

14 Hymenoptera Allergens

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INTRODUCTION

Many insects can cause allergy in man (Table 1) (1). People can be exposed to insect body parts or their secretions by inhalation, to their venoms by stinging, and to their salivary gland secretions by biting. Examples of these routes of sensitization are, respectively, allergies to cockroaches of the order Orthoptera, to ants, bee, and vespids of the order Hymenoptera, and to flies and mosquitoes of the order Diptera.

The importance of venoms as the allergen source in Hymenoptera allergy has been known for some time (2,3). All known insect venom allergens are proteins of 10 to 50 kDa containing 100 to 400 amino acid residues. The one exception is that the bee venom allergen melittin is a 26-residue peptide. But melittin is a minor allergen, active in less than one-third of bee allergic patients (4). Nearly all these allergens have been sequenced and/or cloned. Several of these allergens have been expressed in bacteria, insect, or yeast cells.

This chapter will review the immunochemical properties of known Hymenoptera venom proteins and peptides, and their relevance to our understanding and treatment of insect allergy.

TAXONOMY, GEOGRAPHIC DISTRIBUTION, AND IDENTIFICATION OF HYMENOPTERA INSECTS

Essentially all insects responsible for causing insect sting allergic reactions belong to the order Hymenoptera. This is a large and diverse order composed of over 70 families (5) with over 100,000 species (6). Although many Hymenoptera are capable of stinging, only species belonging to three families sting people with a high degree of frequency. The usual perpetrators are social insects and belong to the Apidae (bees), Formicidae (ants), or Vespidae (wasps). The medically important genera in the United States are outlined in Table 2. Four of these insects are shown in Figure 1. Many of the bees and vespids listed in Table 2 or their closely related species are distributed worldwide. In addition to the fire ants that are in the Americas only, bulldog, jumper, and Samson ants are of medical importance in Asia and Australia.

Accurate identification of social stinging Hymenoptera to species level is a difficult task even for most entomologists. Although not definitive, there are several behavioral characteristics that can help provide clues as to a specimen's identity. For example, honeybees have a unique sting anatomy that causes worker bees to leave their sting apparatuses in the victim's skin. Although sting autotomy is almost exclusively attributed to honeybees, other stinging Hymenoptera will occasionally lose their sting. And conversely, honeybees will occasionally sting without autotomizing (7). Annoying wasps foraging around picnic foods, garbage, or fallen fruit are usually yellow jackets and belong to the genus *Vespula*. Large colonies of wasps living in subterranean nests are also usually of the genus *Vespula* (1). Since there are notable exceptions to the above, the only reliable means of obtaining a positive identification is to collect a specimen and have its identity determined by an entomologist with expertise in the social Hymenoptera.

Table 1 Insects Reported to Cause Allergy in Man

Order Coleoptera—beetles
Order Diptera—flies and mosquitoes
Order Ephemeroptera—mayflies
Order Hemiptera—aphids, bed bugs, and kissing bugs
Order Hymenoptera—ants, bees, and vespids
Order Lepidoptera—moths and caterpillars
Order Orthoptera—cockroaches
Order Siphonaptera—fleas
Order Trichoptera—caddis flies

Source: From Ref. 1.

Table 2 Geographic Distribution and Medical Importance of Some Insects of the Order Hymenoptera

Family/subfamily	Genus and species	Common name	Geographic distribution within U.S.	Medical importance
Apidae/Apinae	<i>Apis mellifera</i>	Honeybee	Entire U.S.	Major
	<i>Bombus</i> spp.	Bumble bee	Entire U.S.	Moderate
Formicidae/Myrmicinae	<i>Solenopsis invicta</i>	Fire ant	SE, SW	Major
	<i>Solenopsis richteri</i>	Fire ant	Mississippi, Alabama	Minor
Vespidae/Vespinae	<i>Vespa crabro</i>	European hornet	NE, SE	Minor
	<i>Dolichovespula maculata</i>	Whitefaced hornet (baldfaced hornet)	Entire U.S.	Major
	<i>Dolichovespula arenaria</i>	Yellow hornet (aerial yellow jacket)	NE, NW, SW	Major
	<i>Vespula flavopilosa</i>	Yellow jacket	NE, SE	Major
	<i>Vespula germanica</i>	Yellow jacket	NE, NW	Major
	<i>Vespula maculifrons</i>	Yellow jacket	NE	Major
	<i>Vespula pensylvanica</i>	Yellow jacket	NW, SW	Major
	<i>Vespula vulgaris</i>	Yellow jacket	NE, NW, SW	Major
	<i>Vespula squamosa</i>	Yellow jacket	NE, SE	Major
	<i>Polistes</i> spp.	Paper wasp	Entire U.S.	Major
Polistinae				

Only insects with known venom allergens are listed.

