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Immunology of Bee Venom

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Abstract Bee venom is a blend of biochemicals ranging from small peptides and enzymes to biogenic amines. It is capable of triggering severe immunologic reactions owing to its allergenic fraction. Venom components are presented to the T cells by antigen-presenting cells within the skin. These Th2 type T cells then release IL-4 and IL-13 which subsequently direct B cells to class switch to production of IgE. Generating venom-specific IgE and crosslinking FcεR1(s) on the surface of mast cells complete the sensitizing stage in allergic individuals who are most likely to experience severe and even fatal allergic reactions after being stung. Specific IgE for bee venom is a double-edged sword as it is a powerful mediator in triggering allergic events but is also applied successfully in diagnosis of the venom allergic patient. The healing capacity of bee venom has been rediscovered under laboratory-controlled conditions using animal models and cell cultures. The potential role of enzymatic fraction of bee venom including phospholipase A2 in the initiation and development of immune responses also has been studied in numerous research settings. Undoubtedly, having insights into immunologic interactions between bee venom components and innate/specific immune cells both locally and systematically will contribute to the development of immunologic strategies in specific and epitope-based immunotherapy especially in individuals with Hymenoptera venom allergy.

Keywords Allergen · Anaphylaxis · Bee venom · Immune response · Immunotherapy

Introduction

The insect order Hymenoptera (meaning "veil wings") includes the bees, wasps, and ants [1]. Bees, the pollinating insects, belong to a monophyletic group of 16,000 species [2, 3] classified in seven recognized families [3]. The family Apoidae includes the social honeybees, solitary bees, and bumblebees. While honeybees are herbivorous and choose nectar and pollen to live on, wasps, hornets, and yellow jackets are known as predacious carnivores living on other insects and sweet substances including sap [4]. Honeybees possess a barbed stinger which is pulled out of the abdomen along with the venom sac during stinging. They can only sting once and soon after die unlike the wasps, hornets, and yellow jackets [1, 4] (Fig. 1). There are three major adaptations observed in venom delivery system of the honeybee: self-embedding into the flesh, venom pumping up to 1 min to reach more sensitive tissues, and finally releasing communicative alarm pheromones such as isopentyl acetate, which has an odor described as similar to banana [5]. Bee venom (apitoxin) [6] is a complex mixture of biologically active components such as proteins, enzymes, and amines. Insect sting effects on the host are classified as local and systemic. Local effects are similar within various groups of Hymenoptera and occur due to toxins in the venoms, whereas the more serious effects are allergen-related systemic responses [4]. Interestingly, the venom of worker honeybee is rich in major allergens and is unlike the venom of queen honeybees being

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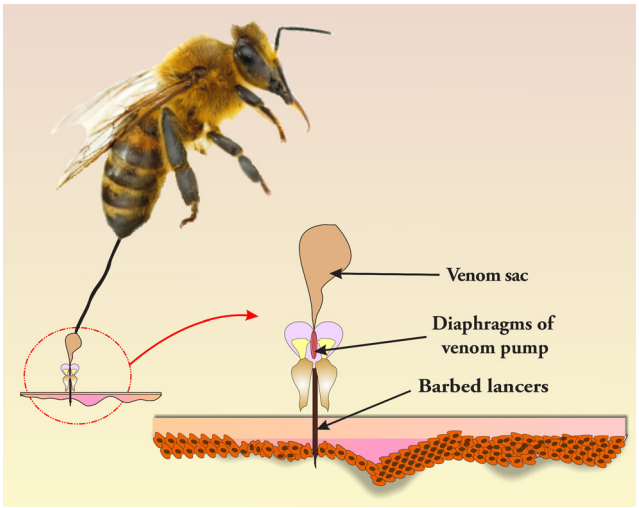

Fig. 1 Unique structure of the venom delivery apparatus in honeybee makes it possible to deliver the venom even after apparatus is being pulled out of the abdomen

used to repel other queens [1]. Although venom sacs may contain up to more than 300 μg of venom, an average of 50 to 140 μg of venom protein is delivered with each sting [7].

