

Allergens in Hymenoptera venom XV: The immunologic basis of vespid venom cross-reactivity

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RAST-inhibition studies were performed by use of whole venom sac extracts to inhibit binding to purified venom allergens from various vespid wasps. Immunodiffusion studies were also performed with rabbit antisera raised against purified venom proteins. Immunologic cross-reactivity was demonstrated among the hyaluronidases, phospholipases, and antigen 5s from both subgenera of yellow jackets, white-faced hornets, and paper wasps. The paper wasp hyaluronidase and antigen 5 were less related to those of the other three species than those of yellow jackets and hornets. It appears that immunologic cross-reactivity is the major mechanism of multiple allergic sensitivity to vespid venoms. Therapy with only the primary venom should be sufficient to protect against reactions from cross-reacting venoms. (J ALLERGY CLIN IMMUNOL 75:611-13, 1985.)

Most patients who are allergic to vespid wasp venoms react by skin test and RAST to multiple venoms.¹⁻⁴ Although in some cases this reactivity may have been caused by insect stings from multiple species,¹ it is generally thought to be due to immunologic cross-reactivity among the venom allergens from the various species of vespids.^{2,4} Previously we have demonstrated the occurrence of multiple sensitization in a few cases and more commonly immunologic cross-reactivity by use of RAST-inhibition studies.⁴ Recently we demonstrated that in some cases HYAL from *Vespula maculifrons*, the eastern yellow jacket, can cross-react with honeybee venom HYAL.⁵

The availability of purified allergens from several species of vespids including *V. maculifrons*, *V. squamosa*, southern yellow jacket, *Dolichovespula maculata*, white-faced hornet, and *Polistes exclamans*, paper wasp (one species),^{5,7} allows the investigation of cross-reactivity among the various protein allergens for a representative selection of species of vespid wasps. Since a large degree of variability has been noted in the allergen reactivity pattern of different sera,^{5,7} all studies were carried out with serum from individual patients.

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Abbreviations used

HYAL: Hyaluronidase
PL: Phospholipase
Ag: Antigen 5

MATERIAL AND METHODS

Venom allergens. The preparation of the protein fractions from the four species of wasps studied has been described in detail.^{5,6}

Allergen disks. Cyanogen bromide activated paper disks were prepared and coupled as previously described for both purified allergens and venom sac extracts.^{5,6,8}

RAST inhibition. RAST inhibition was performed as previously described.^{4,8} The sera were incubated with inhibitor for 1 hr before disks were added. Results are expressed as percent inhibition.

Patient sera. Sera were collected from patients allergic to vespids and stored frozen until their use. The individual sera in this study were selected from the panels used in our previous study⁷ in order to have strong reactivity with the allergen being studied. All sera used elicited from 5.0% to 20.0% specific binding at the dilution used for RAST inhibition. Since no sera were found that bound strongly to *Polistes* HYAL, it was not possible to study this antigen.

Rabbit antisera. New Zealand white rabbits were immunized intradermally with purified protein fractions emulsified in Freund's complete adjuvant as we have described previously.⁵ Immunodiffusion and immunoelectrophoresis were carried out by standard methods.⁵

TABLE I. RAST inhibition of binding to purified vespid venom allergens by venom sac extracts

Serum	Inhibitor	% Inhibition
<i>D. maculata</i> HYAL		
3440	<i>V. squamosa</i>	66
	<i>V. maculifrons</i>	47
	<i>D. maculata</i>	42
	<i>P. exclamans</i>	0
5802	<i>V. squamosa</i>	82
	<i>V. maculifrons</i>	80
	<i>D. maculata</i>	94
	<i>P. exclamans</i>	63
<i>D. maculata</i> PL ₁		
2377	<i>V. squamosa</i>	88
	<i>V. maculifrons</i>	89
	<i>D. maculata</i>	85
	<i>P. exclamans</i>	99
8568	<i>V. squamosa</i>	88
	<i>V. maculifrons</i>	83
	<i>D. maculata</i>	82
	<i>P. exclamans</i>	76
<i>D. maculata</i> PL ₂		
8568	<i>V. squamosa</i>	75
	<i>V. maculifrons</i>	90
	<i>D. maculata</i>	95
	<i>P. exclamans</i>	86
<i>D. maculata</i> Ag 5		
9230	<i>V. squamosa</i>	34
	<i>V. maculifrons</i>	26
	<i>D. maculata</i>	96
	<i>P. exclamans</i>	99
9775	<i>V. squamosa</i>	74
	<i>V. maculifrons</i>	84
	<i>D. maculata</i>	99
	<i>P. exclamans</i>	57
<i>P. exclamans</i> PL		
6750	<i>V. squamosa</i>	10
	<i>V. maculifrons</i>	84
	<i>D. maculata</i>	-10
	<i>P. exclamans</i>	46
6642	<i>V. squamosa</i>	29
	<i>V. maculifrons</i>	73
	<i>D. maculata</i>	21
	<i>P. exclamans</i>	94

PL₁ = phospholipase isozyme 1; PL₂ = phospholipase isozyme 2.**RESULTS**

Rabbit antisera raised against the purified protein fractions of *V. squamosa*, *D. maculata*, and *P. exclamans* venoms were tested by immunodiffusion against the purified protein antigens from *V. maculifrons*, *V. squamosa*, *D. maculata*, and *P. exclamans* venoms. Cross-reactions were observed among the HYALs from both *Vespula* and *Dolichovespula* but