Data for geographic distribution and medical importance are taken from Ref. 1.

BIOCHEMICAL STUDIES OF HYMENOPTERA VENOM PROTEIN ALLERGENS

In Table 3 are listed some of the venom allergens of bees, vespids, and fire ants, which have been sequenced and/or cloned.

Honeybee venom has six allergens of known sequences. Four are proteins, acid phosphatase, hyaluronidase, phospholipase A₂, and protease, the fifth one is a cytolytic peptide melittin, and the sixth one Api m 6 is miniprotein of about 70 amino acid residues. Bumble bee venom has two protein allergens of known sequences: phospholipase A₂ and a protease. Bumble bee and honeybee venom phospholipases A₂ as well as their homologs from Asian bees have high degree of sequence identity with each other.

Each of vespid venoms contains three to four known protein allergens. Three of them have been isolated from all vespids studied, and they are antigen 5, hyaluronidase, and phospholipase A₁. And the fourth one is a protease that has been characterized only from paper wasps. The biological function of antigen 5 is not known, but it may be a protease because of sequence homology with a protease in cone snail (37). Vespid phospholipase A₁ differs from bee phospholipase A₂ in its structure and enzymatic specificity (15,24). Vespid and bee hyaluronidases are homologous with about 55% sequence identity (17,24,38).

Fire ant venom contains four known protein allergens: Sol i 1 to 4. Sol i 1 and 3 are homologous with vespid phospholipase and antigen 5, respectively (12,20).

Only one allergen of ~12kDa is known from jumper ant (*Myrmecia pilosula*) venom, and it was identified by immunoblot with mercaptoethanol-reduced venom (39). The presence of

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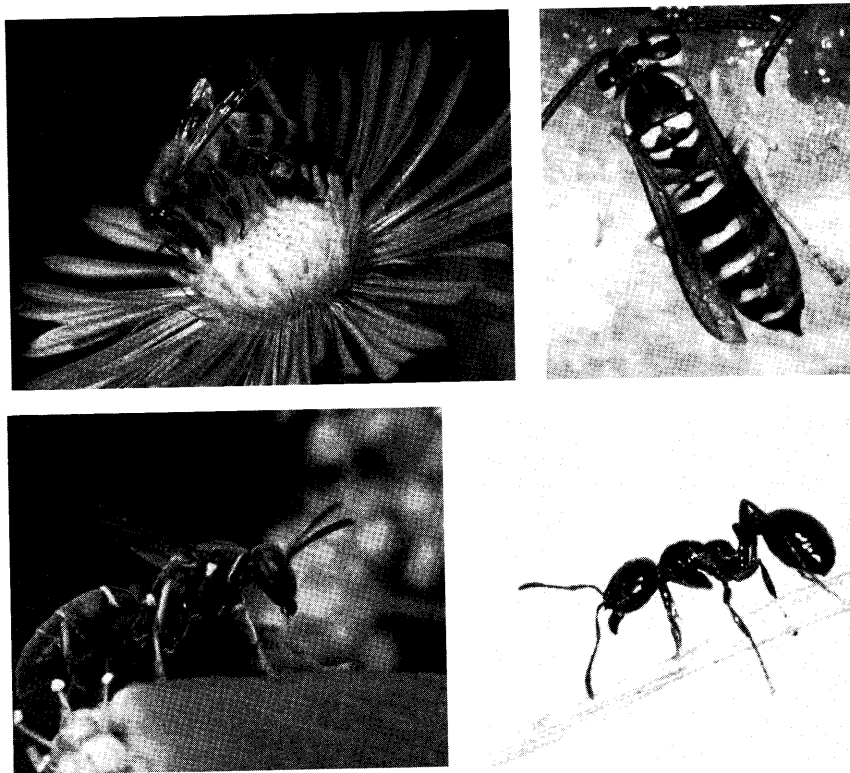


Figure 1 (See color insert.) Common stinging insects. The photos, starting from top left and going clockwise, show respectively honeybee (*Apis mellifera*), yellow jacket (*Vespula maculifrons*), paper wasp (*Polistes fuscatus*), and fire ant (*Solenopsis invicta*). The approximate lengths of these insects in the order given are 16, 10, 19, and 3 mm, respectively. The photos are of different magnifications.

hyaluronidase, phosphatase, phospholipase, and other enzymes in jumper and bulldog ant venoms have been reported (40), and it is possible that these venom proteins also are allergens.