Chemical Composition of Bee Venom

The active components of honeybee venom include wide range of enzymes, peptides, low-weight proteins, and amines [8]. Adolapin [9], melittin, apamin [10], and peptide 401 are well-studied peptides in bee venom [11–13]. Melittin, a helical [14] hydrophobic polypeptide, causes cell lysis through altering membrane permeability [15]. Furthermore, this highly basic polypeptide [16] is responsible for pain by affecting either neurons or releasing pain-inducing chemicals [17]. Melittin induces cell lysis and fusion in addition to activation of phospholipase A2 and adenylate cyclase [18]. Both melittin and phospholipase A2 are able to increase the blood clotting time in vitro [19, 20]. Peptide 401 causes mast cells to degranulate and therefore triggers inflammatory reactions. Apamin, the smallest neurotoxin in bee venom is composed of only ten amino acids and two disulfide bonds [21] and is capable of blocking Ca^{2+}-dependent K^{+} channels [22]. Phospholipase A2 (PLA2), a calcium-dependent enzyme, hydrolyzes the sn-2 ester of glycerophospholipids-releasing fatty acids and lysophospholipids [23]. PLA2 is classified into three broad classes based on the cellular distribution: secreted PLA2 (sPLA2) which is present in snake and bee venoms, cytosolic (cPLA2), and $Ca2^{+}$-independent PLA2 (iPLA2) [24]. PLA2 leads to the disruption of lipid bilayer integrity through destroying membrane phospholipids. Interestingly, the biochemicals produced during membrane disruption (lysophosphatidylcholine, lysophosphatidic acid (LPA), and sphingosine 1-phosphate) have cytotoxic or immunostimulatory effects on a wide range of cell types causing inflammation and immune responses. LPA activates eosinophils, and PLA2 induces CD69 expression on the surface of these cells [25] (Fig. 2). Phospholipase A2 is not toxic when pure but, in proximity to melittin, changes to a hemolytic factor. PLA2, a major venom allergen, is responsible for inducing immunoglobulin E (IgE)-mediated anaphylaxis [1]. Phospholipase B (lysophospholipase), found in low concentrations in some venoms, is able to cleave acyl chains from both sn-1 and sn-2 positions of phospholipids [24, 26, 27]. Choo et al. revealed that bee venom serine protease has fibrinolytic activity in mammals [28]. Hyaluronidase (spreading factor) is the second most common allergen in honeybee venom which causes changes in cell membranes [29]. It is initially derived from a preproenzyme [30] and contributes to invasion of venom toxins through the gaps between cells during the destruction of extracellular matrix. Moreover, hydrolyzed hyaluronan fragments induce faster systemic envenomation since they possess pro-inflammatory, pro-angiogenic, and immunostimulatory properties [26]. The enzymatically active fraction of bee venom also includes α-D-glucosidase, α-galactosidase, and arylamidase [19]. There are significant enzymatic activities reported in honeybee venom including acid phosphatase α-glucosidase, esterases, and peptidases. The acid phosphatase presented as both monomer and dimer is capable of triggering histamine release from sensitized human basophils. Wheal-and-flare reaction can be observed after intradermal injection of acid phosphatase into the skin of

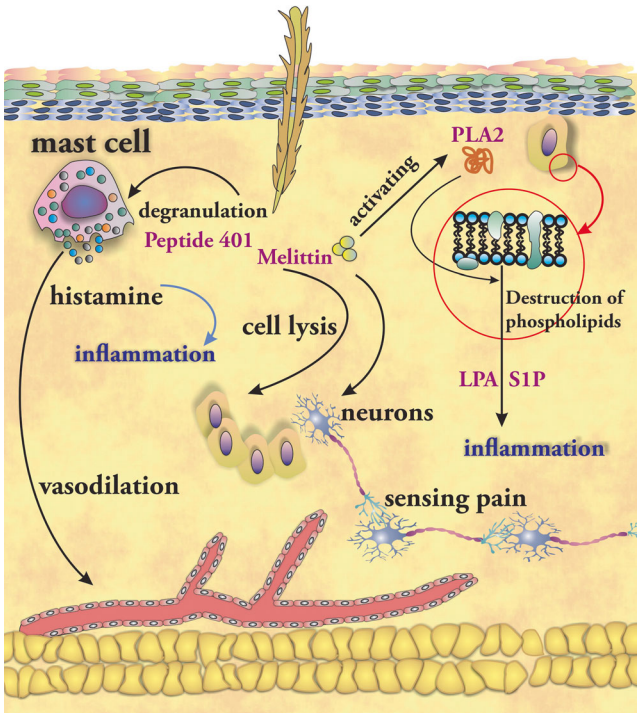

Fig. 2 Main pathophysiologic effects of bee venom biochemicals on a variety of cells following a sting

