
Allergens of Hymenopteran Venoms

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Human reactions to Hymenoptera stings range from transient local redness, swelling, and pain to generalized pruritus, urticaria, bronchospasm, and shock. Because venoms contain toxic and irritating materials, it was at one time suspected that their effects were not immunologic at all, and that reactions that seemed allergic might really be due to histamine and vasoactive peptides present in venoms.¹ Elucidation of the mechanism of systemic reactions to stings did not occur until the importance of IgE to immediate hypersensitivity was recognized²; only after sufficient quantities of venoms were made available to clinical and laboratory studies was it possible to demonstrate the allergic nature of systemic sting reactions.³

For some years the only material available to physicians for treating insect sting allergy were extracts prepared from the whole, crushed bodies of insects. Several investigators pointed to the diagnostic inadequacy of such extracts,^{4,5} while others described their therapeutic inadequacy.^{6,7} Venoms were shown to trigger histamine release from the basophil leukocytes of sting-allergic patients *in vitro*, and passive sensitization and absorption experiments confirmed that the reaction depends on IgE antibodies.² Subsequently, high titers of IgG antibodies to honeybee venom were found in frequently stung professional beekeepers, and immunization of honeybee-allergic patients with honey bee venom resulted in an increase of IgG antibodies to venom.³ Leukocyte histamine release and skin test studies showed that allergy to Hymenoptera venoms was more or less specific at the genus or subgenus level (Table 1),^{8,9} although, as noted below, recent studies have shown cross-reactivity between certain venoms. After the superior diagnostic and therapeutic efficacy of venoms was demonstrated,^{10,11} a limited panel of venoms was approved for use in man in 1979. Paradoxically, the outmoded whole body extracts (WBE) are still for sale. This is presumably because, although ineffectual, they are relatively inexpensive; and because they lack appreciable venom content, their administration is usually free from allergic reactions. The mistaken notion that the WBEs are therapeutically effective appears to be based on 2 factors: 1) the previously unappreciated natural decline in venom sensitivity that

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Table 1. Taxonomy of Honeybees, Hornets, Wasps, and Yellowjackets

Order	Hymenoptera			
Superfamily	Vespoidea		Apoidea	
Family	Vespidae			Apidae
Subfamily	Vespinae		Polistinae	Apinae
Genus	<i>Vespula</i> Thomson		<i>Vespa</i>	<i>Polistes</i>
Subgenus	<i>V sensu strictu</i>		<i>Dolichovespula</i>	<i>Apis</i>
Species*	<i>V germanica</i> <i>V maculifrons</i> <i>V vulgaris</i> <i>V squamosa</i>	<i>D maculata</i> <i>D arenaria</i>	<i>P annularis</i> <i>P exclamans</i> <i>P fuscatus</i>	<i>A mellifera</i>
Common name	Yellow jackets	Hornets	Wasps	Honeybees

*A partial list of known species is given above.

occurs in most venom-allergic patients, even without venom immunotherapy; and 2) the fact that the risk of repeat sting reaction in patients with histories of prior reactions and positive skin tests is not 100%, but closer to 50% or 60%.^{11,12}

The venom antigens discussed in this paper are found in the commercially available venoms: honeybee, yellow jackets, yellow hornet, white-faced hornet, and *Polistes* paper wasps. Although most reactions to insect stings are due to these insects, other Hymenoptera venoms (eg, imported fire ant and bumble-bee) have clinical importance but are neither available in sufficient quantity nor approved for clinical use. Of the insect venoms that cause allergy in man, only the venom antigens from honeybees and vespids have been sufficiently characterized for immunochemical studies. In the following paragraphs, the known immunochemical properties of these venom antigens will be reviewed.