Several venom allergens have partial sequence identity with other proteins from diverse sources, and this is summarized in Table 4. As an example, the sequence identities of three vespid antigen 5s, fire ant antigen 5 (Sol i 3), human and mouse testis proteins, human glioma protein, and proteins from tomato, nematode, and lizard, in their C-terminal 50 residue region, are given in Figure 2. We may note in particular the partial sequence identity of venom allergens with proteins of male reproductive functions; antigen 5s with a mammalian testis protein (18), hyaluronidases with those from mammalian sperm and other tissues, phosphatase with a prostate enzyme (20), and protease with mammalian acrosin (15).

X-ray crystallography was used to determine the structures of honeybee venom hyaluronidase (11) and phospholipase A₂ (9), and those of antigen 5 and hyaluronidase from yellow jacket, *V. vulgaris* (26,27). Vespid phospholipase A₁ has sequence homology with porcine pancreatic lipase (46). As the structure of porcine lipase is known, the structure of vespid phospholipase can be obtained by modeling. Using the modeling approach, the structures of nearly all the proteins in Table 3 can be obtained.

The structures of a number of allergen proteins from different sources have been determined. No unusual structural features of these protein allergens are known (49).

RECOMBINANT HYMENOPTERA VENOM PROTEIN ALLERGENS

Several of the allergens in Table 3 have been expressed in bacteria, insect, or yeast cells to yield recombinant proteins. The recombinant proteins that are expressed in the cytoplasm of bacteria are usually unfolded as they lack the disulfide bonds of the natural proteins and do not have the native conformation of the natural proteins. The cytoplasm of bacteria is a

Table 3 Some Insect Venom Allergens with Known Sequences and Structures

Allergen name ^a	Common name	Glycoprotein	Molecular size ^b	Structure ^c	Recombinant protein ^d	References
<i>Honeybee, Apis mellifera</i>						
Api m 1 ^e	Phospholipase A ₂	Yes	16 kDa	Direct	Unfolded/folded	8,9
Api m 2	Hyaluronidase	Yes	39 kDa	Direct	Folded	10,11
Api m 3	Acid phosphatase		43 kDa			12
Api m 4	Melittin	No	3 kDa	Direct	Folded	4
Api m 5	Protease		28 kDa			13
Api m 6			8kDa			14
<i>Bumble bee, Bombus pennsylvanicus</i>						
Bom p 1	Phospholipase A ₂		16 kDa	Modeling		15
Bom p 4	Protease		28 kDa			15
<i>White face hornet, Dolichovespula maculate</i>						
Dol m 1	Phospholipase A ₁	No	34 kDa	Modeling	Unfolded	16
Dol m 2	Hyaluronidase	Yes	38 kDa	Modeling	Unfolded	17
Dol m 5 ^f	Antigen 5	No	23 kDa	Modeling	Unfolded/folded	18,19
<i>European hornet, Vespa crabro</i>						
Ves c 1	Phospholipase A ₁		34 kDa	Modeling		20
Ves c 5	Antigen 5	Yes	23 kDa	Modeling		21
<i>Paper wasp, Polistes annularis</i>						
Pol a 1	Phospholipase A ₁	No	34 kDa	Modeling	Unfolded	22
Pol a 2	Hyaluronidase	Yes	38 kDa	Modeling	Unfolded	22
Pol a 5	Antigen 5	No	23 kDa	Modeling	Unfolded/folded	18,23
	Protease ^g		28 kDa			13
<i>Yellow jacket, Vespa vulgaris</i>						
Ves v 1 ^h	Phospholipase A ₁	No	34 kDa	Modeling	Unfolded	24
Ves v 2 ^h	Hyaluronidase	Yes	38 kDa	Modeling	Unfolded	25,26
Ves v 5	Antigen 5	No	23 kDa	Modeling	Unfolded/folded	23,27
<i>Fire ant, Solenopsis invicta</i>						
Sol i 1 ⁱ	Phospholipase A ₁	Yes	37 kDa	Modeling		28
Sol i 2			30 kDa			29
Sol i 3 ^j	Antigen 5		23 kDa	Modeling		30
Sol i 4			20 kDa			30

^aAllergen names are designated according to an accepted nomenclature system (31).

