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Pharmacological and Immunological Properties of Wasp Venom

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Additional information is available at the end of the chapter

1. Introduction

Animal toxin envenomations have medical as well as ecological significance. Toxin-producing animals are categorized under either venomous group or poisonous group. Venomous animals are capable of producing and delivering the toxin during a biting or stinging act whereas poisonous animals are those whose tissues, either in whole or in part, are toxic. [1] About 75% of the world's animal species are arthropods—a few of which have appreciable interaction with humans and is capable of causing significant medical problems. [2] Hymenopterous insects, snakes and spiders are the three animal groups most often responsible for human deaths attributable to venomous animals. [1] However, the evolution of venom in these animals has its own purpose of balancing the ecosystem and maintaining its position in the food chain. Hymenoptera is an insect order under phylum arthropoda. It is the third largest of all insect order, and perhaps the most beneficial to humans. The order Hymenoptera comprises approximately 115,000 described species which includes wasps, bees, ants, ichneumons, calchids, sawflies etc. Collectively, the Hymenoptera are most important to humans as pollinators of wild and cultivated flowering plants, as parasites of destructive insects and as makers of honey and beeswax. Nonetheless, the order poses significant public health concern as well. [3] The three medically important group of stinging insect of the order Hymenoptera belong to the families of Apidae (bees), Vespidae (paper wasps, hornets and yellow jackets, commonly referred as *wasps*) and Formicidae (ants). [4] The sting from these social wasp become clinically significant if the patient is allergic to Hymenoptera venom or if the patient is exposed to large quantity of the venom due to massive or multiple stings. Most deaths related to wasp stings are the result of immediate hypersensitivity reactions causing anaphylaxis. A single sting is sufficient to cause fatal anaphylaxis in hypersensitive patients. Massive enve-

nomation can, likewise, cause death in non-allergic individuals, probably due to the toxic effects of the venom. A wide range of clinical sequelae is observed during wasp stings—from simple allergic skin manifestations to severe systemic reactions and toxic reactions leading to death. [5] Wasps, being highly diverse insects, are solitary or social, parasitic or predatory, phytophagous or carnivorous or omnivorous.
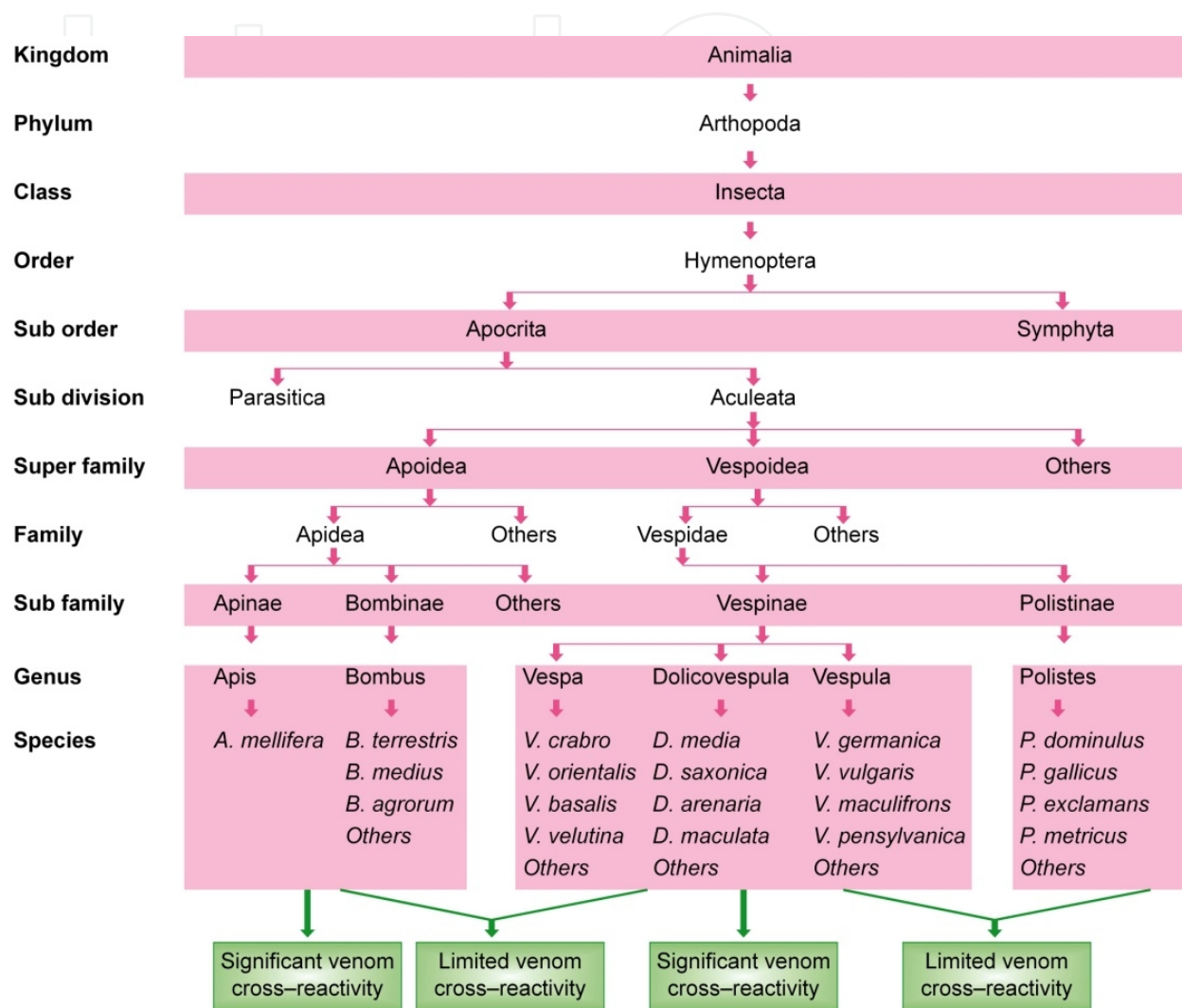

Figure 1. Taxonomy of Hymenoptera and cross reactivity of their venoms.

2. Epidemiology of stings

The insect order Hymenoptera is established on every continent except Antarctica. [6] In countries with predominantly moderate climate, they are present in the environment for a larger part of the year. [7] The season for wasp starts from spring and lasts till early fall. The stinging incidents are high during late summer and early fall with the numbers reaching a peak in August. [5] Various environmental factors such as temperature, humidity, solar radiation, rainfall and

wind speed influences the wasp activity. Activities occurred at all temperatures above 7°C and below 41°C with maximum activity occurring between 20 – 35°C. [8]

The epidemiology of hymenopteran stings occur often throughout the world; more prevalent in adult males involved in outdoor occupations or hobbies. Among the hymenopterans, the sting is more often from vespids, particularly paper wasps and yellow jackets (not hornets), than apids, such as bumblebees and honeybees. [9] Single stinging events usually occurs when large number of hungry wasps are attracted to the food of humans eating outdoor or if it is accidentally stepped on, swatted or otherwise disturbed. In contrast, mass stinging events occur when wasps respond to a human intruder as a threat to their colony, for example, when someone inadvertently stumbles into a colony or otherwise disturbs their hives by throwing rocks at or shooting at or chopping a tree containing the colony. [10] Many times the incident takes place when the adults set fire on the colony to collect the larva which is considered very nutritious. Such practice is very common in the countryside.

Limited and under-estimated data exist on the epidemiology of hymenopteran stings. Depending upon the climate, 56.5 – 94.5 % of the general adult population remember receiving hymenoptera sting at least once in his life. The prevalence of sensitization, which is indicated by a positive skin test and/or detection of specific IgE in patients with no previous case history, is estimated between 9.3 – 28.7 % in adults. The prevalence of large local reactions in the general population ranges from 2.4 – 26.4 %, up to 38 % in beekeepers. European epidemiological studies reports a prevalence of systemic reactions between 0.3 – 7.5 % among the adults whereas in the USA, the prevalence ranges form 0.5 – 3.3%. [7,11]

3. Components present in the wasp venom: Classification, list, structure and function

Wasp venom components are generally categorized as: a) high molecular weight proteins that includes phospholipases, hyaluronidases, antigen 5 etc.; b) low molecular weight peptides that includes mastoparans, wasp kinins and chemotactic peptides, and c) bioactive molecules such as histamine, serotonin, catecholamines, acetylcholine, tyramine etc..

Vespid venom is more variable in their composition among the species, different to that of apid (bee) venom. They are complex mixture of powerful allergens and pharmacologically active compounds, primarily made up of proteins. The vespid venom contains three major proteins that act as allergens and a wide variety of vasoactive amines and peptides. The important allergens are antigen 5, phospholipases and hyaluronidase. Antigen 5 is the major allergen in all vespid venom and has been most thoroughly studied among the others. [4] Two additional proteins, Vmac 1 and Vmac 3 from *V. maculifrons*, with allergenic activity have been described, but are incompletely characterized. [4, 12] Similarly, serine-protease has been identified as an important allergen for vespid-allergic individuals in European *Polistes* [13, 14] venom and dipeptidylpeptidase IV [15] and vitellogenin [16] in *V. vulgaris* venom. The vasoactive amines in vespid venom includes serotonin, histamine, tyramine and catechola-mines. Wasp kinins and mastoparans are the peptides unique to vespid venom.

