

Structure and Biology of Stinging Insect Venom Allergens

Te Piao King Michael D. Spangfort

The Rockefeller University, New York, N.Y., USA, and ALK-Abelló Inc., Horsholm, Denmark

Key Words

Stinging insect venom allergens

Abstract

Bees, fire ants and vespids cause insect sting allergy. These insects have unique as well as common venom allergens. Vespids, including hornets, paper wasps and yellow jackets, have common allergens. Bees and vespids have one common allergen with hyaluronidase activity; they also have unique allergens with different phospholipase activities. Fire ants and vespids have one common allergen, antigen 5 of unknown biologic activity. The common venom allergens with < 70% sequence identity have barely detectable levels of antigenic cross-reactivity. Possible uses of modified allergens for immunotherapy are described.

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Introduction

Insects of the order Hymenoptera are mainly responsible for causing insect sting-allergic reactions. Many hymenopterans are capable of stinging, but only species belonging to three families sting people with a high degree of frequency. They are either the Apidae (bees), Formicidae

(ants), or Vespidae (wasps). The medically important genera and species are outlined in table 1 [1].

The importance of venoms as the allergen source has been known for some time [2, 3]. The known insect venom allergens are proteins of 10–50 kD containing 100–400 amino acid residues. The one exception is the bee venom allergen melittin which is a 26-residue peptide. But melittin is a minor allergen, active in less than one-third of bee-allergic patients [4]. The sequences and structures of nearly all these allergens have been determined. Many of these allergens have been expressed in bacteria, insect or yeast cells. This article will review the immunochemical properties of known hymenopteran venom allergens.

Biochemical Studies of Hymenoptera Venom Allergens

The venom allergens of bees, fire ants and vespids of known sequences and structures are listed in table 2 [5–27]. The vespids include hornets, yellow jackets and paper wasps. Honeybee venom has four allergens of known sequences: acid phosphatase, phospholipase A₂ (PLA₂), hyaluronidase and a cytolytic peptide melittin. With the exception of acid phosphatase, the structures of these bee allergens are known from X-ray crystallography [6, 8, 28].

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Correspondence to: Dr. Te Piao King
The Rockefeller University
1230 York Avenue
New York, NY 10021-6399 (USA)
Tel. +1 212 327 8212, Fax +1 212 327 8878

Table 1. Stinging hymenoptera of medical importance

Family ¹	Some known species
Vespidae	
Subfamily Vespinae	
Genus <i>Vespula</i> (yellow jackets)	
<i>Vespula vulgaris</i> species group	<i>V. flavopilosa</i> , <i>V. germanica</i> , <i>V. maculifrons</i> , <i>V. pennsylvanica</i> , <i>V. vulgaris</i>
<i>Vespula rufa</i> species group	<i>V. acadica</i> , <i>V. consorbina</i> , <i>V. vidua</i>
<i>Vespula squamosa</i> species group	<i>V. aquamosa</i>
Genus <i>Dolichovespula</i> (aerial yellow jackets) ²	<i>D. maculata</i> , <i>D. arenaria</i>
Genus <i>Vespa</i> (hornets) ³	<i>V. crabro</i> , <i>V. mandarinia</i>
Subfamily Polistinae	
Genus <i>Polistes</i> (paper wasps)	
Subgenus <i>Aphanilopterus</i>	<i>P. annularis</i> , <i>P. apachus</i> , <i>P. exclamans</i> , <i>P. fuscatus</i> , <i>P. metricus</i>
Subgenus <i>Polistes</i>	<i>P. dominulus</i>
Apidae	
Subfamily Apinae	
Genus <i>Apis</i> (honey bees) ⁴	<i>A. mellifera</i> , <i>A. cerana</i> , <i>A. dorsata</i>
Genus <i>Bombus</i> (bumble bees)	<i>B. pennsylvanicus</i>
Formicidae	
Subfamily Myrmicinae	
Genus <i>Solenopsis</i> (fire ants)	<i>S. invicta</i>
Genus <i>Pogonomyrmex</i> (harvester ants)	
¹ This table has been adapted from reference 1. ² Common names of <i>Dolichovespula maculata</i> and <i>D. arenaria</i> are bald-faced or white-faced hornet and yellow hornet respectively. ³ <i>V. crabro</i> is in Europe and N. America and its common name is European hornet; <i>V. mandarinia</i> is in Asia. ⁴ <i>Apis mellifera</i> is the important honey bee species. <i>A. cerana</i> and <i>A. dorsata</i> are two known Asian honey bees.	