^bSeveral allergens are glycoproteins, and the molecular size given refers only to the protein portion.

^cStructures were determined directly or by modeling of structures of homologous proteins.

^dAvailability of recombinant proteins in folded or unfolded forms is indicated.

^eSequences of phospholipases A₂ from *A. crena*, *A. dorsata* (32), and *B. terrestris* (33) are known.

^fOther vespid antigen 5s with known sequences are *D. arenaria*, *P. exclamans*, *P. fuscatus*, *P. dominulus*, *V. flavopilosa*, *V. germanica*, *V. maculifrons*, *V. pennsylvanica*, *V. squamosa*, *V. vidua*, and *V. mandarinia* (12).

^gCloning of proteases from *P. dominulus*, *P. exclamans*, and *P. Gallicus* were reported (34).

^hSequences of phospholipase A₁ from *V. germanica*, *maculifrons*, *pennsylvanica*, *flavopilosa* and *squamosa* and of hyaluronidase *V. germanica* are known (35).

ⁱSequences of homologs from *S. invicta* are known (36).

reducing environment and any disulfide bonds that do form are reduced through the action of disulfide reducing enzymes. Several recombinant proteins with disulfide bonds have been obtained in mutants of *Escherichia coli* with decreased disulfide reducing enzymes in their cytoplasm (50). In some cases the unfolded recombinant proteins can be folded and oxidized in vitro into their native conformation, e.g., bee venom phospholipase A₂ (38,51,70) and yellow jacket venom hyaluronidase (26).

Recombinant proteins from insect or yeast cells have the native conformation of the natural proteins as they are folded during secretion into medium, e.g., Api m 2 (10), Sol i 2 (29), and vespid antigen 5s (19,23).

Recombinant allergens have different applications. One obvious application is for use as diagnostic reagents. For example, recombinant yellow jacket antigen 5 was used to show the frequency of patient response to three yellow jacket venom allergens. Ninety percent of the 26 patients tested were positive to antigen 5, and 70% to 80% positive to hyaluronidase and phospholipase (52).

Table 4

Insect alle

Antigen 5

Hyaluronidase
Phospholipase
Phospholipase
Phospholipase
Protease

^aHomolog^bHomolog^cSperm pr

Dol m 5

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Table 4 Sequence Identity of Insect Allergens and Other Proteins

Insect allergens	Other proteins	Residues compared	% Identity	References
Antigen 5s	Mammalian testis protein	130	35	41
	Human glioma PR protein	124	23	42
	Hookworm protein ^a	130	28	43
	Plant leaf PR protein ^b	130	28	44
	Mexican lizard toxin	130	28	44
	Cone snail protease			37
Hyaluronidase	Mammalian sperm protein ^c	331	50	45
Phosphatase	Mammalian phosphatase	343	16	20
Phospholipase A ₁	Mammalian lipases	123	40	46
Phospholipase A ₂	Mammalian phospholipases	129	20	
Protease	Mammalian acrosin	243	38	20
	Horseshoe crab enzyme	243	41	20

^aHomologous worm proteins are present in other nematodes (47).

^bHomologous plant PR proteins are present in tobacco, tomato, barley and maize (44).

^cSperm protein is one of six known mammalian hyaluronidases from different tissues (48).

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Dol m 5  VGHYTMVWG KTKEIGCGSI KYIE.DNWT H...YLVCN YGPGGNDFNQ
Pol a 5  IGHYTMVWG KTKEIGCGSL KYME.NMQN H...YLICN YGPAGNYLGQ
Ves v 5  TGHYTMVWA NTKEVGCGSI KYIQ.EKWHK H...YLVCN YGPGGNFMNE
Sol i 3  VEHYTQIWA KTSKIGCARI MFKEPDNWK H...YLVCN YGPAGNVLGA
human tpx VGHYTQLVWY STYQVGCIA YCPNQDSLKY ....YVVCQ YCPAGNNMNR
mouse tpx VGHYTQLVWY SSFKIGCGIA YCPNQDNLKY ....FYVCH YCPMGNNVMK
hum glioma CGHYTQVWA DSYKVGCAVQ FCPKVSGFDA LSNAGHFICN YGPGGNYPTW
tomato pr CGHYTQVWR NSVRVGCARV QC....NNGG Y....VVSCN YDPPGNYRGE
hookworm IGHYTMAWD TTYKLGCAVV FC....NDFT ....FGVCQ YGPGGNYMGH
lizard IGHYTQVWY RSYELGCAIA YCPDQPTYKY ....YQVCQ YCPGGNIRSR