3.1. Antigen 5

Animal tests have shown that antigen 5 is not a toxin. [17] It is a member of a conserved family of proteins found in eukaryotes, including yeasts and have sequence identity with other proteins of diverse origin and tissues, such as mammalian cystein-rich secretory proteins in salivary and reproductive organs, secretory proteins of helminths produced during sexual maturation, human brain tumor proteins, reptile venom, pathogensis-related proteins of plants and fire ant venom. [17, 18] The mature antigen 5 from yellow jacket and hornet have 201 and 205 amino acids respectively, with several highly conserved regions. Almost all of the sequence variations seen in hymenoptera antigen 5 were found on the surface. The highly cross reactive groups within the genera have few changes. The antigen 5 homolog from ants do not exhibit antigenic cross reactivity with those from vespid wasps due to the low degree of surface conservation and changes in loop lengths. [18] However, in hyperimmune sera, occasionally, antigenic cross reactivity has been observed between vespid antigen 5 and homologs from other animals. [19]

3.2. Phospholipases

The wasp venom phospholipase (PL) belongs to a different superfamily than those of bee venom phospholipase. Vespid wasp phospholipases have PLA_1B specificity and are members of GX class lipase, lipoprotein lipase superfamily, pancreatic lipase homologous family and RP2 sub-group of phospholipase. [18, 20] The PLs from vespid wasp venom usually do not contain carbohydrate and have highly homologous regions surrounding the active sites. The cross reactivities of the PLs generally follow the phylogeny: closely related species are highly cross reactive and those that are further removed are less cross reactive.
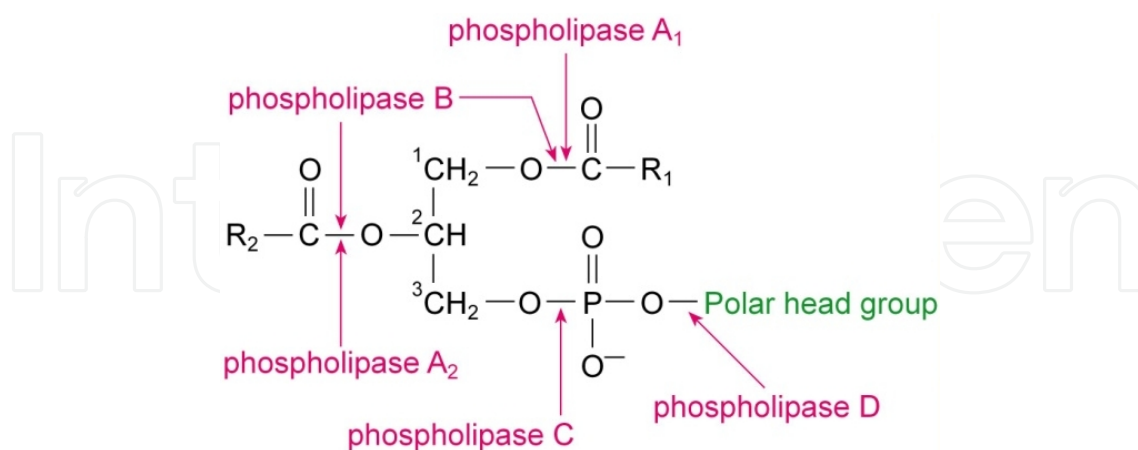

Figure 2. Site of Phospholipase action.

The characterized PLs in vespid venom are PLA_1, PLA_2 and PLB. The vespid venom PLs has an offensive as well as defensive role. The venom is not only used as toxins for preyed insects,

but also digests their cell wall components of diacylphospholipids such as phosphatidylcholine, phosphatidylserine and phosphatidylethanolamine to fatty acids and lysophospholipids by the containing PLs. PLBs are more universally digestive enzymes than PLA_1 and PLA_2, which seems to be a kind of enzymatic adaptation of the carnivorous insects, i.e, vespids against vegetarian insects, i.e, apids that contain only PLA_2 in the venom. Besides the enzymatic activity, PLBs also possesses the haemolytic activity and cardiotoxicity. [21]

Phospholipase A_1 (PLA_1) is an enzyme that hydrolyzes ester bonds of phospholipids at the *sn*-1 position and produces 2-acyl-lysophospholipids and fatty acids. Vespid-venom PLA_1s belong to the pancreatic lipase family and exhibit PLA_1 activities but do not show any lipase activities. The tertiary structure of lipases have two surface loops, the lid and the β9 loop, which covers the active site and are implicated in substrate recognition. The amino acid sequence alignment of the pancreatic lipase family members (eg, phosphatidylserine-specific PLA_1 (PS-PLA_1), membrane-associated phosphatidic acid-selective PLA_1 (mPA- PLA_1), vespid PLA_1, hepatic lipase (HL), endothelial lipase (EL), pancreatic lipase, and pancreatic lipase-related protein 2 (PLRP2)) revealed two molecular characteristics of PLA_1s: first, lipase members exhibiting PLA_1 activity have short lids; and second, lipase members exhibiting only PLA_1 activity have short β9 loops. Thus, pancreatic lipase and LPL which exclusively exhibit triacylglycerol lipase activity have long lids and long β9 loops, while PS- PLA_1, mPA- PLA_1, vespid PLA_1 which only shows PLA_1 activity have both short lids and short β9 loops whereas EL and PLRP2 which exhibit both PLA_1 and triacylglycerol lipase activity have short lids but intact β9 loops [22, 23] PLA_1, thus, possess direct cytolytic effects, besides their role in allergic and inflammatory processes.

PLA_2 catalyzes the specific hydrolysis of ester bonds at the *sn*-2 position of 1,2-diacyl-3-*sn*-glycerophospholipids into their corresponding lyso compounds with release of free fatty acids. Thus, it is able to disrupt the phospholipid packings from several types of biological membranes leading to pore formation and/or cell lysis. [20, 24] Vespid PLA_2 has very potent cytolytic actions.

3.3. Hyaluronidase

Hyaluronidases (Hyal) are a widely distributed glycoside hydrolases that cleaves β-1,4-glycosidic bonds between N-acetylglucosamine and D-glucuronic acid of hyaluronic acid (HA) [14], one of the primary components of the extracellular matrix in all the vertebrates. They are also present in almost all venoms, acting as a "spreading factor" by facilitating the penetration of the other harmful venom components and enhancing their action in various tissues into the bloodstream. They are the "allergenic factors" in vespid and apid venom and are able to induce severe and fatal anaphylactic IgE-mediated reactions in humans. [25] They are the phylogenetically most strongly conserved Hymenoptera allergens. Sequence homologies between *Vespula* and *Dolichovespula* species hyaluronidases are 90% or greater, whereas those for antigen 5 and PLA_1 are only around 60% to 65%. In agreement with this, immunologic cross-reactivity between different vespid genera is strong with hyaluronidases but more restricted with antigen 5 and PLA_1. Vespid hyaluronidases are significantly similar with honey bee hyaluronidase which shows 50% sequence homology with vespid homologs Ves v 2, Ves

g 2 and Dol m 2. In accordance with this, hyaluronidases have been identified in inhibition studies using patients' sera as the most important cross reactive allergens in yellow jacket and honeybee venom. [14]

Hyaluronidase of wasp venom is an allergen. The asparagine-linked carbohydrate often appears to constitute the common IgE-binding determinant. Irrespective of the nature of the protein, protein-linked glycans can bind IgE, which turns many proteins, especially those of higher molecular mass, into apparent allergens. Hyaluronidase is the dominating glycoprotein in the wasp venom and contains α-1,3-fucose-containing N-glycan which is responsible for allergenicity. The cross-species survey performed by Kolarich et al shows that venom from six wasp species (*V. vulgaris, V. germanica, V. flavopilosa, V. maculifrons, V. pensylvanica* and *V. squamosa*) contained the difucosylated paucimannosidic N-glycans MUF³F⁶ and MMF³F⁶ as the major glycan structures. [26] The allergic response is initiated by the epitope that cross-links the Fc-receptor-bound IgE antibodies on the surface of mast cells. This is followed by rupture of mast cell membrane and the release of stored mediators, such as histamine, which are responsible for the immediate type hypersensitivity reaction. [27]