allergic individuals [31]. Physiologically active amines in composition of honeybee venom include histamine, dopamine, and norepinephrine [11]. In wasp venom, histamine, serotonin, and acetylcholine play a role in affecting neurons and severe pain initiation [1], alarm pheromones including isopentyl acetate, 2-nonanol, and n-butyl acetate [32] after evaporation from the surface of the sting alert, and attract other bees to the marked target [1]. One of the bee venom components, the mast cell degranulating peptide (MCD), is a 22 amino acid residue peptide which structurally resembles to apamin. It is a potent anti-inflammatory agent; however, at low concentration, MCD mediates mast cell degranulation and histamine release [33], while it has been reported to inhibit histamine release in the presence of IgE at high concentration [34]. MCD peptide, tertiapin, and secapin have been demonstrated to possess neurotoxic properties [35]. Two main receptors B1 and B2 have been determined to mediate MCD interactions [33]. Kinins via B1 receptors successfully stimulate tumor necrosis factor (TNF) and IL-1 formation by macrophages [36]. Although there are common biologically active chemicals in the composition of bee and wasp venom, some peptides are exclusive to each insect [26]. Venom electrophoretic profiles of 25 different hymenopteran species and comparison at the global level revealed significant differences in protein patterns from one species to another [37] (Table 1).

Medical Applications

Bee venom because of its diverse pharmacological activities has been used widely in oriental medicine [45]. Moreover, it has been applied as a cosmetic ingredient possessing antiaging, anti-inflammatory, and antibacterial properties in many products [10]. Bee venom therapy as an alternative medicine approach has been utilized to relieve pain and to treat rheumatoid arthritis. Park et al. studied the antinociceptive effects of bee venom acupuncture on inflammatory pain in the rat model of collagen-induced arthritis and reported α2-adrenoceptors to mediate the effect [46]. Yang et al. [47] reported capability of bee venom to attenuate neuroinflammatory events and extending survival in Amyotrophic lateral sclerosis (ALS) in animal model. After diluting bee venom in saline and preparing 0.1 μg/g doses, they injected venom bilaterally (subcutaneously) into 14-week-old (98 days old) male $hSOD1^{G93A}$ transgenic mice. The treatment positively increased survival rate and motor activity [47]. Melittin has been reported to inhibit the replication of a number of viruses including murine retrovirus and herpes simplex virus [48]. Additionally, it possesses antimicrobial activity against a wide range of gram-positive and gram-negative bacteria [49]. The antimicrobial activity in hymenopteran venom serves to prevent the contamination of the

Table 1 Exclusive and common chemicals in bee and wasp venoms

Allergen	Source	General and biologic properties	Reference
Melittin	Bee venom	Hemolytic peptide, anti-microbial, anti-tumor, anti-inflammatory peptide	[38]
		Bee venom main component (≥50% *w*/*w*)	
Apamin		Neurotoxic peptide, consists of 22 amino acid residues	[39]
		Possesses three possible isomers and contains two intramolecular disulfide bonds	
MCD		Histamine releaser at low concentrations	[40]
		Possessing anti-inflammatory activity at higher concentrations	
		Increases the free cytoplasmic Ca^{2+} concentration	
Mastoparan	Wasp venom	Consists of 14 amino acid residues	[41]
		Allosteric regulator of heterotrimeric G proteins	[42]
		Mast cell degranulating peptide	
Bradykinin		Possesses inflammatory activities	[43]
		Plays an important role in paralyzing prey	[44]
Common biologically active chemicals in bee and wasp venoms			
Hyaluronidase			[26]
Serotonin			
Histamine			
Phospholipase A2			
Phospholipase B			
Dopamine			
Noradrenaline			
Adrenaline			