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Figure 2 Sequence identity of vespid antigen 5s and other proteins in their C-terminal region. The sequences shown from top to bottom are for antigen 5s from hornet, paper wasp, yellow jacket and fire ant venoms, human and mouse testis-specific proteins, human glioma protein, tomato leaf pathogenesis-related protein, hookworm protein, and lizard venom protein, respectively. References for these proteins are given in Table 4. Bold characters indicate residues identical to those of vespid antigen 5s and dots indicate blanks added for maximal alignment of sequences. The underlined peptide region was found to contain a dominant T-cell epitope of vespid antigen 5 (see text).

Another application is to prepare allergen hybrids with reduced allergenicity but retaining its immunogenicity. The hybrids contain a small segment of the guest allergen of interest and a large segment of a host protein. The host protein is homologous to the guest allergen, and they are poorly cross-reactive as antigens. The host protein functions as a scaffold to hold the segment of the guest allergen in its native conformation as homologous proteins of >30% sequence identity can have closely similar structures. In this way, the hybrids retain the discontinuous epitopes of the guest allergen but at a reduced density.

The above approach was demonstrated with hybrids of yellow jacket and wasp antigen 5s (53). These two antigens 5s have 59% sequence identity, and they are poorly cross-reactive in patients or in animals. Hybrids with one-fourth of yellow jacket antigen 5 and three-fourth of wasp antigen 5 showed 10^2 to 10^3 fold reduction in allergenicity when tested by histamine release assay in yellow jacket-sensitive patients. These hybrids retained the immunogenicity of antigen 5s for antibody responses specific for the native protein and for T-cell responses in mice. Therefore, the hybrids may be useful vaccines as they may be used at higher doses than the natural allergen.

B-CELL EPITOPES OF HYMENOPTERA VENOM ALLERGENS

The entire accessible surface of a protein is believed to represent a continuum of B-cell epitopes (54). The B-cell epitopes are divided into the continuous and discontinuous types, and their sizes range from 6 to 17 amino acid residues. The continuous type consists of only contiguous amino acid residues in the molecule, while the discontinuous type consists of contiguous as well as noncontiguous residues, which are brought together in the folded molecule. The

Table 5 Cross Reactivity of Native and Reduced Vespid Allergens Detected with Specific Mouse Sera

Solid-phase antigen 5	Anitgen 5-specific sera					
	Hornet		Yellow jacket		Wasp	
Hornet	++	++	+	+	+	-
yellow jacket	+	+	++	++	±	-
Wasp	+	+	+	+	++	+
Solid-phase hyaluronidase	Hyaluronidase-specific sera					
	Hornet		Yellow jacket		Wasp	
Hornet	++	±	++	+	±	-
yellow jacket	++	-	++	++	±	-
Wasp	++	-	+	+	++	-
Solid-phase phospholipase	Phospholipase-specific sera					
	Hornet		Yellow jacket		Wasp	
Hornet	++	±	-	-	-	-
Yellow jacket	±	-	++	±	±	-
Wasp	±	nd	±	nd	++	-

For each sera there are two columns of results. The first column is from ELISA on solid phase natural allergens, and the second column is from immunoblot of reduced allergens.

The ++, +, ±, and - signs refer to relative titer of sera on ELISA, or intensity of bands of immunoblots. "nd" denotes not done.

majority of protein-specific antibodies, 90% or more, are of the discontinuous type. This is the case for venom allergen-specific IgEs in patients by comparative tests with natural or disulfide bond reduced allergens, e.g., bee venom phospholipase A₂ (51) and fire ant Sol i 2 (29). Studies have shown that the same B-cell epitopes can induce both IgE and IgG responses.