Hyaluronidases, on the basis of mechanism of action, are classified into three classes: a) the group of endo-β-N-acetyl-D-hexosaminidases that hydrolyse the high molecular weight substrate (HA) to tetrasaccharide as the main end product, represented by the testicular enzymes; b) the β-endoglucuronidases group represented by hyaluronidases from leeches and hookworm, and; c) the group of lyases that act via β-elimination, yielding disaccharides as the main products represented by the bacterial hyaluronidases. The enzymes of the first class also catalyses transglycolation reactions, producing hexa-, di- and octa- saccharides during hydrolysis of HA. Hymenoptera venom hyaluronidases belong to the first class. Unlike the latter two classes of hyaluronidases, this first class acts not only on HA, but also on chondroitin 4-sulfate and chondroitin 6-sulfate. [25]
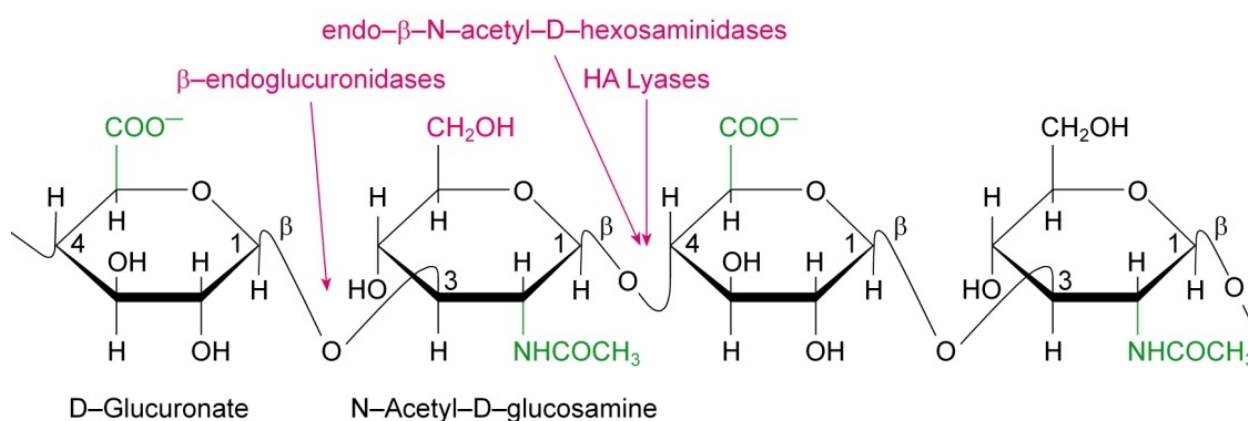

Figure 3. Hyaluronic Acid and Site of Action of Different Hyaluronidases.

3.4. Mastoparan

Mastoparans are low molecular weight peptides, generally tetradecapeptides, extracted from the venom sac of social wasps that act in the defense system of these insects. They are rich in hydrophobic and basic residues which are distributed in the peptide chain in such a way that, in adequate environment, they form amphipathic helical structures [28] which favours electrostatic interactions with the negatively charged phospholipid head groups of the biological membranes. This characteristic may lead to peptide insertion into the membrane bilayer and thus interact directly with G proteins on the cytoplasmic face attacking the transmembrane signalling [29] and sometimes to membrane destabilization with its consequent lysis. [30, 31] These peptides, thus, present important biological activities such as antimicrobial, mast cell degranulation, haemolytic activities, [28] activation of G-protein mediated mechanisms, stimulation of phospholipase A_2, C and D, mobilization of Ca^{2+} from mitochondria and sarcoplasmic reticulum, activation of ryanodine receptor, modulation of various enzymes, such as Na^+-K^+-ATPase of rat brain, induction of the mitochondrial permeability transition and cell death by necrosis and apoptosis. [32]

Mastoparans are discovered in wasp venom in a screening test for mast cell degranulating agents. They are also the potent stimulants of purified PLA_2 from different sources. They bind to phospholipids making them the better substrates. They facilitate the PLA_2 of both venom and victim, thereby promoting generation of arachidonic acid, the precursor of prostaglandins and leukotrienes which are mediators of adverse reactions associated with immediate type hypersensitivity. The high affinity binding of mastoparan to calmodulin has led to speculate the role of the peptides in inhibition of calmodulin-mediated reactions. [33] Mastoparan is a potent stimulator of exocytosis from diverse mammalian cells. It causes secretion of histamine from mast cells, serotonin from platelets, catecholamines from chromaffin cells and prolactin from the anterior pituitary. In case of histamine secretion, the effect of mastoparan is mediated by an increase in cytoplasmic Ca^{2+} that is itself caused by an increase in the intracellular second messenger inositol-1,4,5-triphosphate (IP_3). Such IP3-mediated increase of intracellular Ca^{2+} is controlled at the level of phospholipase C (PLC) by one or more of a group of GTP-binding regulatory proteins, or G proteins. [29] Mastoparan induced apoptosis or oncosis is initiated by Ca^{2+} release from intracellular release from intracellular stores via PLC and IP3, and the disruption of plasma membrane integrity occurs secondarily. [34] Mastoparan, an activator of G_i and mast cells, selectively stimulates an PLD_2, independently of G_i, ADP-ribosylation factor-1 (ARF-1), protein kinase C and calcium, in intact cells and in isolated preparations enriched in plasma membranes where PLD_2 is located. [35] PLD catalyses the hydrolysis of the major membrane phospholipid, phosphatidylcholine (PC) to generate phosphatidic acid (PA) and choline. PLD is involved in the exocytosis of secretory granules from mast cells and neutrophils. One possible function of PLD that would rationalise its role in exocytosis may be related to the ability of PA to regulate PI(4)P 5-kinase. PI(4)P 5-kinase is one of two kinases required for the synthesis of PIP_2 (PI3). This unique lipid is essential for many membrane trafficking events including exocytosis. The product of PC hydrolysis by PLD is PA, which therefore has the potential to dynamically regulate the synthesis of PIP_2 in specific membrane compartments, ie, where PLD is active. [36]

Mastoparans: Vespinae

Origin	Name	Amino acid sequence
Paravespula lewisii	Mastoparan	INLKALAALAKKIL·CO-NH$_2$
Vespa mandarinia	Mastoparan-M	INLKAIAALAKKLL·CO-NH$_2$
Vespa xanthoptera	Mastoparan-X	INWLQIAAMAKKLL·CO-NH$_2$
Vespa analis	Mastoparan-A	IKWKAILDAVKKVL·CO-NH$_2$
Vespa tropica	Mastoparan-T	INLKAIAAFAKKLL·CO-NH$_2$
Vespa orientalis	Mastoparan-II	INLKALAALVKKVL·CO-NH$_2$
Vespa basalis	Mastoparan-B	LKLKSIVSWAKKVL·CO-NH$_2$
Mastoparans: Polistinae		
Polistes jadwigae	Polistes mastoparan	VDWKKIGQHILSVL·CO-NH$_2$
Parapolybia indica		INWAKLGKLALEVI·CO-NH$_2$
Ropalidia sp.		INWSKLLSMAKEVI·CO-NH$_2$
Protonectarina sylveirae	Protonectarina mastoparan	INWKALLDAAKKVL·CO-NH$_2$
Agelaia pallipes pallipes	Agelaia-MP	INWLKLGKAIIDAL·CO-NH$_2$
Polybia paulista	Polybia-MPI	IDWKKLLDAAKQIL·CO-NH$_2$
Protopolybia exigua	Protopolybia MPI	INWLKLGKKVSAIL·CO-NH$_2$
Protopolybia exigua	Protopolybia MPII	INWKAIIEAAKQAL·CO-NH$_2$
Protopolybia exigua	Protopolybia MPIII	INWLKLGKAVIDAL·CO-NH$_2$
Polistes rothneyi iwatai	Polistes-mastoparan-R 1 [Pm-R1]	INWLKLGKKILGAI·CO-NH$_2$
Polistes rothneyi iwatai	Polistes-mastoparan-R 2 [Pm-R2]	LNFKALAALAKKIL·CO-NH$_2$
Polistes rothneyi iwatai	Polistes-mastoparan-R 3 [Pm-R3]	INWLKLGKQILGAL·CO-NH$_2$

Table 1. Amino Acid Sequence of Mastoparans in the Venom of Vespinae and Polistinae

3.5. Wasp kinins

Wasp kinins are of interest because two kinins- bradykinin (BK) and lysyl-bradykinin or kallidin occur in humans, produced by plasma kallikreins and tissue kallikreins respectively. The peptides are generated and act locally in humans but are stored in venom. They are important mediators of inflammatory responses, potent pain producers and increase vascular permeability and vasodilatation. [33, 37, 38] Bradykinin is a nonapeptide usually found in body secretions such as urine, saliva and sweat. They are also found in several tissues such as heart, vasculature, blood, kidney, colon and liver. [39]

Wasp kinins are polypeptides (9-18 amino acid residues) containing a bradykinin-like sequence at the C-terminal. In some cases the whole nonapeptide sequence of bradykinin is present within the wasp kinin sequence. The primary sequence of most kinin related peptides

from animal venom are longer with potent pharmacological actions and long lasting effects compared to bradykinin. Due to a greater taxonomic diversity, a series of different bradykinin-related peptides (wasp kinins) have been identified in the venom from different species of wasps. Neurotoxic kinins, such as threonine-bradykinin (Thr[6]-BK) and megascoliakinin (Thr[6]-BK-Lys-Ala) and glycosylated wasp kinins have been described. Wasp kinins are experimentally involved in constriction and relaxation of muscles, activation of leukocytes followed by a release of cytokines, prostaglandins, leukotrienes, reactive oxygen species and the blockage of the cholinergic transmission in the insect central nervous system. [38]

Peptides	Amino acid sequence
Bradykinin	**RPPGFSPFR**
Protopolybiakinin-I	DKNKKPIRVGGR**RPPGF**T**PFR**
Protopolybiakinin-II	DKNKKPIWMAGFP**GF**TPI**R**
[Thr[6]] Bradykinin	**RPPGF**T**PFR**
Vespakinin-M	G**RP**X**GFSPFR**ID
Vespakinin-X	A**RPPGFSPFR**IV
Vespakinin-A	G**RPPGFSPFR**VI
Vespakinin-T	G**RP**X**GFSPFR**VV
Polisteskinin-3	pETNKKKLRG**RPPGFSPFR**
Polisteskinin-R	AR**RPPGF**T**PFR**
Polisteskinin-J	RR**RPPGFSPFR**
Polisteskinin-C	SK**RPPGFSPFR**