venom apparatus by the presence of opportunistic pathogens in stung prey. Interestingly, venom components are also present on epicuticle, the most external layer of the insect cuticle, as a chemical barrier to protect against microorganisms [50]. Wachinger et al. studied the inhibitory effects of melittin on HIV-1 and reported that the production of infectious and cell-free virus was inhibited in a dose-dependent manner with ID_{50} values in the range of 0.9–1.5 μM for melittin. Moreover, the levels of Gag antigen and HIV-1 mRNAs were found to be reduced in the presence of melittin [51]. Neuroprotective effects of bee venom have been in the center of attention in recent years. Ye et al. [27] based on their previous study showing that phospholipase A2 of bee venom (bvPLA2) could significantly increase the regulatory T (Treg) population studied the neuroprotective effects of this enzyme in the 3xTg AD mouse model of Alzheimer's disease. After administrating bvPLA2, the levels of amyloid beta (Aβ) deposition in the hippocampus, glucose metabolism of the brain, microglia activation, and $CD4^+$ T cell infiltration were analyzed. The obtained results were surprising: (1) cognitive function of the 3xTg-AD mice enhanced; (2) Aβ deposition in the hippocampus was dramatically decreased in association with microglial deactivation and reduction in CD4+ T cell infiltration; and (3) neuroprotective effects of bvPLA2 were abolished in Treg-depleted mice [27]. Liu et al. in an experiment showed the capability of bee venom to inhibit K1735M2 mouse melanoma cells in vitro. Flow cytometric measurements revealed that arresting the cell cycle at the G1 stage was the reason of such inhibition [52].

Allergens

Since honeybee venom is a very complex blend of uncharacterized chemical compounds such as allergens, a wide range of immunologic and biochemical techniques have been applied to determine its allergen composition. 2D gel electrophoresis in combination with mass spectrometry has been accepted as a powerful tool for proteome mapping [53]. Api m 1, Api m 2, Api m 4, Api m 6, Api m 7 [54], Api m 10 [55], and Api m 5 [56] are well known as the main allergens found in bee venom (Table 2). Blank et al. [55] identified and reported the 200-kDa high molecular weight allergens from vitellogenins family in the venoms of the hymenoptera species *Apis mellifera* and *Vespula vulgaris* [55]. While principle allergens of honeybee venom include phospholipase A2 and hyaluronidase, Vespidae are known to possess phospholipase A1, antigen 5, and hyaluronidase. Two major royal jelly proteins (MRJP) 8 and 9 which are two isoforms of Api m 11 and novel pan-allergens, the vitellogenins Api m 12 and Ves v 6, have recently been introduced as honeybee allergens [57]. Since only a few allergens are present in substantial amounts in the bee venom, purification process is not only difficult but also there is a high risk of contamination with other allergens and cross-reactive carbohydrate determinants (CCDs) [58]. Such determinants are the most frequent cause of multiple reactivities between allergens present in yellow jacket and honeybee venoms. For instance, α-1,3-linked core fucose residues are considered essential for establishing the cross-reactive glycan-based epitopes. The presence of impurities negatively affects diagnostic applications at the molecular level. Recombinant technologies have been successfully applied to bypass these problems. Seismann et al. [59] could produce full-length Api m 2, Ves v 2a, and Ves v 2b by baculovirus infection of *Trichoplusia ni* (HighFive) and *Spodoptera frugiperda* (Sf9) insect cell lines [59]. Generally, some allergens have been produced in bacteria, but in some cases, conformational IgE epitopes are affected because of lacking proper posttranslational modifications and correct folding [58].

Allergy and Anaphylaxis

Within 10 min after sting, generalized hypersensitivity reactions such as pruritus, urticaria, angioedema, nausea, vomiting, diarrhea, rhinoconjunctivitis, bronchospasm, hypotension, cardiovascular collapse, and unconsciousness can be observed in allergic individuals [60]. Anaphylaxis due to Hymenoptera sting is considered as one of the most severe consequences of IgE-mediated hypersensitivity immunologic reactions caused by the crosslinking of receptor-bound IgE antibodies on the surface of mast cells and basophils [58, 61]. Massive envenomation, by the more aggressive Africanized bees, triggers immediate and delayed toxic reaction and even death [60]. There are five classes of reactions to Hymenoptera stings including normal local reactions, large local reactions, systemic anaphylactic reactions, systemic toxic reactions and unusual reactions. Large local and systemic anaphylactic reactions are the most frequent. Swelling in large local reactions exceeds a diameter of 10 cm and lasts longer than 24 h. While some patients' skin and in vitro tests show an IgE-mediated mechanism, others show a cell-mediated allergic pathogenesis or even a combination of both. Systemic anaphylactic reactions generally are IgE mediated; however, other rare mechanisms such as short-term sensitizing IgG antibodies or complement activation by IgG–venom complexes have been reported [7]. Previous history of systemic sting reaction [62], positive skin test, and detection of venom-specific IgE antibodies are considered important in diagnosis of Hymenoptera venom allergy. Skin prick test (SPT), intradermal test, sIgE test, and the basophil activation test are generally advised. The sensitivity of venom sIgE is shown to be lower than that of skin intradermal testing. sIgE can be performed for the whole extract or for natural and recombinant components in venom [63]. RAST inhibition was the first