Bumblebee venom has two allergens of known sequences: PLA₂ and a protease. The two bee venom PLA₂ have extensive sequence identity with each other and they have no sequence identity with vespid phospholipase A₁ (PLA₁) [17]. PLA₁ and PLA₂ differ in their specificity in catalyzing the hydrolysis of fatty acid residues at positions 1 and 2 of phospholipids respectively. In contrast to the phospholipase, bee venom hyaluronidase has about 55% sequence identity with vespid hyaluronidases and they have the same enzymatic specificity of endo-N-acetylhexosaminidase [17].

Vespid venoms each contain three known allergens. They are antigen 5, hyaluronidase and PLA₁. The presence of a fourth allergen, a protease in the venoms of *Polistes dominulus* and *Polistes exclamans* was reported recently [29]. The biologic function of antigen 5 is not known although it had been reported that antigen 5 from *Vespa mandarinia* to be a neurotoxin active at the neuro-

muscular junctions of a lobster walking leg [30]. The homologous venom allergens from hornets, yellow jackets and paper wasps have varying degrees of sequence identity ranging from about 60% for phospholipases and antigen 5s to about 80% for hyaluronidases [12–14, 16, 17]. Allergens of different species within a species group of each genus generally have a higher degree of sequence identity than those of a different species group. For example, antigen 5s from 5 species of yellow jackets of the *Vespula vulgaris* group in table 2 have about 95% sequence identity within the group and they have about 73% identity with antigen 5s of *Vespula squamosa* and *Vespula rufa* groups [16]. PLA₁ from two species of yellow jackets, *Vespula maculifrons* and *Vespula vulgaris*, have 95% sequence identity and they have about 67 and 55% identity with white face hornet and paper wasp proteins, respectively [25].

Table 2. Insect venom allergens with known sequences and structures

Allergen name ¹	Common name	Molecular size ² , kD	Structure ³	Recombinant protein ⁴		References
				unfolded	folded	
Honey bee, <i>Apis mellifera</i> ⁵						
Api m 1	Phospholipase A ₂	16	++	+	+	5, 6
Api m 2	Hyaluronidase	39	++	+	+	7, 8
Api m 3	Acid phosphatase	43	–	–	–	9, 10
Api m 4	Melittin	3	++	–	–	4
Bumble bee, <i>Bombus pennsylvanicus</i>						
Bom p 1	Phospholipase A ₂	16	+	–	–	11
Bom p 4	Protease	28	–	–	–	11
White-faced hornet, <i>Dolichovespula maculata</i> ⁶						
Dol m 1	Phospholipase A ₁	34	+	+	–	12
Dol m 2	Hyaluronidase	38	+	+	–	13
Dol m 5	Antigen 5	23	+	+	+	14, 15
European hornet, <i>Vespa crabro</i> ⁷						
Vesp c 1	Phospholipase A ₁	34	+	–	–	16
Vesp c 5	Antigen 5	23	+	–	–	16
Paper wasp, <i>Polistes annularis</i>						
Pol a 1	Phospholipase A ₁	34	+	–	–	Unpubl. ⁸
Pol a 2	Hyaluronidase	38	+	–	–	Unpubl. ⁸
Pol a 5	Antigen 5	23	+	+	+	14, 18
Yellow jacket, <i>Vespula vulgaris</i>						
Ves v 1	Phospholipase A ₁	34	+	+	–	17
Ves v 2	Hyaluronidase	38	+	+	–	17
Ves v 5	Antigen 5	23	++	+	+	18, 19
Fire ant, <i>Solenopsis invicta</i>						
Sol i 1	Phospholipase A ₁	37	+	–	–	20
Sol i 2		30	–	–	+	20, 21
Sol i 3	Antigen 5	23	+	–	+	20, 22
Sol i 4		20	–	–	–	20

¹ Allergen names are designated according to an accepted nomenclature system [23].