Table 2. Amino Acid Sequence Alignments of Bradykinin, Protopolybiakinins and Some Wasp Kinins

The general pharmacological effects of kinins have been attributed through two G protein coupled receptors—B_1 or B_2 receptors. Intact BK is the characteristic agonist for the B_2 receptors, whereas kinin metabolites such as Lys-des-Arg[9]-BK or des-Arg[9]-BK produced by neuronal endopeptidase action activate the B_1 receptor which is far less expressed in normal tissues. [40]

Wasp kinins impart its pharmacological effects via B_2 receptor activation. [41] When injected into the vertebrate predators by stinging, they produce severe pain, thus producing a significant role in their defense system. On the other hand, they are used to irreversibly paralyse the prey. [42]

3.6. Other bioactive molecules

There are many other bioactive molecules in the wasp venom such as histamine, 5-HT, acetylcholine, tyramine, catecholamines, and various peptides. Bioamines such as histamine, 5-HT, acetylcholine, tyramine, catecholamines etc are only a minor portion of the wasp venom, however their pronounced reaction is due to the endogenous release initiated in the victim by

other factors such as mastoparans, wasp kinins and phospholipases. [38] Peptides carry out important biological processes in venom. Their activites are very diverse and range from neurotoxic and inflammatory to antibacterial. Besides, mastoparans and wasp kinins discussed above, several other peptides includes protonectins, mandaratoxins and chemotactic peptides. Chemotactic peptides recruit macropahages and polymorphonuclear leukocytes near the site of stinging. Protonectins are the mast cell degranulating peptides responsible for histamine release. Several other peptides have been identified with antibacterial properties. [43]
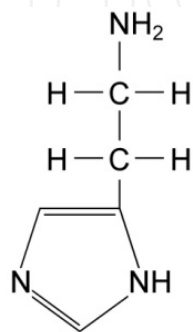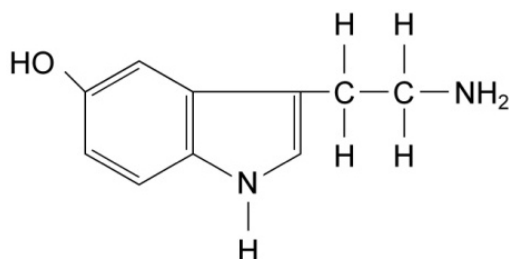
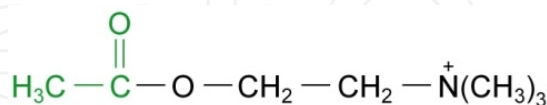**Figure 4.** Histamine.
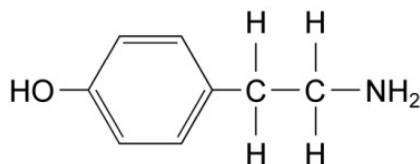
Figure 5. Serotonin.
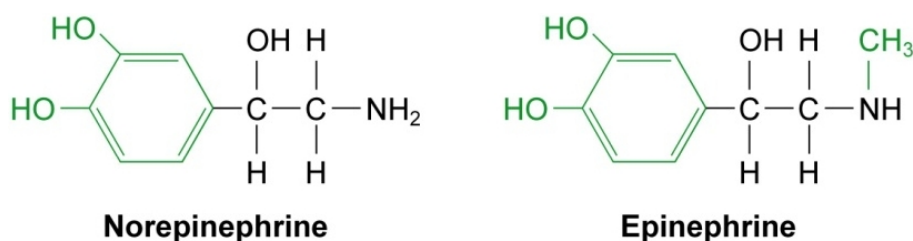
Figure 6. Acetylcholine.

Figure 7. Tyramine.

Figure 8. Catecholamines.

Protonectins: Polistinae		
Origin	**Name**	**Amino acid sequence**
Protonectarina sylveirae	Protonectin	INWLKLGKKILGAI·CO-NH$_2$
Polybia paulista	Polybia-CP	LNFKALAALAKKIL·CO-NH$_2$
Polistes rotheyi iwatai	Polistes-protonectin	INWLKKLGKQILGAL·CO-NH$_2$

Table 3. Amino Acid Sequence of Protonectins in the Venom of Polistinae

4. Venom allergens, hypersensitivity reactions and lethality

Antigens that elicit clinical allergic reactions are typically referred to as allergens. [44] The major allergens identified in the vespid venom are phospholipase A$_1$, hyaluronidase and antigen 5. The minor allergens described are Vmac 1 and Vmac 3 from *V. maculifrons*, [18] serine protease from *Polistes spp*. [44] Vmac 3, the band between hyaluronidase and phospholipase on SDS-PAGE, appears to be a variant form of hyaluronidase on protein sequencing results, however the high molecular weight fraction Vmac 1 which is yet not well characterized contains significant amounts of carbohydrate. [18] Mastoparan, a tetradecapeptide, also elicit some immunological properties. Immunochemical studies with the vespid venoms have elucidated that the major immunogens or allergens are those of large molecular weight venom components such as hyaluronidases, phospholipases and antigen 5. Small peptides are generally poor immunogens. [45] Both carbohydrate-based epitopes (glycotopes) and protein-based epitopes are able to inflict immune responses. [46] Phopholipases and hyaluronidases are glycoprotein where cross-reactive carbohydrate determinants (CCDs) play major role in inflicting immune responses whereas antigen 5 does not contain carbohydrates and inflicts immune responses via its peptide-epitopes. Epitopes are a particular portion of an allergen that specifically binds serum IgE. Structurally, epitopes can either be linear or conformational. Linear (continuous) epitopes are defined by the primary amino acid sequence of a particular region of a protein. The sequence that interact with the antibody are situated next to each other sequentially on the protein. Conformational epitopes consist of amino acids which are in close proximity while the protein is folded correctly. They may be continuous or discontinuous, i.e,

the amino acids may be situated next to each other or are found on multiple regions in the primary structure. Most allergenic epitopes are conformational. [44]

There are four types of hypersensitivity reactions, viz:

a.   Type I hypersensitivity reaction (IgE mediated),

b.   Type II hypersensitivity reaction (IgG mediated),

c.   Type III hypersensitivity reaction (Immune complex mediated), and

d.   Type IV hypersensitivity reaction (Cell mediated)

Hymenoptera sting reactions are classified into a) normal local reactions, b) large local reactions, c) graded systemic reactions, d) systemic toxic reactions and e) unusual delayed reactions. [47, 48] Hypersensitivity reactions of type I, III and IV have been found to involve in sting reactions. Local reactions are type IV hypersensitivity IgG-mediated reactions and are either focal or large local. Systemic reactions are IgE mediated type I hypersensitivity reactions and are graded from I to IV depending upon the presence of one of the following respectively: urticaria, angioedema, airway obstruction, or anaphylaxis. Unusual delayed reactions are IgG- and IgM-mediated type III reactions which include serum sickness, vasculitides, central nervous system signs and symptoms, haemolytic events, myocardial infarction, disseminated intravascular coagulation, and acute kidney injury. Immune complex mediated type III reactions are the indirect cause of systemic toxic reactions in wasp envenomation. [48, 49]

Wasp stings are usually not fatal. The commonest manifestations are related to allergy and low grade systemic reactions characterized by pain, swelling, urticaria and redness at the sting site that usually lasts for 1-2 days. [50] Large local reactions are late phase manifestations that may involve a large area and persist up to a week. They are not life threatening unless they involve the airway. They may result in considerable morbidity because of temporary loss of function, such as occurs when the sting involves a foot or hand or is near eye. [51] These local reactions generally resolves without any treatment. [52] Death from wasp envenomation is a rare event – most often it is caused by IgE mediated type I anaphylaxis attributed even to a single sting in venom allergic individuals. Hence, these local reactions and anaphylaxis are not dose dependent or related to number of stings. Less commonly, in non-allergic individuals, death occurs from the toxic effects of massive envenomation involving hundreds to thousands of stings. Such toxic reactions are venom volume dependent. However, it is noteworthy that wasp venom toxicity varies among and even within the species which is due to the variation in the composition and the quantity of the venom released. *Vespula*, *Dolichovespula* and *Polistes* stings release 1.7 – 3.1 µg, 2.4 – 5.0 µg and 4.2 – 17 µg of venom proteins, respectively. [47] Mammalian toxicity tests on mice has revealed that wasp venom has more deleterious effect than that of bee venom despite of the fact that wasps inject less venom per sting than bee (50 µg – 140 µg). Organ dysfunction, eg, renal failure, and death may occur in the range of ~20 – 200 wasp stings and ~150 – 1000+ bee stings. [10]