Table 2 Bee venom allergens and their main properties

Allergen	Other name	Properties	References
Api m1	Phospholipase A2	Binds directly to the CD206 mannose receptor	[60]
		Secreted PLA2 (sPLA2) has a key role in a wide range of cellular responses such as phospholipid metabolism, signal transduction and regulation of inflammatory and immune responses	[24]
		Induces IL-4 release from murine mast cells and IgE response on low-dose immunization	[61]
Api m2	Hyaluronidase	Catalyzes the hydrolysis of hyaluronan (HA)	[61]
		Activity potentiates infiltration by dissolving the extracellular matrix	[31]
Api m3	Acid phosphatase	Present as both monomers of about 48 kDa and dimers	[31]
		Possesses the ability to cause histamine release from sensitized human basophils thus, produces a wheal-and flare reaction after intradermal injection	
Api m4	Melittin	Main lethal component	[62]
		Promelittin during biosynthesis is converted to 22 amino acid peptide, melittin	
		Possesses predominantly hydrophobic N-terminal region and a hydrophilic C-terminal region	
Api m5	Allergen C	Homolog with Ves v 3 in yellow jacket	[62]
Api m6		Unglycosylated allergen, exists as four isoforms of 7190, 7400, 7598, and 7808 Da	[63] [64]
Api m7	CUB serine protease 1		[65]
Api m8	Carboxylesterase		[66]
Api m9	Serine carboxypeptidase		[66]
Api m10	Icarapin	Phosphorylated allergen	[65] [67]
Api m11	Major royal jelly protein 9		[65]
Api m12	Vitellogenin	200 kDa peptide belonging to vitellogenin family	[65] [65]

in vitro method that helped to identify the causative venom, but later molecular allergy (MA) diagnostics was applied to detect specific sIgE to single venom allergens [64]. Moreover, the ImmunoCAP solid-phase assay and Immulite liquid allergen testing systems are mainly used for the quantitative detection of recombinant venom IgE antibodies in routine practice [65].

Basophil activation testing could be helpful when intradermal test results are negative while sIgE testing has a positive result. The sensitivity of SPT is lower than that of intradermal testing. The SPT generally is performed at a concentration between 1.0 and 100 μg/mL, and the initial intradermal concentration should be in the range of 0.001–0.01 μg/mL [63]. However, there are complications with such diagnostic protocols in which there are patients with a convincing history of anaphylaxis but negative diagnostic tests and, furthermore, up to half of the patients show positive tests with more than one venom [58]. Investigation of Hymenoptera venom allergy in patients with mastocytosis has revealed interesting facts both in terms of diagnosis and immunotherapy. Mastocytosis is a group of mast cell disorders characterized by an increase of mast cells in skin and/or internal organs including the bone marrow, spleen, liver, lymph nodes, and gut. Mast cells normally require stem cell factor (SCF) binding to their surface receptor KIT (CD117) for proliferation and development. The presence of (D816V) mutation in KIT receptor in adults with systemic mastocytosis leads to receptor activation even in the absence of SCF [66]. Mastocytosis patients generally have elevated serum tryptase, histamine, prostaglandin D2, and leukotriene C4 levels [67]. Patients with Hymenoptera venom allergy who suffer from mastocytosis experience severe life-threatening reactions since Hymenoptera venom is considered a trigger of anaphylaxis in these patients [68]. Moreover, venom immunotherapy (VIT) in these patients is associated with a higher rate of severe side effects, which may force physicians to stop immunotherapy [69]. Skin tests in mastocytosis patients who demonstrate high tryptase (mast cell activation marker) levels may provoke systemic reactions [70]. The