Classification of Hymenoptera

The taxonomy of the honeybee and the vespids is given in Table 1.^{13,14} The vespids include hornets, wasps, and yellow jackets. In this review, we designate as hornets those insects belonging to the genera *Dolichovespula* and *Vespa*; as wasps, those belonging to the genus *Polistes*; and as yellow jackets,

those of the genus *Vespula*. However, there is disagreement on the use of these common names for insects of different genera.¹⁵

Venom Constituents

The known proteins and peptides in the venom of honeybees^{16–18} and vespids^{19–22} are listed in Table 2. All venom proteins can elicit specific IgE responses in sensitive individuals, although to varying degrees. No venom peptide is known to be immunogenic in man, except for melittin in bee venom. The venom peptides have kinin,²³ mast cell degranulating, or neurotoxic activities,^{16,24} while the venom proteins have acid phosphatase, hyaluronidase, and phospholipase activities.^{16–22}

Honeybee Venom Allergens

The components of honeybee venom have been most thoroughly characterized.¹⁶ About 80% of the venom solids can be accounted for as the proteins and the peptides given in Table 2.

All bee venom-sensitive persons have IgE and IgG antibodies specific for these bee venom proteins: phospholipase A₂, hyaluronidase, and acid phosphatase. In most patients the antibodies with the highest concentrations are specific for the phospholipase A₂ component. About one-third of bee venom-sensitive persons also have antibodies specific for the peptide component melittin.^{17,25}

Bee venom phospholipase A₂ is a basic glycoprotein of 128 amino acid residues, and its primary structure is known.²⁶ Phospholipase A₂ is widely distributed in all living systems. The amino acid sequences of phospholipase A₂ from several sources, such as bovine, human, and porcine pancreas and snake venoms, are known.^{27,28} In Table 3 the amino acid sequences of phos-

Table 2. Known Protein and Peptide Components in the Venoms of Honeybee and Vespids

	Honeybee Venom		Vespid Venoms	
	MW	% of Venom	MW	% of Venom
Proteins				
Phospholipase A ₂	15,800	12		0
Phospholipase A ₁		0	37,000	6–14
Hyaluronidase	45,000	2	43,000	1–3
Antigen 5		0	23,000	3–10
Acid phosphatase	49,000	NA	49,000	NA
Peptides				
Melittin	2840	50		0
Apamin	2038	2		0
Mast cell degranulating peptide	2593	2	1600	NA
Kinin		0	1200	NA

NA = not available, and MW = molecular weight.

Data for honeybee venoms are taken from references 16 to 18, and for vespid venom proteins from references 19 to 22.

Table 3. Amino Acid Sequences of Phospholipases A₂ from Bovine, Human Pancreas, and Honeybee Venom.^a

	10	20	30	40	50
Ox	A L W Q F N G M I K C K I P S S E P L L D F N N Y G C Y C G L G G S G T P V D D L D R - - - - - C				
Man	A V W Q F R K M I K C V I P G S D P F L E Y N N Y G C Y C G L G G S G T P V D E L D K - - - - - C				
Bee	I I - - - Y P G T L - - - - - W C G H G N K S S G P N E L G R F K H T D A C				
					
		60	70	80	90
Ox	C Q T H D N C - - - - - Y K Q A K K L D S C K - - - V L V D N P Y T N N Y S Y S C S N				
Man	C Q T H D N C - - - - - Y D Q A K K L D S C K - - - F L L D N P Y T H T Y S Y S C S G				
Bee	C R T H D M C P N V M S A G E S K H G L T D T A S R L S - C N N N D F Y K N S A D T I S S Y F V G K				
					
		110	120	130	140
Ox	T E I - - - - T C S S E N N A - - - C E A F I C N C D R N A A I C F S K V P Y N K E H K N L D - K - K N C				
Man	S A I - - - - T C S S K N K E - - - C E A F I C N C D R N A A I C F S K A P Y N K A H K N L D T K - Y S C Q				
Bee	M Y F N L I N T K C Y K L E H P V T G C G - - - - E R T E G R C L H Y T V D K S K P K V Y Q W F D L R K Y				

^aData are taken from references 26 to 29. Amino acid residues common to human and bee enzymes are indicated by bullets.

pholipase A₂ from bovine and human pancreas and from bee venom are provided. The bovine enzyme contains 122 amino acid residues, while the human enzyme has 123 residues. To have maximum alignment of the sequences of the bovine, human, and bee enzymes, it is necessary to assume that portions of their sequences have either been added to or deleted from that of a common ancestral enzyme during evolution.²⁹ When compared on that basis, about two-thirds of the amino acid residues of the human and the bovine enzymes are common to each other, while slightly less than one-fifth of the residues of the human and the bee enzymes are in common.