Classification of reactions	Hypersensititvity reactons	Onest times	Reacting Igs	Clinical manifestations
Local	IV	4-48 h	Cell-mediated IgG	Painful, pruritic, and edematous sting lesions, 2.5-10cm in diameter, lasting <24 h
Large local	IV	4-48 h	Cell-mediated IgG	Painful, pruritic, and edematous sting lesions, >10cm in diameter, lasting >24h
Systemic grade I: urticarial	I	10-20 min up to 72 h	IgE	Anxiety, malaise, generalized urticaria, itching
Systemic grade II: angioedema	I	10-20 min up to 72 h	IgE	Any grade I signs above, plus ≥ 2 of the following: angioedema (grade II if alone), dizziness, vomiting, diarrhea, chest tightness, abdominal pain.
Systemic grade III: airway obstruction	I	10-20 min up to 72 h	IgE	Any grade II signs above, plus ≥ 2 of the following: stridor, dyspnea, wheezing (grade III if any of these alone), hoarseness, dysarthria, dysphagia, weakness, confusion
Systemic grade IV: anaphylactic	I	10-20 min up to 72 h	IgE	Any grade III signs above, plus ≥ 2 of the following: unconsciousness, hypotension, cardiovascular collapse, cyanosis, urine and /or fecal incontinence
Unusual delayed reactions	III	2-14 day	IgM, IgG	Serum sickness, generalized vasculitis, rhabdomyolysis, acute tubular necrosis, Central nervous system involvement (seizures, neuritis, peripheral neuropathy or radiculopathy, cerebrovascular accident), hemolysis, thrombotic thrombocytopenic purpura, Disseminated intravascular coagulation, Myocardial infarction

Table 4. Classification of Hymenoptera Sting Reactions [48]

4.1. Venom allergens and their systematic nomenclature

The venom allergens have been assigned official names by the International Union of Immunological Studies, Allergen Nomenclature Subcommittee. Vespid venom proteins are assigned

1 to phospholipase A_1B, 2 to hyaluronidase, 3 to dipeptidylpeptidase IV, [15, 53] 4 to venom protease, 5 to allergen 5, and 6 to vitellogenin. [16, 18, 54] Allergens are designated according to the accepted taxonomic name of their source as follows: the first three letters of the genus, space, the first letter of the species, space and an Arabic number. Numbers are assigned to allergens in the order of their identification, and the same number is generally used to designate homologous allergens of related species. [55] For example, Ves v 1 refers to the first venom allergen identified from *Vespula vulgaris*.

4.2. Cross reactivity among vespid (wasp) venom

The allergic manifestation is the commonest feature of wasp sting. It includes the typical dermatologic expression incorporating edema, erythema, pruritus, urticaria and pain at and around the sting site, usually. These clinical features generally subside within a few days to a week without any treatment or simple treatment such as ice-packs and analgesics. Such allergic manifestations are not fatal and majority of the populations have such simple allergy to wasp venom. These allergic reactions become serious when the individual is hypersensitive to the wasp venom and produces the venom specific IgE. Such specific IgE binds for a particular portion(s) of an allergen, referred to as an epitope(s). Cross-reactivity is the recognition of similar or identical epitopes on proteins from different sources. The presence of the cross-reactive epitopes can make an individual appear allergic to an insect venom protein which they have not encountered. This is merely due to the structural homology between the venom proteins. Treatment of insect allergies requires the identification of the sensitizing species and the cross-reactive epitopes make the identification of the sensitizing species difficult. This error in identification of the sensitizing species could lead to incomplete or partial protection of the patient during the treatment. [44] The allergenic cross-reactivity can, both, be due to protein-based epitopes or carbohydrate-based epitopes (glycotopes). Cross-reactive carbohydrate determinants (CCDs) are the key carbohydrate molecule attached to the glycoprotein. They share significant structural homologies and are thus prone to extensive cross-reactivity between the products from various sources. [56] Generally, the N-glycans found on most hymenoptera venom proteins possess a number of non-mammalian features rendering them potentially immunogenic. However, the hallmark of CCDs on insect venom allergens comprise carbohydrates carrying α-1,3-linked core fucose residues. IgE with specificity for such CCDs are key players in allergen cross-reactivity and represents a major concern for diagnostic and therapeutic approaches, however, the role of CCDs for occurrence of allergic symptoms is still controversial. [53] These CCDs are the reason for multiple sensitivity observed in the hyper-sensitive individuals.

Cross-reactivity generally follows phylogeny: closely related/homologous species are highly cross-reactive and those that are further removed are less cross-reactive. For example, the yellow jacket antigen 5 has 69% and 60% sequence identity with the white-faced hornet and *Polistes* wasp, respectively. Similarly, yellow jacket hyaluronidase and phospholipase show 92% and 67% sequence identity, respectively with their homologs of white-faced hornets. This sequence homology confers considerable cross-reactivity with an order of cross-reaction of the three vespid allergen as hyaluronidase > antigen 5 > phospholipase. The cross-reactivity among

the yellow jacket and white-faced hornet allergen is greater than the cross-reactivity among yellow jacket or white-faced hornet and *Polistes* wasps. [57]

4.3. Physiological manifestation of stings (single sting versus multiple sting)

Stinging events involving wasp are rare and the death due to wasp sting is infrequent. However, in wasp venom hypersensitive individual, a single sting is sufficient enough to cause the clinically significant case or death, which often is caused by IgE-mediated type I anaphylaxis. These single stings occur when a single insect is disturbed while searching for food. Besides, mass stinging events by wasps also occur when someone inadvertently stumbles into their colony or otherwise disturbed their hive by throwing stones at, shooting at, or chopping trees containing their colony. In such case, hundreds or thousands of wasps may sting resulting in massive envenomation to a person leading to the venom intoxication. In case of non-allergic individuals, the toxic effects of venom due to high venom load can result into physiological changes developing in clinical sequelae. [10]

Wasp toxin anaphylaxis is a typical immediate-type allergic reaction. Specific IgE antibodies directed against components of the toxin mediates the activation of mast cells and basophilic granulocytes, leading to the release of mediators that cause acute manifestations of disease. In the great majority of cases, a single sting is the cause. The reaction usually arises 10 to 30 minutes after the sting, although the latency may be shorter or longer. The severity of anaphylaxis is graded on the basis of clinical manifestations. Most patients recover without any permanent sequelae. The main causes of death due to anaphylaxis are airway obstruction and cardiovascular failure; rarer causes are disseminated intravascular coagulation (DIC), acute kidney injury and epinephrine overdose. Myocardial infarction, stroke and thrombotic events can cause permanent morbidity. [58, 59] Besides, the immune-mediated reactions upon single or multiple stings, direct toxic effects of venom are also observed which generally are the features of mass envenomation. Such direct toxic effects are venom-volume dependent and the venom components pose for the cytolytic or cytotoxic effects. The clinical manifestations for such toxic effects include intravascular Haemolysis, rhabdomyolysis, pigment nephropathy, renal impairment, acute kidney injury, liver impairment, disseminated intravascular coagulopathy, central nervous system damage and direct toxicity to multiple organ system. [5, 10, 60]

5. Diagnostic method for sting allergy

Hymenoptera venoms are known to cause life-threatening IgE-mediated anaphylactic reactions in allergic individuals. Proper diagnosis of hymenoptera venom allergy is severely affected by molecular cross-reactivities resulting into double or multiple positive tests. [61] Double or even multiple positive tests can be caused by true double sensitisation indicating potential systemic allergic reactions to the next sting by either insect species, if not treated by immunotherapy with both venoms; or by cross-reactive IgE antibodies which recognize either peptide based epitopes of venoms or carbohydrate-containing epitopes (CCDs) in glycoprotein allergens. [62]

It is important to distinguish between cross-reactivity and true double sensitization for the choice of venom(s) for immunotherapy. [47] Since, many patients fail to identify the stinging insect, skin testing and *in vitro* detection of venom specific IgE antibodies are the only tools to detect the culprit insect involved in the allergic reaction and are used to select the appropriate venom immunotherapy depending upon the severity of clinical symptoms. [63] Cross-reactivity within the vespid venoms is strong due to similarities of venom composition and structure of single allergens. The hyaluronidase enzyme of the honeybee and wasps show 50 % sequence identity and hence has been identified as the major cross-reactive component. [47]

The sera of 20% – 50% of patients with hymenoptera venom allergy show *in vitro* reactivity with both honeybee and wasp venom. [62] This IgE positivity to both hymenoptera (honey bee and wasp) venoms is referred to as true double sensitization leading to immunotherapy against both venoms. However, there are other reasons for IgE-double positivity: a) true independent sensitization (co-sensitization) to different allergens, which is a very rare phenomenon; b) immunochemical cross-reactivity due to sequence homologies between allergens from different sources; c) cross-reactive carbohydrate determinants (CCDs) in the glycoproteins from various sources; and d) non-specific absorption of IgE to the allergosorbent, a phenomenon that is particularly relevant when total serum IgE is extremely elevated. CCDs are important antigen targets for specific IgE (sIgE) binding providing at least two different IgE-binding sites. [63] 5% of non-allergic individuals and 10% of non-pollen allergic subjects have CCD-sIgE antibodies. [64] 10%-15% patients with grass pollen allergy have CCD-sIgE antibodies which increases up to more than 60% with concomitant sensitization to pollen from trees, grasses and weeds, [63] 23% patients with honeybee venom allergy and 11% patients with yellow jacket venom allergy have CCD-sIgE antibodies. [65] IgE inhibition studies by various methods can help distinguish true double sensitization from cross-reactivity. [62] Recent advancement on diagnostic procedure incorporates the recombinant allergen based IgE testing that effectively distinguishes true double sensitization from cross-reactivity. [66]

5.1. Diagnostic strategy

5.1.1. History

Informations regarding number, date and site of stings and its reactions, sort and severity of symptoms, interval between sting and the onset of symptoms, emergency treatment, risk factors of a particular severe reactions, risk factors for repeated re-stings, tolerated stings after the first systemic reactions and other allergies, if any, should be collected. [47]