² Several allergens are glycoproteins, and the molecular size given refers only to the protein portion.

³ ++ and + signs refer respectively to structures determined directly, or indirectly by modeling of structures of homologous proteins.

⁴ + and – signs refer to the availability of recombinant proteins.

⁵ Sequences of phospholipases from *A. crena* and *A. dorsata* are known [24].

⁶ Sequence of phospholipase from *V. maculifrons* is known [25]. Sequences of antigen 5s from several other vespids are known: *D. arenaria* [14], *P. exclamans* and *P. fuscatus* [14, 16], *P. dominulus* [26], and *V. flavopilosa*, *V. germanica*, *V. maculifrons*, *V. pensylvanica*, *V. squamosa* and *V. vidua* [16].

⁷ Sequence of antigen 5 from *V. mandarinia* is known [27].

⁸ Unpublished data, accessible from Genbank.

Structure of antigen 5 from *V. vulgaris*, Ves v 5, was determined recently by X-ray crystallography [19]. Structures of vespid hyaluronidase and PLA₁ are on hand indirectly from the structures of their homologs. Vespid hyaluronidases and phospholipases are homologous to bee hyaluronidase and porcine pancreatic lipase respectively and X-ray crystallography was used to determine their structures [8, 31].

Fire ant venom contains four known allergens: Sol i 1 to 4. Sol i 3 has about 50% sequence identity with vespid antigen 5. Only partial sequence data is available for Sol i 1, and it shows partial sequence identity with vespid PLA₁ [9].

The venom allergens in table 2 have different biological functions, sequences and structures. Their one common property is their partial sequence identity with other proteins from diverse sources. The venom allergen that has partial sequence identity with the largest number of other proteins is vespid antigen 5. It has sequence identity with mammalian testis proteins [32], human glioma and neutrophil proteins [33, 34], pathogenesis-related proteins from different plants [35], and proteins from hook worm [36], lizard [37], and fruit fly [38]. We may note in particular the partial sequence identity of venom allergens with proteins of male reproductive functions; antigen 5s with mammalian testis proteins [32], hyaluronidases with a mammalian sperm protein [7], phosphatase with a prostate enzyme [9] and protease with mammalian acrosin [11].

Several of the allergens in table 2 have been expressed in bacteria or in insect cells. The recombinant proteins which are expressed in the cytoplasm of bacteria lack the disulfide bonds of the natural proteins, and they are unfolded and do not have the native structure of the natural proteins. In some cases, the unfolded proteins can be refolded in vitro into their native structure, e.g. bee venom PLA₂ [5]. Recombinant proteins from insect or yeast cells have the native conformation of the natural proteins as they are folded into their native structure during secretion into culture medium, e.g. bee venom hyaluronidase [39], fire ant Sol i 2 and 3 [21, 22] and vespid antigen 5s [15, 18].

B-Cell Epitopes and Antigenic Cross Reactivity of Venom Allergens

Insect-allergic patients often have sensitivity to multiple insects by skin test or RAST with venoms [3, 40]. The multiple sensitivity can be due to exposure to multiple

insects and/or antigenic cross-reactivity of insect venoms. Bees, fire ants and vespids each have unique as well as homologous venom allergens. One of the four known bee allergens is homologous to vespid hyaluronidases with about 50 % sequence identity. Two of the four known fire ant allergens are homologous to vespid antigen 5s and phospholipases. Fire ant antigen 5 has about 35% sequence identity with vespid antigen 5s. Data to be given below suggest that proteins of <60% sequence identity generally do not have significant degrees of cross-reactivity.