TABLE I. Cont'd

Serum	Inhibitor	% Inhibition
<i>P. exclamans</i> Ag 5		
3913	<i>V. squamosa</i>	56
	<i>V. maculifrons</i>	66
	<i>D. maculata</i>	76
	<i>P. exclamans</i>	95
5339	<i>V. squamosa</i>	53
	<i>V. maculifrons</i>	67
	<i>D. maculata</i>	86
	<i>P. exclamans</i>	86
<i>V. squamosa</i> HYAL		
8254	<i>V. squamosa</i>	41
	<i>V. maculifrons</i>	48
	<i>D. maculata</i>	67
	<i>P. exclamans</i>	4
<i>V. squamosa</i> PL		
7994	<i>V. squamosa</i>	58
	<i>V. maculifrons</i>	65
	<i>D. maculata</i>	-15
	<i>P. exclamans</i>	-18
12041	<i>V. squamosa</i>	55
	<i>V. maculifrons</i>	31
	<i>D. maculata</i>	18
	<i>P. exclamans</i>	-2
<i>V. squamosa</i> Ag 5		
8317	<i>V. squamosa</i>	90
	<i>V. maculifrons</i>	88
	<i>D. maculata</i>	94
	<i>P. exclamans</i>	76
9079	<i>V. squamosa</i>	89
	<i>V. maculifrons</i>	86
	<i>D. maculata</i>	57
	<i>P. exclamans</i>	35

not with *Polistes*. The PLs were cross-reactive among all four species. There was an incomplete identity between the two isozymes of *D. maculata* PL. The Ag 5s demonstrated cross-reactions among the *Vespula* and *Dolichovespula* but not with *Polistes*. The other fractions of the venoms were found to contain distinct proteins and in some cases to contain proteins related to PL or Ag 5.

RAST inhibition was performed at only a single point for each combination because of limited availability of materials. The point chosen was 20 µg of venom sac extract, which typically could inhibit binding to whole venom from 80% to 95% in previous studies.⁴ The disks were prepared with the purified allergen fractions at 0.2 µg per disk to minimize interference by impurities. The complete inhibition results are illustrated in Table I. All four venom HYALs exhibited cross-reactivity with serum 5802, but only the *Vespula* and *Dolichovespula* exhibited cross-reac-

tivity with sera 3440 and 8254. The PLs exhibited cross-reactivity with all four venoms for three sera with just the *Vespula* in two sera and between *Vespula maculifrons* and *Polistes* in one serum. This is strongly suggestive of the presence of multiple determinants on the PL molecules. It appears that a determinant present on *V. maculifrons* PL is absent from *V. squamosa* PL. The Ag 5s exhibited a variable degree of cross-reactivity among all four species with all six sera studied.

In some cases the homologous venom was not the most potent inhibitor. This could be the result of several factors. The concentrations of individual components differ widely among the venom sac extracts; the inhibitors were standardized only by total protein content. It is possible that the primary specificity of the serum was incorrectly assigned and that the sensitizing insect was really the one that is the more potent inhibitor. The cross-reactive determinant may be present in higher effective concentration in a venom other than the sensitizing venom. Problems of this nature make the definitive assignment of specificities impossible among the vespids.

It was possible to obtain over 50% inhibition of binding to each of the purified allergens with each of the sera studied at the concentration of venom sac inhibitor used. The differences in inhibition results for different sera with the same purified allergen illustrate the heterogeneity of the human IgE response.

DISCUSSION

The results presented in this article demonstrate that there is an immunologic basis for the cross-reactivity observed among vespid wasp venoms in allergic patients. The HYALs, PLs, and Ag 5s can all exhibit cross-reactivity with human IgE antibodies. The variable patterns of reactivity observed in this and previous studies^{1, 4, 5-7} demonstrate that there are multiple allergenic determinants on these molecules. We did not investigate the cross-reactivities among the various other allergenic protein fractions of vespid venoms in this study.

Although the immunodiffusion studies with rabbit antibodies are not strictly relevant to the specificities of human IgE antibodies, in this case the results are similar to those obtained from RAST inhibition. The immunodiffusion studies demonstrate the relatively close relationship among the HYALs, PLs, and Ag 5s from vespids. The rabbit studies did indicate that the

Polistes HYAL and Ag 5 were somewhat different from the *Vespula* and *Dolichovespula* antigens.

In a few of the cases in Table I, negative inhibition percentages are reported. This is usually due to the presence of soluble aggregates in the inhibitor that bind to the free sites of antibody attached to the disk and contain other allergens not present on the disk.

The observation of true immunologic cross-reactivity of human IgE antibodies with a variety of vespid allergens clearly demonstrates that the multiple reactivity commonly observed in vespid allergic patients is usually a result of cross-reactivity and only rarely a result of multiple independent sensitization. The natural conclusion from these observations and the studies previously reported^{4, 9, 10} is that therapy with only the major venom should be sufficient to protect the patient from reactions caused by stings from insects whose venom is cross-reactive.⁹

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