5.1.2. Skin test

Skin tests are performed by skin prick or intradermal injection of venom. It is recommended at least 2 weeks after the reaction to a sting to avoid the possibility of false negative results during a refractory period. Stepwise incremental venom skin tests are recommended. The test is stopped when the patient has a conclusive reaction at a set venom concentration. Venom concentrations of 0.01 – 100 μg/ml are generally used for skin prick test, and 0.02 ml of venom concentration ranging from 0.001 – 1 μg/ml is intradermally injected into the volar surface of

the forearm for intradermal testing. Skin prick tests have lower sensitivity than intradermal tests even at 100 μg/ml concentration; hence the patients with negative skin prick tests should be confirmed by intradermal tests. The sensitivity of intradermal test is estimated at about 90% or higher for 1 μg/ml concentration. [47]

5.1.3. In vitro tests

In vitro tests specifically diagnoses or assists in diagnosis of venom allergy, depending upon the tests performed. Number of variables can be checked *in vitro* such as allergen-specific IgE, allergen-specific IgG, baseline serum tryptase, basophil activation test, basophil histamine release test, leukotriene release test and immunoblotting.

a. *Allergen-specific IgE Assay*

Venom-specific IgE (sIgE) usually increases within days or weeks after a sting. Following this initial phase specific IgE declines slowly with a large individual variation. The test should be repeated after a few weeks in patients with no detectable specific IgE to the presumptive relevant venom. *In vitro* radioallergosorbent test (RAST) and various derived methods are used to assay the allergen-specific IgE. [47] A sIgE value of ≥ 0.35 kU/L is regarded as positive. [67] Venom sIgE tests are, however, less sensitive and specific than intradermal skin test in the patients with a history of systemic sting reactions, especially after the first year following a reaction. [47] Skin test reactivity and levels of sIgE also do not correlate with clinical reactivity and hence must be interpreted in conjunction with clinical history. [68] Similarly, double positive or multiple positive tests are conferred either by true double sensitization or by cross-reactivity of venom sIgE with certain carbohydrate ligand. The RAST inhibition test is helpful in distinguishing between true double sensitization and cross-reactivity. The sIgE assay is thus modified by including an initial inhibition phase. [47] Contrast to sIgE, serum total IgE is generally regarded as a non-specific and global marker for atopy. [69] Recently, the component-resolved analysis using recombinant species-species major allergens (rSSMA) are incorporated to improve the differentiation between true double sensitization and cross-reactivity. [70]

b. *Allergen-specific IgG Assay*

The serum level of specific IgG (sIgG) primarily reflects the exposure to allergen. Venom-sIgG increases after a sting and does not correlate with the presence or absence of an allergic sting reaction. Venom immunotherapy is accompanied by an increase in allergen-specific IgG, however neither concentration of sIgE and sIgG nor sIgE/sIgG ratio closely correlates with the clinical improvement to immunotherapy. [47]

c. *Baseline Serum Tryptase*

Tryptase is a predominant protease of human mast cells that exist in three forms: α-tryptase, pro-β-tryptase and β-tryptase. α-tryptase and pro-β-tryptase are enzymatically inactive and released continuously. A persistent rise, therefore, in serum α-tryptase is an indicator of an elevated number of mast cells and thus may indicate for mastocytosis. β-tryptase is the enzymatically active tetramer stored in mast cell granula and released during acute allergic

reactions resulting into extensive mast cell degranulation. The serum concentration of β-tryptase is thus a measure for mast cell activation. If an elevated level of tryptase is observed outside an acute allergic reaction, it indicates an increased total body mast cell load, while within the context of an allergic reaction suggests mast cell activation. [67, 71] A significant proportion of patients presenting with anaphylaxis to hymenoptera sting have an elevated (>11.4 µg/L) baseline tryptase. Such patients fall into the spectrum of "mastocytosis" and further investigations including bone marrow examination to exclude systemic mastocytosis or monoclonal mast cell activation syndrome may be necessary. It has been reported that patients with elevated baseline tryptase with or without systemic mastocytosis develop significantly more severe reactions, especially cardiovascular anaphylactic reactions, as opposed to those with normal baseline tryptase. [68]

d. *Basophil Activation Test (BAT)*

Basophils are a rare population of peripheral leukocytes which play an important role as effector cells in allergic disease. CD63 and CD203c are the two markers utilized in assessing the basophil activation via flow cytometry. [72] This investigative tool is known as basophil activation test and hold promise as an alternative confirmatory assay for monitoring sensitization to wasp and other hymenoptera venoms. It can aid in clarifying history positive cases that have negative skin and serological tests for venom-specific IgE. [73] BAT correlates well with serum-specific IgE and have comparable sensitivity and specificity to skin tests and serum-specific IgE. [68]

5.1.4. Sting challenge test

Sting challenge test is performed in venom-allergic patients using live insects. As a matter of fact, some patients well-tolerate venom immunotherapy, but still have systemic reactions to a sting from the same insect. In such case, challenge tests with subcutaneously or intracutaneously administered venom are not reliable. Sting challenges are, thus, recommended in patients on maintenance VIT to identify those who are not yet protected. This test has the utility to assess the effectiveness of VIT in those patients under the increased risk of re-sting. The test has been tried for its prognostic value by using in untreated patients with or without a history of anaphylactic reactions in order to identify those who need immunotherapy, but was not successful enough to make the predictions. [47]

6. Risk factors for allergic reactions

Contrast to the higher prevalence of IgE sensitization in adults with no previous case history of an allergic reaction, systemic reactions occur in a small percentage for the reasons not known well. [74] There are, besides, various factors that determines the severity of reaction to wasp sting such as older age, male sex (male:female ratio, 2:1), insect type (honey bee stings are more dangerous than vespid stings), sting site (stings in the head, neck or throat are more severe than stings at extremities), atopy, pre-existing cardiovascular and respiratory disease and use of some medications such as β-blockers and angiotensin-converting enzyme inhibitors. [7]

Similarly, time interval between stings and number of stings also influences the natural history of the reaction. A short interval between stings increases the risk of systemic reaction to the later one. With increasing interval, the risk generally declines but remains in the range of 20 – 30 % even after 10 years. On the contrary, being stung very frequently appears to induce the tolerance, for eg., 45 % of beekeepers who are stung < 25 times a year had a history of systemic sting reactions when compared to those with > 200 stings per year. [47] However, patients who have had multiple stings at one time may have experienced true anaphylaxis and not a toxic response. [74]

Mastocytosis, even in non-allergic patients, may predispose subjects to a severe reaction after an insect. A high baseline tryptase, a marker enzyme of mast cells, represents a risk factor for an anaphylactic reaction after a sting in allergic patients. [7] Mast cell product, β-tryptase, degrades the allergens and IgE antibodies protecting the patients from venom toxicity. However, this theoretically beneficial effects of mast cells on downregulating allergic immediate type reactions are insufficient to protect patients with mastocytosis from severe anaphylaxis. [75] Mastocytosis has also been observed as a risk factor for venom allergy, side effects of VIT and VIT treatment failure. [76]

Geography, climate, temperature, insect behaviour and certain outdoor occupations, hobbies and activities influences the risk of receiving a sting. Beehives and wasp nests located in the near vicinity of residences and work places are also accounted as the risk factors. [47]

7. Current treatment and management strategies

There are, in general, four treatment and management strategies for hymenoptera venom allergic patients, viz., a) avoidance, b) pharmacotherapy, c) immunotherapy, and d) anti-IgE therapy. [73] Undoubtedly, venom immunotherapy (VIT) is a highly effective and the only specific treatment which aims to maintain a low risk of a systemic reaction during and after treatment, prevent morbidity and mortality and improve health-related quality of life. The treatment for systemic allergic reactions to wasp venom consists of emergency treatment and specific allergen immunotherapy. [77, 78] Anaphylaxis is a life-threatening emergency needing immediate treatment in wasp stings. The first steps of treatment are cardiopulmonary resuscitation (severity grade IV), epinephrine administration (grade II or above; usually given intramuscularly on the scene), and as soon as possible, shock positioning and the placement of intravenous catheter (all grades). Further component of basic treatment are:

• Oxygen administration (grade II or above)

• Intravenous glucocorticoid administration, and

• The administration of H_1 blocker (all grades).