Data in agreement with the above generalization were obtained with vespid allergen-specific mouse antisera, which contain mainly specific IgGs. Comparison of the data in Table 5 shows that vespid allergen-specific antisera bind natural allergens, and they bind poorly, if at all, reduced and unfolded allergens that lack the discontinuous epitopes of the folded molecules. This is particularly the case for vespid hyaluronidases and phospholipases (17,24) and to a lesser extent for vespid antigen 5s (18,55). Another general conclusion is that cross-reactivity is readily detectable for homologous venom proteins of >90% sequence identity, and barely detectable for homologous venom proteins of <50% sequence identity, and variable extents of cross-reactivity are detectable for homologous proteins with about 50% to 90% sequence identity. The variable extents of cross-reactivity of homologous proteins probably reflect the degree and the area of identity on the protein surface.

The continuous B-cell epitopes of a protein can be mapped readily by testing polyclonal antibodies with a series of overlapping peptides of 7 to 20 residues in length. Multiple epitopes were found for the 204-residue hornet antigen 5, and only one was found for the 26-residue bee venom melittin (56). No unusual pattern of amino acid sequence was observed for these B-cell epitopes. The discontinuous B-cell epitopes can be mapped with monoclonal antibodies for proteins of known structures (57).

Several insect venom proteins are glycoproteins. Their oligosaccharide side chains have been demonstrated to function as B-cell epitopes for IgE and IgG responses in patients as well as in animals (58,59). In contrast to the multitude of amino acid sequences of different insect allergens, the sequence differences in their carbohydrate side chains are limited. The carbohydrate side chain is N-linked to the asparagine residue of the protein by its innermost *N*-acetylglucosamine residue. The predominant carbohydrate side chain has the sequence of Man α 1 $\rightarrow$ 6(Man α 1 $\rightarrow$ 3)Man β 1 $\rightarrow$ 4GlcNAc β 1 $\rightarrow$ 4GlcNAc with one or two fucoses (α 1,6 and/or α 1,3) linked to the innermost *N*-acetylglucosamine residue. This was shown for bee phospholipase (60) and bee and yellow jacket hyaluronidases (25). Similar or identical carbohydrate side chains are present in other plant and animal proteins. The glycan in plant proteins contains, in addition to the fucoses, xylose(β 1,2) linked to the mannose in the middle position. Tests with patient or animal sera specific for glycans from different sources suggest that the specificity of the cross-reactive carbohydrate determinant resides mainly in the fucose residues for insect proteins and in the fucose and xylose residues for plant proteins (61). Indeed

horseradish peroxidase can be used to establish whether patient sera cross-reactive to bees and yellow jackets are due to their cross-reactive carbohydrate determinant (62).

T-CELL EPITOPES OF HYMENOPTERA VENOM ALLERGENS

T-cell epitopes are of interest because of the central role of T cells in regulating the antibody class switch event of B cells. They are also of interest as possible reagents for immunotherapy as shown with T-cell peptides of bee venom phospholipase A₂ (63).

T-cell epitopes are peptides of about 15 residues in length formed following intracellular processing of antigens by antigen-presenting cells, and they do not depend on the secondary or tertiary structure of the antigen. This is the case with venom allergens as shown by the identical T-cell stimulating activities of natural or recombinant allergens or reduced allergens; e.g., vespid antigen 5s, hyaluronidases, and phospholipases A₁ (24,55).

Bee venom phospholipase A₂ and hornet antigen 5 are found to have multiple T-cell epitopes distributed throughout the entire molecule by tests with a series of overlapping peptides in patients (64-66) or in mice (67,68). Because of major histocompatibility complex (MHC) class II restriction, patients of different polymorphic background, or mice of different haplotypes, differ in their pattern of peptide recognition. Nonetheless, both insect allergens were found to have several dominant T-cell epitopes recognized by both patients or mice tested.

One T-cell epitope-containing peptide of bee venom phospholipase was found to require the presence of its carbohydrate side chain for its activity (69).

One of the dominant T-cell epitope peptides of hornet antigen 5 was found to cross-react with paper wasp and yellow jacket antigen 5s as well as with a homologous peptide of a mouse testis protein (Fig. 2). The cross-reactivity with mouse protein is not reciprocal as the corresponding peptide from mouse testis protein did not cross-react with hornet antigen 5-specific cells (68).