In the aligned sequences shown in Table 3, the 8 cysteine residues of bee venom enzyme occupy exactly the same positions as 8 of 12 cysteine residues of the mammalian venom enzymes. However, only 2 of the 8 cysteine residues of bee venom enzyme have the same disulfide bond-pairing scheme (residue 8 to 120) as that of the mammalian venom enzymes, while the remaining 6 cysteine residues have different pairing schemes. Therefore, the bee venom enzyme may have a conformational structure that is different from that of the human enzyme.

Because of these structural differences, the bee venom phospholipase A₂ will appear foreign to the immune system of man. Therefore, it is not surprising that the bee venom phospholipase A₂ can be a potent immunogen in man. Porcine insulin differs from human insulin in only 1 amino acid residue out of a total of 51 residues. This degree of structural difference between the antigen and the homologous host protein is sufficient to evoke an immune response in susceptible persons.

The antigenic regions of bee venom phospholipase A₂ that are involved in specific IgE and IgG antibody responses in man have not yet been identified. Most antigenic determinants of bee venom phospholipase A₂ are probably dependent on its native conformation because the denatured enzyme that was obtained on reduction and carboxymethylation of the disulfide bonds retained less than 10⁻⁴-fold the activity of the native enzyme to induce histamine release from leukocytes of sensitive humans.¹⁷ Studies with chemically modified derivatives of bee venom phospholipase A₂ suggest that individuals vary in their antibody responses to the different antigenic determinants of the protein.¹⁷

Melittin is a peptide of 26 amino acid residues. It has cell surface-active properties, eg, those of inducing lysis of red blood cells and of potentiating phospholipase A₂ digestion of red blood cells.¹⁶ Its amino acid sequence as well as its recently determined x-ray crystallographic structure are shown in Figure 1.³⁰ Because of its unique distribution of amino acid residues, the amino terminal half of melittin is highly hydrophobic, whereas the carboxyl half is highly hydrophilic. The crystal structure of melittin shows that its conformation is mainly an α -helix with a bend in the mid-portion, and that there is a unique distribution of hydrophobic residues on the surface of the α -helix cylinder. Melittin in aqueous solution is an equilibrium mixture of monomer and tetramer. In the tetramer structure the hydrophobic regions of each melittin molecule are in contact with each other and they are removed from the solvent (Fig. 1).