The majority of protein-specific antibodies, 90% or more, are specific for the epitopes of the discontinuous type. Our findings with venom allergen-specific mouse antibodies support this notion; venom allergen-specific antibodies bound native allergens well but they bound poorly, if at all, unfolded allergens. This is particularly the case for vespid hyaluronidases and phospholipases [17] and to a lesser extent for vespid antigen 5 [14, 41]. Similar observations were made with venom allergen-specific IgEs from patients by comparative tests with natural or disulfide bond-reduced allergens, e.g. bee venom PLA₂ [4, 42].

Antigen 5s of hornets, yellow jackets and wasps have 58–67% sequence identity, their phospholipases have 54–68% sequence identity, and their hyaluronidases have 73–92% sequence identity. We observed that cross-reactions of vespid hyaluronidases were readily detectable with mouse antibodies, those of vespid antigen 5s were variably detected and those of vespid phospholipases were barely detectable. We may note the different extents of cross-reactions for homologous antigen 5s or phospholipase, although their homologous proteins have similar ranges of sequence identity.

Cross-reactions are more likely to be detected with antibodies specific for the unfolded proteins than those specific for the native proteins [17]. This difference in the specificity of antibodies is probably associated with the spatial distributions of the amino acid residues in native and unfolded proteins. Homologous proteins of >30% sequence identity can have the same secondary and tertiary structures [43] but their surface topographies differ. The majority of sequence variations of homologous proteins, as well as their B-cell epitopes, are located on their surface [44].

The amino acid residues of discontinuous B-cell epitopes of a protein can be determined only by studies with the protein-antibody complex. This requires the availability of monoclonal antibodies. One discontinuous epitope of birch pollen allergen Bet v 1 was established in this manner by X-ray crystallography [45]. The continuous B-

cell epitopes can be mapped readily with a series of overlapping peptides of 7–20 residues in length. Multiple epitopes were found for the 204-residue hornet antigen 5 [46], and only one was found for the 26-residue bee venom melittin [47]. No unusual pattern of amino acid sequence was observed for these B-cell epitopes.

Bee venom PLA₂ is a glycoprotein. Its oligosaccharide side chain has been demonstrated to function as a B-cell epitope for IgE and IgG responses in patients as well as in animals [48]. Oligosaccharide side chains of closely similar sequences to those of bee venom protein are present in plant proteins, and this may be one explanation for the cross-reactivity of glycosylated allergens from diverse sources.

T-Cell Epitopes of Venom Allergens and Their Possible Use in Immunotherapy

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 native or denatured venom allergens as shown by their identical T-cell stimulating activities, e.g. vespid antigen 5s, hyaluronidases and PLA₁.

Bee venom PLA₂ 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 [49, 50] or in mice [51, 52]. Because of MHC class II restriction, patients of different polymorphic background, or mice of different haplotypes, differ in their pattern of peptide recognition. Both bee PLA₂ and hornet antigen 5 were found to have several dominant T-cell epitopes recognized by nearly all patients or mice tested. Normal and atopic people were found to recognize the same T-cell epitope peptides of bee venom phospholipase [50].

One T-cell epitope-containing peptide of bee venom phospholipase was found to require the presence of its carbohydrate side chain for its activity [53].

One of the dominant T-cell epitope-containing peptides of hornet antigen 5 was found to cross react with the homologous peptides of yellow jacket and wasp antigen 5s and a mouse testis protein. The cross-reactivity with the mouse protein is not reciprocal as the corresponding peptide from mouse testis protein did not cross-react with hornet antigen 5-specific cells [52]. When male or female mice were immunized with hornet antigen 5, indistinguishable antibody titers were obtained. The cross-react-

ing T-cell epitope-containing peptide of hornet antigen 5 has a high degree of sequence identity with other antigen 5s, human and mouse testis-specific proteins, and hookworm, plant and lizard proteins.