No unusual features were observed for the dominant T-cell epitope peptides of insect venom allergens. Others have reported similar findings for T-cell epitopes of allergens from grass and tree pollens, cat dander, mites, and chicken ovalbumin (71). Both normal and atopic people were found to recognize the same T-cell epitope peptides of bee venom phospholipase (65) and the major birch pollen allergen (72).

ANTIGENIC CROSS-REACTIVITY OF HYMENOPTERA VENOMS

Insect allergic patients often have sensitivity to multiple insects by skin test or RAST with venoms (3). This multiple sensitivity can be due to exposure to different insects and/or antigenic cross-reactivity of different venoms. This issue of multiple exposure or antigenic cross-reactivity is of importance in the choice of single or multiple venoms for immunotherapy of patients. RAST inhibition carried out with multiple venoms is one possible approach to resolve this issue of multiple sensitivities (73). As noted earlier, the cross-reactivity can be due to their common peptide and carbohydrate determinants.

Bees, fire ants, and vespids each have unique as well as homologous venom allergens. Two of the six known bee allergens are homologous to vespid allergens; hyaluronidase and protease with about 50% sequence identity. Two of the four known fire ant allergens are homologous to vespid allergens, antigen 5 and phospholipase. Fire ant antigen 5 has about 35% sequence identity with vespid antigen 5s. For the vespids, the sequence identity of their homologous antigen 5s, hyaluronidases, and phospholipases ranges from about 40% to 99% for different species of hornets, yellow jackets, and wasps. As noted in section "B-Cell Epitopes of Hymenoptera Venom Allergens," cross-reactivity of B-cell epitopes is readily detectable for homologous venom proteins of >90% sequence identity, and barely detectable for homologous venom proteins of <50% sequence identity, and variable extents of cross-reactivity are detectable for homologous proteins with about 50% to 90% sequence identity.

The above considerations would indicate that patients with sensitivity to multiple insect can be due to cross-reactivity of a single allergen, hyaluronidase, as in the case for bees and vespids, or of multiple allergens in other cases. For cross-reactivity of fire ants and vespids, or

of different vespids, hyaluronidase again has the major role with antigen 5 and phospholipase having secondary and negligent roles, respectively.

Some insect allergens have common peptide and carbohydrate determinants. However, peptide and carbohydrate determinants differ in their epitope density and antibody affinity. For peptide determinants, the entire accessible surface of a protein represents a continuum of epitopes (54). For carbohydrate determinants, the number of epitopes is restricted to a few potential glycosylation sites, and these sites are not necessarily glycosylated. As an example, yellow jacket hyaluronidase has five potential sites but only two to three sites are glycosylated (26).

Mediators are released from IgE-bound mast cells or basophils on allergen challenge. This biological activity requires the allergens to have multiple determinants/epitopes so that they can cross-link the bivalent IgE antibodies. Thus, the low-density carbohydrate determinants are likely to be of less biological importance than are the high-density peptide determinants. Also the biological activity of allergens to cause mediator release depends on the affinity of allergen-specific IgEs (74,75). For these reasons, there is debate as to the importance of glycan-specific IgE in allergic diseases (76).

Several authors have reported that a sizable group of normal people who showed no clinical sensitivity to insects tested positive with insect venoms (77,78). These false-positive results may possibly represent cross-reactivity of insect venoms with other proteins to which people have been exposed. As noted earlier in Table 4, insect allergens have variable extents of sequence identity with proteins from diverse sources.

Investigators have observed that more men than women, in a ratio of about 2 to 1, had insect allergy as judged by their systemic and large local reactions or by their death statistics (79). It has been assumed that these results were primarily due to greater exposure because of work habits of men and women. Whether or not the partial sequence identity of venom allergens with proteins of male reproductive functions (Table 4) plays a role in these observations is not known.

BIOCHEMICAL STUDIES OF HYMENOPTERA VENOM PEPTIDES

In addition to proteins, Hymenoptera venoms contain peptides, biogenic amines such as histamine and dopamine, and other low molecular weight components (80). Table 6 lists the biological activities and the names of these venom peptides (81). These biological activities include mast cell degranulation, chemotaxis, kinin, and others. The most abundant peptides in bee and vespid venoms are melittin and mastoparan, respectively, and they have mast cell degranulating activity. Bee venom contains another mast cell degranulating peptide, known as MCD, with a greater mast cell degranulating activity than melittin. Bee venom induced mast cell degranulation leads to the release of biologically active products: some of these products cause inflammatory responses resulting in morbidity or mortality but some, such as proteases, are in fact of protective value by their action on venom components (82).

