instance, that there was no cross-reactivity between these two antigens.¹⁰ The sensitivity to both antigens, in this case, was explained by their common existence in ragweed pollen and the uniform exposure of the patients.

Our results in these experiments are more difficult to explain. In carrying out quantitative correlations between the concentration of the various vespid venoms required for 50% histamine release, there was essentially no relationship between any of the venoms with the exception of a modest association between white and yellow hornet venoms. This parallels the taxonomic relationship among this group of insects. Following our previous criteria, therefore, we would conclude that there is little or no allergenic cross-reactivity among the antigens of the different vespid venoms. How then do we explain the fact that most individuals are sensitive to more than one venom? We cannot, in this instance, invoke a common exposure to the multiple antigens since this requires not only that the patients were stung by, but also became sensitive to, multiple non-cross-reacting insect venoms. This seems unlikely.

A possible explanation is that these individuals were originally sensitive to only one insect but, as a result of immunotherapy with mixed whole body extract, were sensitized to the other venoms. (Seven of the 13 patients sensitive to 3 or more venoms had been treated with whole body extract.) Whole body extracts differ from lot to lot and from manufacturer to manufacturer with respect to venom content.¹ It is likely, however, that most preparations contain at least some venom proteins. It is clear that immunization with proteins can lead to sensitivity in virtually all individuals. The degree of sensitization produced inadvertently is likely to be of little significance in pollen immunotherapy where the total seasonal antigenic challenge is nanograms spread over several weeks and delivered to mucosal surface. It may well be, however, of clinical significance in Hymenoptera sensitivity in which the insect sting delivers microgram doses of antigen in a rapid, parenteral fashion.

Perhaps the best explanation for these results is that there is some cross-reactivity among the allergens in the various venoms but that this is multifactorial. That is, if each venom contains multiple antigens to each of which the patients can develop differential sensitivity, one might obtain the type of results depicted in Fig. 2. This would mean that the vespid venoms are more complex than honey-

bee venom, which contains one principal allergen, phospholipase-A, a single secondary antigen, hyaluronidase, and mellitin, which is at best very weakly antigenic. This question, unfortunately, can be definitely answered only when each of the venoms has been analyzed by chromatographic techniques to isolate and purify each of the allergenic proteins. Since this will require a considerable volume of work over many years, it is appropriate to address ourselves to the question of how we can utilize these data at the present time. As indicated above, we believe that immunotherapy with venoms is likely to prove superior to that with whole body extracts. A number of patients who were treatment failures with whole body extract were protected after venom therapy, and this correlates well with their increase in blocking antibody against the venom antigens.⁵ In those instances in which venom immunization is to be carried out, it is clearly necessary that diagnostic studies of a quantitative nature be done with each of the venoms to which the patient is potentially sensitive. Since our data show that more than half of the individuals are not sensitive to all four venoms and, indeed, that some are sensitive to only a single venom, a specific immunization mixture must be prepared for each patient. Even if immunotherapy with whole body extracts is to be utilized, the possible clinically important sensitization by the use of irrelevant insect preparations demands appropriate diagnostic studies.*

*Appropriate diagnostic reagents for testing Hymenoptera-sensitive patients are being prepared for the Allergic Diseases Centers under a contract let by the National Institute of Allergy and Infectious Diseases, National Institutes of Health.

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