As far as is known, a peptide that is homologous to melittin in structure

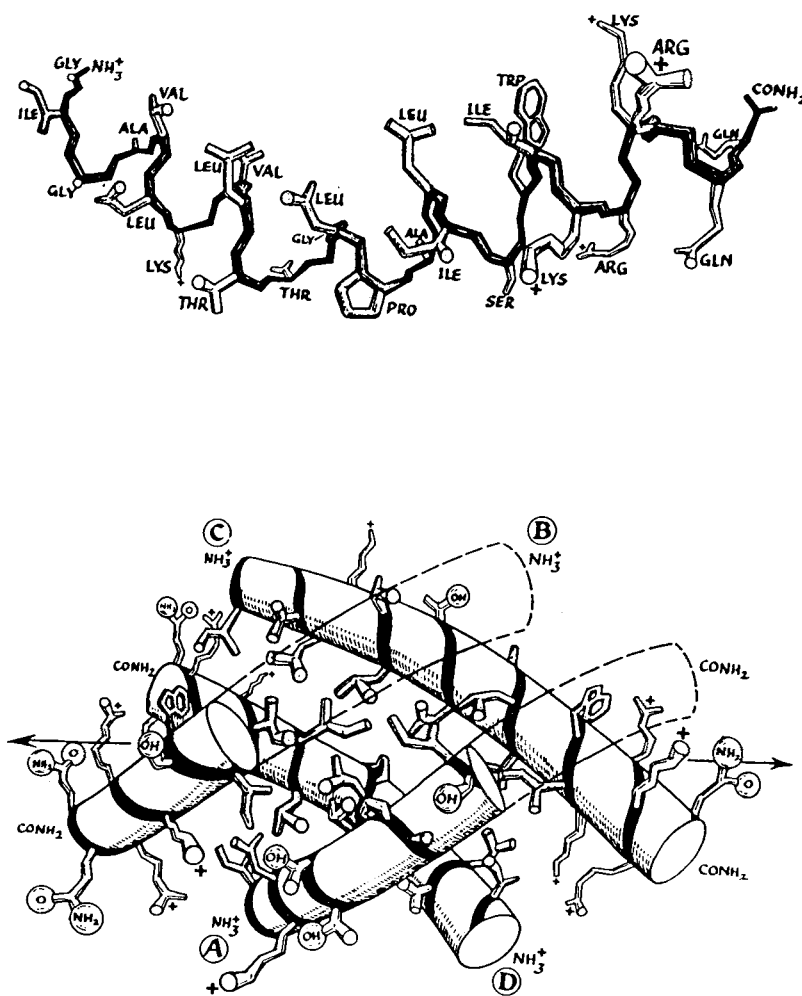


Figure 1. Tertiary structure of melittin monomer and tetramer. Data are taken from reference 30.

or function does not exist in man. Therefore, melittin is foreign to man, and it can be an immunogen. Usually peptides are poor immunogens, eg, apamin in bee venom—which is of the same size as melittin—is not allergenic in man. The immunogenicity of melittin may be related to its property of association to yield a tetramer of the size of a small protein. Alternatively, its immunogenicity may be related to its cell surface active property. The alternate hypothesis is compatible with the finding that the antibodies in sera of mice immunized with melittin are specific mainly for the carboxyl terminal heptapeptide region of the molecule. It is also observed that melittin is immunogenic only in selected strains of mice.³¹ The findings serve to illustrate the importance of genetic control of immune response. In the case of ragweed allergy in man, it is known that there is a correlation between a person's

histocompatibility antigens and his IgE response specific for ragweed antigen Ra5.³²

Vespid Venom Allergens

Three of the vespid venom proteins indicated in Table 2 have been purified to near homogeneity for several species of hornets, wasps, and yellow jackets. They are antigen 5, hyaluronidase, and phospholipase A₁. The venoms from hornets, wasps, and yellow jackets each contain the same homologous proteins, although in different relative amounts. All vespid venom-sensitive persons tested had IgE and IgG antibodies specific for these proteins.¹⁹⁻²² Because the dialyzed vespid venom extracts are fully active as allergens, the peptide components are probably not of importance as allergens.

The biochemical function of antigen 5 is not known now. Its amino acid composition as well as its molecular size are very similar to those of a neurotoxin from the venom of an Asian hornet, *Vespa mandarinia*, which is specific for crustacean neuromuscular junction.³³ The amino acid sequence for the first 20 residues of vespid antigen 5s was recently determined. There is significant sequence homology of white-faced hornet, yellow hornet, yellow jacket, and wasp antigen 5s. There is also partial sequence homology of vespid antigen 5s and scorpion neurotoxins (T. P. King, manuscript in preparation).

There are 2 types of phospholipase, A₁ and A₂, that catalyze the hydrolysis of the acyl group at the 1- or 2-position of lecithin, respectively. Both phospholipases A₁ and A₂ are widely distributed in living systems.³⁴ In bee venom only phospholipase A₂ is present, while in vespid venoms only phospholipase A₁ is present.²² The phospholipase A₁ in vespid venom, like those present in bovine pancreas or in microbial sources, also has phospholipase B activity (lysophospholipase), which catalyzes the hydrolysis of the acyl residue of 1- or 2-lysolecithin.