Melittin and mastoparan are basic peptides with 26 and 14 to 15 residues, respectively. Both peptides are synthesized in insects as precursor proteins and are released on proteolysis (83). Both peptides are immunogenic in mice for antibody responses (84-86). But melittin, not mastoparan, was reported to be an allergen (4).

Table 6 Bioactive Peptides in Hymenoptera Venoms

Species	Kinin	Chemotaxis	Mast cell degranulation ^a	Others ^a
<i>Apis mellifera</i>	No	No	MCD	Melittin Apamin
<i>Bombus</i> spp.				Bombolitin
<i>Polistes</i> spp.	Yes	Yes	Mastoparan	
<i>Vespa</i> spp.	Yes	Yes	Mastoparan	Crabolin
<i>Vespula</i> spp.	Yes	Yes	Mastoparan	

^aPeptide names are listed.

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Mastoparan was discovered for its activity to induce release of histamine and other mediators from mast cells (87). It binds to cell membranes (88,89), and it can act as a strong secretagogue for different cell types. For examples, mastoparan is reported to stimulate the release of the inflammatory mediators, $\text{TNF-}\alpha$, $\text{IL-1}\beta$, nitrous oxide, and prostaglandin E_2 into peritoneal exudates of mice (90). These mediator releases are related to its diverse range of biochemical activities. They include stimulation of phospholipases A_2 (91), C (92) and D (93,94), and G-protein activation (95).

Mastoparan was found to have a weak adjuvant activity to enhance IgG1 and IgE responses to yellow jacket antigen 5 in mice (96). This adjuvant action may be related to its activity to induce the release of $\text{T}_\text{H}2$ cell-associated mediators from basophils/mast cells, macrophages, and possibly other antigen-presenting cells. Melittin was not found to have adjuvant activity for Ves v 5-specific antibody response, although melittin has biological properties similar to mastoparan. Others found melittin to be an adjuvant for ovalbumin-specific IgE response in mice (84). The two different findings on melittin may reflect the experimental conditions used as IgE responses in mice are antigen, dose, and mice strain dependent.

Yellow jacket venom was found to be lethal in mice when injected intraperitoneally but not subcutaneously (96). The toxic action was shown to require the synergistic action of the venom peptide mastoparan and the venom protein phospholipase A_1 .

STING REACTIONS

There are three types of reactions that individuals may experience from a Hymenoptera sting. The normal response is a *local cutaneous reaction* characterized by redness, swelling, and pain confined to the sting site. This is a toxic response. A *large local reaction* is thought to be IgE mediated and involves an extensive area of warmth, redness, and swelling contiguous with the site of the sting. Large local reactions typically develop in one to three days, may involve an entire extremity, and may persist for up to 5 days. An *allergic systemic reaction* usually occurs within half an hour of envenomization and includes symptoms remote from the site of the sting. Systemic allergic reactions may involve the skin, the respiratory system, the vascular system, or any combination thereof.

Minimal treatment is necessary for local cutaneous reactions. The sting site should be kept clean to avoid secondary infections, and ice packs may help to reduce local pain and swelling. Large local reactions may cause considerable discomfort and are frequently treated with analgesics, antihistamines, and glucocorticosteroids. Systemic allergic reactions can be quite serious and occasionally fatal. They can be successfully treated with venom immunotherapy to prevent future reactions (see chap. 24).

SALIENT POINTS

- The medically important stinging insects are fire ants, bees, and vespids (wasps). The vespids include hornets, paper wasps, and yellow jackets.
- Insect venom allergens are proteins of 10 to 50 kDa. Nearly all known venom allergens have been cloned and expressed as recombinant proteins in different systems. However, some recombinant proteins are not properly folded.
- Insect venom allergens have different biochemical functions. Their only known common feature is their partial sequence identity with proteins from other sources in the environment.
- Each of these insect venoms has unique allergen(s) as well as homologous allergen(s) with partial sequence identity.
- Multiple sensitivity of patients to different insects or to more closely related vespids can be due to multiple exposures and/or antigenic cross-reactivity of venom allergen(s).
- Detailed immunochemical knowledge of insect venom allergens are useful for monitoring the quality of insect venoms used for diagnosis and treatment, as well as lead to the development of new immunotherapeutic reagents.

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