Treatment of mice with T-cell epitope-containing peptides under appropriate conditions induced T-cell anergy with accompanying downregulation of antibody response [54, 55]. As T-cell peptides are not allergenic, they have potential as safe reagents for immunotherapy. This approach was tested recently in patients with T-cell peptides of bee venom PLA₂ [56].

Bee-allergic patients were treated with a mixture of three dominant T-cell peptides of phospholipase for two months. Three of the five peptide-treated patients were protected on challenge with a live sting. Peptide- or venom-treated patients showed similar cellular changes: their peripheral blood mononuclear cells showed decreased proliferative response and decreased production of cytokines on peptide or phospholipase stimulation. Peptide-treated patients showed no change in their levels of specific IgE or IgG4 while venom-treated patients showed increases of these antibodies. However, both groups of patients showed increased ratios of their specific IgG4 to IgE. Protection to sting challenge in treated patients is believed to be due to decreased basophil releasability as the anergic T cells do not provide adequate amounts of cytokines to prime the effector cells for full activity or mediator release [56].

Similar findings were made with allergic patients treated with dominant T-cell epitope-containing peptides of the major allergens of cat and ragweed pollen [57, 58]. There were changes in the pattern of cytokine release but no changes in antibody levels. The different findings from mice [54, 55] and patients [57, 58] may be due to dose and length of treatment. It may also reflect the difficulty for T cells to modulate memory B cells as compared with naive B cells [59].

Modified Allergens as Vaccines

Immunotherapy is an effective way for treatment of insect allergy [60]. The exact mechanism for symptom reduction following immunotherapy with increasing doses of insect venom is not known. Patients on venom immunotherapy show a rapid change in the proliferative response and cytokine release of their peripheral blood mononuclear cells following allergen stimulation, and allergen-specific IgGs rise rapidly while venom-specific IgEs decline gradually [3, 61]. It is not known whether the

symptom relief is related primarily to decreased inflammatory responses associated with changes in the type and level of cytokines, decreased IgE antibodies, allergen removal by protective antibodies, and/or some other factors.

The amount of venom that can be used on immunotherapy is limited by possible systemic reaction. Modified allergens with low allergenicity yet retaining the desired immunogenic or immunosuppressive properties can be useful vaccines. Allergenicity can be reduced by decreasing the B-cell epitope density of an allergen, as mediator release from basophils or mast cells requires the interaction of a multivalent allergen with cell-bound specific IgE. One approach is to use partially or fully denatured allergens [62] as the dominant B-cell epitope of the discontinuous type depends on the native structure. But denatured proteins do not induce antibodies specific for the native proteins. Cell culture studies showed that denatured bee phospholipase, in contrast to native phospholipase, was exclusively presented by monocytes and induced a Th1 cytokine profile leading to IgG4 production [63]. These observations suggested the use of denatured allergens as vaccines. Treatment of ragweed-sensitive patients with the denatured ragweed allergen did lead to reduced proliferative response but there was no change in antibody response or symptom reduction [64].

Another approach is to use single or multiple residue-mutated allergens. If key residues of a B-cell epitope region are known, or can be inferred from their surface accessibility, mutation of one or more residues is sufficient to suppress its binding of antibodies. This was

shown recently with the major allergen of birch pollen; a single mutation decreased its binding of specific IgEs in a serum pool of allergic patients [45].

We tried a different approach with allergen hybrids. 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 as antigens they are poorly cross-reactive. 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.

We tested the above approach with hybrids of yellow jacket and wasp antigen 5s [65]. These two antigens 5s have 59% sequence identity and they are poorly cross-reactive in patients or in animals. Hybrids with one-quarter of yellow jacket antigen 5 and three-quarters of wasp antigen 5 showed a 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. The hybrids were also useful to map the dominant discontinuous B-cell epitope-containing regions of a protein on inhibition analysis with polyclonal antibodies. At present, the only way to map such epitopes is by X-ray crystallography of antigen-antibody complexes, and it requires the use of monoclonal antibodies.

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