Hyaluronidase can be divided into 3 types, depending on its site of hydrolysis of hyaluronic acid.³⁵ Recent studies have shown that the hyaluronidases from honeybee and vespid venoms have the same enzymatic specificity; they are endo-*N*-acetyl-hexosaminidase.³⁶

Listed in Table 1 are species of vespids from which the venom proteins have been purified and studied in detail. The homologous venom proteins from different species of yellow jackets have very similar chemical properties, so that they can be considered nearly identical.²⁰ This is the case also for the different species of hornets¹⁹ or of wasps.²¹ When the homologous proteins from hornets, wasps, and yellow jackets are compared, small differences in their chemical properties are noticeable; nonetheless, they are still very similar. Studies of the chemical properties include their amino acid composition, their partial amino acid sequence data for antigen 5, and their specific enzymatic activities for hyaluronidases and phospholipases.

When compared by immunodiffusion with rabbit or mouse antibodies specific for the whole venom or the purified protein, the homologous venom proteins from the 3 species of wasps listed in Table 1 are antigenically indistinguishable.²² This is true also for the 4 species of yellow jackets listed in Table 1, with the exception of *V. squamosa*. There is only partial antigenic

identity of the venom proteins from *V squamosa* with those from other yellow jackets.³⁶⁻³⁸ Of the yellow jackets in Table 1, *V maculifrons* and *V germanica* are reported to be common in the eastern half of North America, *V vulgaris* is widely distributed all over North America, and *V squamosa* is common in the southeast United States.¹⁵ The homologous venom proteins from white-faced hornet and yellow hornet show only partial antigenic identity, even though these 2 species of hornets belong to the same genus.

The homologous venom proteins from white-faced hornet, yellow hornet, wasps, and yellow jackets were examined for antigenic cross-reactivity by quantitative inhibition analysis with rabbit or mouse antibodies.³⁹ These results are summarized in Table 4. The most extensive partial cross-reactivity is observed in white-faced and yellow hornet venom proteins. The hyaluronidases—as well as the antigen 5s—of these 2 hornets show much greater partial antigenic identity than the phospholipases. The next most extensive partial cross-reactivity is found in hornets and yellow jackets, and it is limited to the hyaluronidases and the antigen 5s. There is a weak partial cross-reactivity of the venom proteins from hornets and wasps, and again it is limited to the hyaluronidases and the antigen 5s. By this technique there is no detectable cross-reactivity of the wasp and the yellow jacket venom proteins, although techniques such as radioallergosorbent test (RAST) inhibition with human sera suggest that some cross-reactivity is present.⁴¹

Table 4. Antigenic Cross-Reactivity of Homologous Venom Proteins of Hornets, Yellow Jackets, and Wasps^a

	Mouse antibodies specific for purified venom protein				
	White-faced hornet	Yellow hornet	Yellow jacket	Wasp	Honeybee
Hyaluronidase					
WFH	+++	++	—	—	—
YH	++	+++	—	—	—
YJ	++	++	+++	—	—
W	+	++	—	+++	—
HB	—	—	—	—	+++
Antigen 5					
WFH	+++	+	+	—	—
YH	++	+++	+	—	—
YJ	+	—	+++	—	—
W	+	—	+	+++	—
Phospholipase					
WFH	+++	—	—	—	—
YH ^b	(+)	+++	—	—	—
YJ	—	—	+++	—	—
W	—	—	—	+++	—
HB	—	—	—	—	—

WFH = white-faced hornet, YH = yellow hornet, YJ = yellow jacket, W = wasp, HB = honeybee.

^aCross-reactivity was detected by inhibition assay. The plus signs represent the relative extent of inhibition of binding of the reference antibody to solid-phase reference antigen by the test antigen. Data are from reference 40.