mechanism of action in bee sting allergy in mastocytosis is similar to other allergic conditions with respect to both cytokine pattern and cellular activity. Langerhans cells and dendritic cells introduce processed allergens to Th lymphocytes. Several cytokines, chemokines, and co-stimulatory signals determine the immune skewing towards the development of different types of T cell subsets. In atopic individuals, naive T cells after being activated by APCs in the presence of IL-4 and genetic factors differentiate into Th2 cells which secrete IL-4 and IL-13, responsible for class-switching to IgE in B cells. The IgE molecules bind to FcεRI on the surface of mast cells and basophils. Subsequent re-exposure to the sensitizing allergen activates the production and release of biogenic mediators in mast cells and basophils responsible for type-1 hypersensitivity allergic reactions [71].

Immune Response

Subcutaneous bee venom injection produces significant suppression of leukocyte migration and a significant reduction in concentration of TNF-alpha, suggesting that the anti-inflammatory effect of subcutaneous administration is mediated in part by the release of catecholamines from the adrenal medulla. Bee venom inhibits the activity of pro-inflammatory metaloenzymes and matrix metalloproteinase-2 and -9 and increases interferon beta production depending on time, dose, and treated cell type [72]. Adolapin, an effective anti-inflammatory substance, suppresses the activity of cyclooxygenase (COX) enzyme. Allergic compounds of bee venom including histamine and phospholipase A2 induce the production of IL-10 by Th2 cells and suppress T-cell proliferation. Karimi et al. [73] in an animal model of experimental allergic encephalomyelitis (EAE) on Lewis rats studied the immunologic effects of bee venom introduction. After preparing honeybee venom through a stimulating method mediated by electric pulse, they managed to introduce it to two determined groups of rats. Thirty rats randomly placed in three groups of ten. Group 1: named E-S, received normal saline (0.2 mL) every day, while the second group (E-BV1) received 2 mg/Kg honeybee venom every day. Rats in the third group (E-BV2) received 5 mg/Kg of the same venom every day. Meanwhile, EAE was induced via subcutaneous injection of guinea pig spinal cord homogenate (GPSCH) emulsified in 1:1 ratio of complete Freund's adjuvant (GPSCH-CFA) to the adult female Lewis rats. Rats were evaluated daily for any unwanted symptoms and weight loss. Animals were then scored daily using degrees ranging from 0: indicating normal and without symptoms to 6: death. The sections of the brain and spinal cord were obtained and stained with hematoxylin and eosin for the assessment of inflammatory cell infiltration as well as Luxol fast blue (LFB) for demyelination analysis. Bee venom was found capable to decrease the penetration of mononuclear inflammatory cells with observed pathological changes. Demyelination was markedly decreased in groups that received bee venom. The rates of serum TNF-α and nitrate (for considering the antioxidant and anti-inflammatory effects of bee venom) were specified by ELISA and HPLC. The amount of TNF-α had been decreased in the venom-treated groups compared with the E-S group. The amounts of serum nitrates in E-S group had been increased considerably whereas EBV2 group showed the lowest rates [73] (Fig. 3). Hamedani and colleagues in an attempt to determine whether bee venom is an immunosuppressor or immunostimulant used WEHI-164, HT-1080, and K562 cell lines for assessment of toxicity, proliferative response, matrixmetalloproteinase-2 and -9 (MMP-2 and MMP-9) activity, and interferon production under the influence of Australian and Iranian BV (ABV and IBV) at concentrations of 0.025–1 μg/ml. The zymography method was used to evaluate the MMP-2 and MMP-9 activity. Besides, IFN-α and IFN-β production was assessed using enzyme-linked immunoassay technique. In this multivariable research, both venom types had similar effects on the same cell line but, interestingly, the response of each cell line to each venom was different. The ABV and/or IBV concentrations between 0.025 and 0.5 μg/ml to human monocyte cell line (K562) exhibited proliferative response. Moreover, both venoms had the same immunomodulatory effect on MMP-2 and MMP-9 activity in both cell culture media, WEHI-164, and K562. While stimulatory effect of venom on MMP-2 and MMP-9 activities was reported between doses 0 and 0.05 μg/ml, the inhibitory effect on these two MMPs was seen at concentrations of >0.05 μg/ml. The ABV and/or IBV had no influence on IFN-α production in cell culture media, whereas adding the BV to K562 cell line could significantly increase the production of IFN-β only on day 8 post-treatment. They concluded that immunosuppressive and/or immunostimulatory responses to bee venom depend on a time and dose pattern as well as the type of treated cell line [74] (Fig. 4). Sur et al. [75] in their experience of atopy-like induced dermatitis in BALB/c mice, assessed the expression of Th2 cytokines (IL-4, IL-5, and IL-10) in the lymph nodes of trimellitic anhydride (TMA)-treated mice and found them to be elevated. Bee venom acupuncture (BVA) could abrogate TMA-induced Th2 cytokines production. Interestingly, levels of Th1 cytokines such as IL-2, IL-12, IFN-γ, and TNF-α were also increased by TMA treatment, and the increases were suppressed by BVA treatment. BVA-modulating Th1-Th2 balance was shown to suppress the expression of both Th1 and Th2 cytokines [75]. The role of basophils as mediator immune cells in anaphylactic events has been known for a long time although masked by mast cells. This role in two aspects of released mediators and surface activation markers under influence of honeybee venom secretory phospholipase A2 (HBV-sPLA2) in vitro was studied by Mustafa et al. They purified basophils from buffy coats