Depending upon the clinical manifestations, fluid administration, renal replacement therapy and treatment of airway obstruction may be indicated. [58]

Venom immunotherapy, specific or native, should be recommended for adults with systemic reactions and for children with moderate to severe reactions, but there is no need to prescribe it for children who only present skin reactions after an insect sting, especially if the exposure is very sporadic. The recommendations differ by country. Importantly, the risk-benefit relationship should be assessed in each case. [77] In the case of cross-reactions as a cause for double positivity, the treatment with the venom of the primarily responsible insect alone would be sufficient. However, in case of true double sensitization, immunotherapy with both venoms is indicated. [62]

7.1. Venom immunotherapy as a treatment tool—Its indication and contraindication

Venom immunotherapy, being the only specific treatment for wasp sting allergy, is indicated based on clinical history of systemic reaction, positive diagnostic test and knowledge of the history and risk factors for a severe reaction. [78] The immunotherapy is administered with all of the venoms or allergens that tested positive. VIT begins with a very low dose of venom or allergen (0.05 μg) with incremental doses every week until a plateau or maintenance dose of 100 μg (300 μg if mixed vespid venom) is achieved. Maintenance injections are then given monthly in their 1st year and then perhaps every 6 – 8 weeks for subsequent 3 years or until the skin test becomes negative. [55, 79]

Several mechanisms have been proposed to explain the beneficial effects of immunotherapy, such as, the induction of allergen-blocking IgG (IgG1 and IgG4) antibodies, reduction in specific IgE over the long term, reduced recruitment of effector cells, altered T cell cytokine balance (shift from Th2 to Th1), T cell anergy and the induction of regulatory T cells. Though the mechanism is not well clarified, the effects of immunotherapy on allergen-specific T-cell response are well affected. Following successful immunotherapy, there is reduction in allergen-specific T-cell and eosinophil recruitment in response to allergen challenge. Parallel to it, there is a shift in the balance of expression of Th1 cytokines (eg, interferon γ) and Th2 cytokines (eg, IL-4 and 13). Since cytokines formed by the Th2 subset governs the production of IgE antibodies, altered cytokine balance from Th2 to Th1 reduces the production of specific IgE and thus, may contribute to the treatment of allergic symptoms. Venom immunotherapy also induces the T regulatory 1 (Tr1) cells that produce IL-10 which is regarded as an immunosuppressive or regulatory cytokine. [55] Tr1 cells are able to produce high levels of IL-10 and TGF-β. TGF-β is involved in increasing tolerance to aeroallergens. IL-10 is responsible for VIT-induced IL-4 decrease. Tr1 cells are generated *in vivo* in humans during the early course of immunotherapy, suggesting that high and increasing doses of allergen induce Tr1 cells in humans. [80] Increased IL-10 is also responsible for specific T cell anergy. The anergic T cells do not secrete the cytokines required for the priming, survival and activity of the effector cells. [81] Specific immunotherapy is frequently associated with a rise in allergen-specific IgG antibodies and a modest reduction in specific IgE titres. Immunotherapy induced IgG antibodies have IgE blocking activities. However, the change does not always correlate with the clinical improvements. The induction of IgG antibodies with blocking activities can inhibit allergen-induced IgE-mediated release of inflammatory mediators from mast cells and basophils, thus preventing immediate symptoms. They can also inhibit IgE-mediated allergen

presentation to T cells which might reduce Th2 activation and the subsequent release of Th2 cytokines, hence providing protection against the development of the late-phase response. Besides, allergen specific IgG antibodies may also directly affect IgE production by memory B cells, either through competition with IgE for allergen binding or through the crosslinking of inhibitory IgG receptors on the surface. [82] The allergen-specific immunotherapy, thus, with following underlying mechanisms shows various effects. Very early effects are related to mast cell and basophil desensitization, intermediate effects are related to changes in allergen-specific T cells and late effects are related to B cells and IgE as well as mast cells, basophils and eosinophils. [83]

VIT study analyses have shown that the protection rate is better with vespid than honeybee extract and is better in children than in adults. Factors that influence the risk of incomplete protection after VIT include honeybee venom allergy, venom dose which sometimes needs to be increased to up to 200 µg, elevated baseline serum tryptase concentration, mastocytosis, or repeated side effects during VIT. [78]

7.1.1. Indications and contraindications

Venom immunotherapy is indicated both in children and adults with a history of severe systemic reactions including respiratory and cardiovascular symptoms and documented sensitization to the respective insect with either skin tests and/or specific serum IgE tests. It is not indicated when neither skin testing nor specific IgE antibodies showed venom sensitivity, or for unusual reactions such as vasculitis, thrombocytopenia, nephrosis etc. It is not recommended for large local reactions in either children or adults. For systemic non-life threatening reactions such as urticaria, erythemia, pruritus etc., other factors may influence the decision to initiate venom immunotherapy such as risk of high exposure to culprit insects, concomitant cardiovascular disease, mastocytosis etc.. Contraindications are, in general, with the patients using medications such as β-blockers and ACE inhibitors. β-blockers can aggravate anaphylactic side reactions and delay the recovery. Patients who are receiving β-adrenergic blocking medications might be at increased risk if they experience a systemic reaction to an allergen immunotherapy injection. Hence, their use should be adopted cautiously. This is rarely a problem in immunotherapy for respiratory allergies in young patients who are seldom on β-blockers. Life threatening and potentially fatal reactions from insect venom allergy are most often observed in older patients who are frequently suffering from cardiovascular disease and therefore are on β-blockers. Though there is a good theoretical basis for contraindication of β-blockers during immunotherapy, the clinical evidence is modest. No severe reactions to immunotherapy or sting re-exposure were observed in patients receiving β-blockers and hence concluded that combination of β-blockers with venom immunotherapy may be indicated in venom-allergic patients with severe cardiovascular disease because the risk of the stinging insect hypersensitivity is greater than the risk of an immunotherapy-related systemic reactions. [78, 84-86] However, if the β-blockers are taken off for venom immunotherapy, it should be done under careful supervision, including monitoring of blood pressure and electrocardiogram during the dose-increase phase. [81]

Similarly, life-threatening anaphylaxis is being observed in venom-allergic patients who were on ACE inhibitors and receiving venom immunotherapy or had a sting provocation. It is prudent that venom allergic patients should avoid taking ACE inhibitors unless absolutely necessary and in patients in whom venom immunotherapy is indicated, temporary discontinuation of the ACE inhibitors prior (24 hrs before) to each venom injection may prevent subsequent adverse reactions. [86, 87]

8. Parasitic wasp—A biological pest controller

Hymenoptera sub-order Apocrita is sub-divided into Aculeata and Parasitica. Parasitica is essentially all of the parasitic wasps that have an ovipositor with the sole purpose of egg laying, either near the host, on the host (ectoparasitism) or inside the host (endoparasitism). Parasitic wasps are important natural enemies to a vast array of insects that are considered pests in the agricultural system. Hence, they are the unique bioindicators of the diversity of the hosts they attack. They are sensitive to ecological perturbations, especially pesticides and hence are ideal candidates for conservation studies. [3, 88] These parasitoids produce a wide range of venoms that could serve as models for developing new classes of synthetic chemical insecticides. [89]

In order to successfully parasitize the host, parasitoid wasps generate and release gene products at oviposition that alter the physiology of the host. The females internally store poisonous venom in their ovaries and secretory organs. [89] In endoparasitoid species, various stages of the host can be parasitized (egg, egg-larval, larval, pupal and adult) depending upon the species. However, in ectoparasitoid species, venoms often induce paralysis and /or regulate host development, metabolism and immune responses which benefit the externally developing parasite. Venom proteins from endoparasitic wasps are predominantly involved in regulation of host physiology and immune responses alone or in combination with other factors of maternal origin such as polydnavirus (PDVs) or virus-like particles present in the venom itself or produced in the ovaries or ovarian fluids. [90] Parasitoid venom has evolved to produce both immunosuppressive and stimulatory properties to create the optimal host environment for parasitoid offspring. Female parasitoid regulates the host without totally suppressing the host's physiology and creating an unregulated host environment in order to maximize progeny production. The parasitoid's progeny is subjected to unregulated microbial attack and invasion if the host become immuno-compromised. Thus, the female wasp evades the host immune response without compromising the host's immune system by injecting the venomous mixture that includes virus-like particles such as polydnavirus, in the host body at the time of oviposition. [89]

8.1. Mechanism of actions

8.1.1. Immune suppression

Immune system plays a major role in physiological interactions between the hosts and the parasites. Hosts will react to the invasion of foreign agents by producing antimicrobial

peptides and reactive oxygen species by contact epithelia, fat body and hemocytes. Phagocytosis, encapsulation and nodule formation by specialized hemocytes are more directly involved in the defence mechanism. The female wasp avoids this host immune response by introducing a venomous mixture, often together with virus-like particles and/or polydnaviruses, into the host body just before the oviposition. Such venom injection suppresses the host immune system by targeting two major host defense cascades, viz. a) the phenoloxidase cascade and b) the coagulation cascade. [88]

a. Phenoloxidase Cascade

Melanization of pathogens and damaged tissues forms a major innate defence system in invertebrates which is controlled by a multicopper oxidase enzyme, phenoloxidase. The enzyme results in the deposition of melanin around the damaged tissues or intruding object. This physical shield around the intruder prevents or retards its growth. More importantly, during melanin formation, highly reactive and toxic quinine intermediates are also formed which are involved in production of cytotoxic molecules such as superoxides and hydroxyl radicals that could aid in the killing of intruders. Reduction of phenoloxidase activity is, thus, a well-known strategy of parasitoid wasp, although it has so far been reported in braconid and ichneumonid species. [88] Various venom proteins from different parasitoid species are rendered possible inhibitory function on the phenoloxidase pathway, viz. serine protease such as serpin-1J [91], serpin-3 and serpin-6 [92]; cysteine-rich protein such as Cvp1 [93], LMPI-1 and LMPI-2; [94] and Egf1.0. [95]

b. Coagulation Cascade

The clotting reaction of insect hemolymph is a part of insect immunity. Female wasps make a feeding tube after injecting the venom into the host in order to connect the interior of the pupa with the exterior of the puparium. Inhibition of coagulation is of host hemolymph is thus crucial since the wasp feeds on the host fluid drawn up through the feeding tube. Besides, the young parasitoid larva also feeds on the host's body fluids by grabbing and puncturing the host's integument with its mandibles. [88] Besides the action of maternal venom, larval secretions are also known to inhibit clotting system. Various contributory proteins identified for the inhibition of coagulation cascade of the hosts are reprolysin-type zinc metalloproteinase, [96] calreticulin, [97] and serine protease inhibitors. [98]