^bNo partial cross-reactivity of yellow hornet phospholipase with mouse anti-white-faced hornet phospholipase antibodies was detected, but such was detected with rabbit anti-white-faced hornet venom antibodies.

It is also shown in Table 4 that the hyaluronidases or the phospholipases from honeybee or vespids do not share any antigenic identity. The absence of antigenic identity between bee and vespid homologous venom proteins is not surprising because they are not closely related in their taxonomy. Furthermore, their phospholipases are enzymes of different specificities.

Human Immune Response to Stings and Immunotherapy

Stings not only result in sensitization of a portion of the population, but also in the development of appreciable titers of protective, IgG antibody to venom proteins.⁴¹ Immunotherapy with venoms initially stimulates the formation of IgE antibodies, and later, IgG antibodies. The initial IgE response is not maintained, but the IgG response persists as long as booster injections (every 4 to 8 weeks) of venom are given. On prolonged immunization a shift in IgG₄ to IgG₁ antibody ratio occurs, so that an IgG₄-dominated antibody response usually results.^{42,43}

Because sensitization may be relatively selective for specific venom proteins, it would be desirable if immunotherapy were to result in the production of IgG antibodies with the same specificity. It has been shown that in some instances this is not the case,⁴⁴ despite the completeness of the venom solutions used. For this reason, as well as for reasons detailed below, it might be advantageous if individual venom allergens were available for clinical use.

It is well known that most insect venom-allergic persons show positive reactions to more than 1 vespid venom by skin test, RAST, or RAST inhibition.⁴⁵⁻⁴⁹ The multiple reactions can be due to multiple sensitizations by different vespids or to antigenic cross-reactivity of vespid venom proteins. It is not possible to ascertain by tests with venoms which of these conditions apply because venoms are mixtures of cross-reacting antigens. It is likely that tests with individual venom proteins can more easily be interpreted than those with whole venoms because each set of cross-reacting antigens can be tested separately.

The data in Table 4 showing the antigenic cross-reactivity of homologous venom proteins were obtained from animal models. If proved applicable to man, the data suggest that the individual venom proteins may be practical for unambiguous diagnosis of a person's sensitivity to venoms from 1 or more vespids. For example, if a patient is sensitive only to yellow jacket venom, his tests with antigen 5s from yellow jacket and hornets will all be positive because the antibodies specific for yellow jacket antigen 5 cross-react with the hornet antigen 5s. However, the patient's tests with hyaluronidases and phospholipases will be positive only with the proteins from yellow jacket because the antibodies specific for yellow jacket hyaluronidase or phospholipase do not cross-react with the homologous hornet protein. If a patient is sensitive only to the hornets, his test will be positive to antigen 5s and hyaluronidases of hornets and yellow jackets because the antibodies specific for hornet antigen 5 or hyaluronidase cross-react with the homologous yellow jacket proteins. The patient's tests with phospholipases will be positive only with those from hornets because the antibodies specific for hornet phospholipases do not cross-react with the homologous yellow jacket protein. Finally, if the

patient is sensitive to both hornets and yellow jackets, his tests will be positive to all 3 proteins from hornets and yellow jackets. In a preliminary skin-testing trial with purified vespid venom proteins, antigen 5s proved useful as skin test reagents, but it was not possible to diagnose single insect sensitivity.⁵⁰

Summary

Venoms of the Hymenoptera are mixtures of several antigens with nonimmunogenic small peptides and vasoactive amines. Limited local toxicity occurs with stings; systemic reactions to venoms are mediated by IgE antibodies and can be prevented by immunization with the appropriate venom. Skin testing with venoms detects the sensitized state, and a positive test predicts a 50 to 60% risk of an allergic response to a future sting. Because considerable cross-reactivity exists between vespid venom allergens, choice of correct venom(s) for immunotherapy will not always be indicated by skin-testing with whole, unfractionated venom. Further efforts at skin testing with individual venom antigens may help to resolve problems regarding choice of venoms for immunotherapy.

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