8.1.2. Immune stimulation

Parasitic wasps suppress the immune system of its hosts rather than completely shut down. A complete shut down of host's immune system would be potentially deleterious to the parasitoid's developing progeny, which conceivably would be forced to compete with microorganisms for host nutrients, could directly infect/attack the wasp offspring and/or contaminate their nutritional source. This is why the parasitoid's venom selectively suppresses the host's immunity and allows or even stimulates certain antimicrobial defences. Few venom proteins that contribute to immune stimulation in parasitized hosts are chitin-binding like proteins with antibacterial and wound healing properties; β-1,3-Glucan recognition protein (βGRP) whose interaction with β-1,3-Glucan initiates the activation of prophenoloxidase

cascade; dipeptidyl peptidase IV (DPP IV) that could possibly function as a stimulator of venom related processes, including immunity; and, angiotensin I-converting enzyme, a processing enzyme involved in the synthesis of antibacterial peptide. [99]

8.1.3. Developmental arrest

Insect host are modulated in various ways to provide favourable environment for the development of the wasp parasitoid's progeny. Typically, the host undergoes a developmental arrest and later on dies away after the parasitoid has become independent of its host. Teratocytes inhibits frowth, alter development and affect the related physiological parameters. Likewise, polydnavirus coinjected with venom induces a variety of physiological changes in development and immunity through their gene expression. Other molecules such as EpMP3 metalloprotease, lysosomal arylsulphatase, and calreticulin-like venom proteins appears to be a critical agent in developmental arrest. [100]

8.1.4. Increment of lipid levels

Adult parasitoid wasps lack the capacity of lipogenesis. Growth and survival of parasitoid larvae is largely dependent on host. Thus, parasitoid ensures a suitable environment for their developing offspring by manipulating their host's physiology or development—either by influencing the feeding habit of host and its growth (koinobionts) or by arresting the host's development (idiobionts). Parasitism has been found to induce changes in the amount of amino acids, proteins, pyruvate and carbohydrates within the host in both endo- and ectoparasitoids. The female parasitoid wasp strategically creates the resource for its progeny's development which includes an increase in whole body lipid content, an enhanced metabolism of fat body triacylglycerols and a higher level of free fatty acids in the hemolymph. Larva in its early stages mainly consumes host hemolymph while in its later stages directly feeds the host's fat body. These changes in the nutritional content of the host are brought about by a variety of mechanisms such as teratocytes, wasp venom and associated mutualistic viruses. Teratocytes are cells derived from the dissociation of the embryonic membrane of parasitoid wasp which play an important role in nutritional exploitation by parasitoid larvae. They attach themselves to the host's fat body and contribute to its disruption (teratocyte-specific carboxylesterase involved in hydrolysis of host lipids, a fatty acid-binding protein involved in transport of host fatty acids to the developing parasitoid larva, two collagenases that may attack the collagen sheath surrounding the fat body to permit selective release of fat body cells). In addition to the effects of teratocytes, the parasitoid larva itself is capable of bringing about physiological changes, eg in hormone and lipid levels, aiding its own development. [101]

Maternal substances which are transferred along with the egg during ovipoistion provide an additional way of host exploitation. These include different types of viruses such as poly-DNAviruses, non-polyDNAviruses and virus-like particles as well as venom. Parasitoid venom contains various proteins that may disrupt the host's fat body such as matrix metallo-proteinase [102] that causes lysis of cells and release of lipid particles from the fat body. Wasp venom triggers PLC activation resulting in IP_3 formation and subsequent Ca^{2+} release from

mitochondria which in turn activates PLA_2. The activated PLA_2 in turn increases the fatty acid level in host fat body. [30]

Hence, the multitude of mechanisms employed to manipulate host metabolism ensures an abundance of lipid resources during development, providing parasitoid larvae with a unique opportunity to consume host lipids instead of synthesizing them de novo. Manipulation and consumption of host lipids probably provides a selective advantage for parasitoid larvae, because *de novo* lipid synthesis is energetically expensive. [101]

8.1.5. Apoptosis

Venom for parasitoid wasps induces cellular injury and culminates in oncotic death. [88] Crude venom alone has been shown in *in vitro* assays to evoke disruption of plasma membrane integrity, blebbing, rounding, swelling and cell death thought to be linked to a G-protein dependent oncotic mechanism. [103] Various candidates in venom triggers the apoptotic cell death such as venom phenoloxidase, calreticulin, laccase, endonuclease G, and gamma-glutamyl transpeptidase-like venom protein. [88]

8.1.6. Nutritional functions

Nutritional and physiological milieu of the host is manipulated for the better nurturing of the parasitoid's offspring by the venom injected by the female wasp. For example, teratocytes of endoparasitoids have a secretory and nutritive function whereas venom of some ectoparasitoids changes the host metabolism to provide nutrients. [88] The discovery of trehalase in parasitoid wasp venom protein ensures the provision of glucose for developing wasp larvae from trehalose, which is the main reserve sugar in the hemolymph of flying insects. Several other digestive proteins in venom such as trypsin and other serine proteases, trypsin-like enzymes, lipase-like venom protein, and acid phosphatases are involved in assuring the optimal nutrition for its offspring. [88, 104, 105]

9. Conclusion

Wasp is a common name for any insect species of the order Hymenoptera and sub-order Apocrita, excluding bees and ants. A highly diverse group of insects, they are social or solitary, parasitic or predatory, phytophagous or carnivorous or omnivorous. The most primitive Hymenoptera possess ovipositors to insert eggs into plant tissues. In some parasitic groups, this structure and the glands associated with it have been modified to inject venom to paralyse other insects that they use for their developing larvae. These parasitic wasps are extremely beneficial to natural ecosystem and agriculture as biological pest controller. Their stings are not so painful to humans and are of none clinical significance.

Social wasps, however, are evolved with a venom system that is specialized as a defence weapon. The sting produces a range of clinical manifestations in humans—from simple skin allergic manifestation that do not require any medical treatment to fatal anaphylaxis and toxic

reactions where immediate medical intervention is utmost. Yellow jackets, hornets and paper wasps are three medically important stinging social wasps. Stinging social wasp venom comprises of various allergens, toxins and bioactive molecules that imparts physiological and pathological changes upon envenomation in humans. Immune-mediated and non-immune mediated mechanisms, both are involved.

Wasp stings are alkaline and are traditionally addressed by the application of vinegar or lemon juice to neutralize the venom. Besides, there are many local practices being observed, which might need scientific evidence to be proven, such as placing ice packs, freshly sliced cucumber, potato and onion on the sting sites and applying the aloe vera gel, garlic paste, ethanol and curds on the sting sites.

Wasp stings usually manifests allergic symptoms—normal local allergies to systemic anaphylaxis. Allergy, in general, is a public health threat of pandemic proportions today. Respiratory allergies, food and drug allergies and allergic reactions to insect venom are the commonly reported allergic incidents. Allergic patients not only suffer from the debilitating disease resulting in decreased quality of life, career progression and personal development but also constitute a significant burden on health economics and macroeconomics due to the days of lost productivity and underperformance. The symptomatic treatment for allergy are not sustainable which includes short-term symptom relieving or long-term anti-inflammatory drugs—the effect of which are suboptimal, relapse of the symptoms very shortly after ceasing daily use of medication even after years of a continuous and effective treatment, and the possible fear of adverse effect due to the long-term use of drugs. [106] These symptomatic medications as well imparts financial burden. Allergen-specific immunotherapy is an effective treatment used by allergists and immunologists for common allergic conditions, particularly allergic rhinitis/conjunctivitis, allergic asthma and stinging insect hypersensitivity [107] and can achieve substantial results for patients—improves the quality of life, reduces the long-term costs and burden of allergies and prevents the progression of allergic disease. Currently, it is the only curative treatment for Hymenoptera venom allergy. [106] However, various factors should be taken into consideration on a case-by-case basis, taking into account individual patient factors, before proceeding with the allergen specific immunotherapy such as the degree to which symptoms can be reduced by avoidance measures and pharmacological therapy, the amount and type of medication required to control symptoms, the adverse effects of pharmacological treatment and patient preferences. This form of therapy carries the risk of anaphylactic reactions, hence taking into consideration the indications and contraindications, it should be prescribed and practiced by physicians who are adequately trained in the treatment of allergy. Moreover, injections must be given under medical supervision in clinics that are equipped to manage anaphylaxis. [107] These requirements and preparations obviously make the allergen specific immunotherapy not so readily available and expensive but considering the beneficial effects of allergen specific immunotherapy and unsustainability of pharmacotherapy, allergen specific immunotherapy emerges as a reliable curative approach.

Parasitic wasps have their own significant space in this ecosystem and contribute as a biological pest controller. They are not only important in agricultural system, but as well have significant role in controlling the disease spreading, such as the toxicity of venom can inhibit the multiple

developmental stages of several mosquitoes and house flies, both of which are a major vector of human disease. The utility of their venom components provide a promising frontier in development of new classes of bioinsecticides.

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