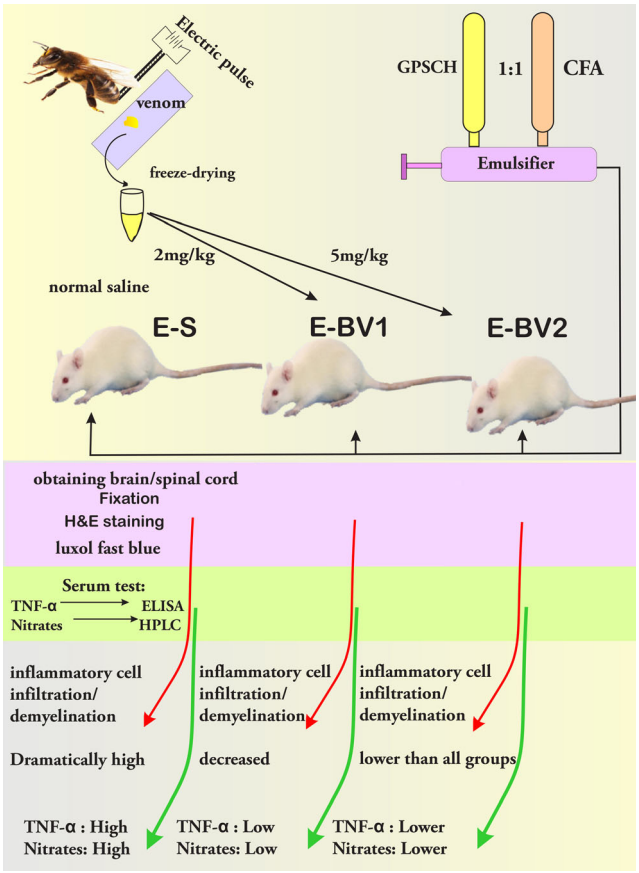

Fig. 3 Efficiency of bee venom in treating EAE in three groups of Lewis rats was assessed by histologic staining of the brain/spinal cord sections and measuring nitrates and TNF-α in venom-treated groups (E-BV1 and E-BV2) and non-treated group (E-S)

and found out that while production and expression of leukotriene C4 (LTC4) was induced in 5 min in the presence of HBV-sPLA2, IL-4 induction took longer (Fig. 5a, b). HBV-sPLA2-inducing effect on LTC4 could be abrogated by introducing 9–12 octadecadiynioc acid which is a cyclooxygenase-1 (COX-1) and 15-lipoxygenase inhibitor (Fig. 5c). The activating effect of HBV-sPLA2 on basophils also was shown by the upregulation of surface activation markers such as CD63, CD69, and CD11b. Phospholipase present in bee venom is capable to produce neolipid antigens after cleaving non-allergenic phospholipids. Neolipid antigens are presented to circulating CD1a-restricted T cell by CD1a which is expressed on epidermal Langerhans cells and dermal DCs (Fig. 6a). Subramaniam et al. [76] revealed the association of lipid antigens and CD1a-reactive T cells with the allergic response to bee venom considering that wasp and bee venom are injected naturally near the site of CD1a expression. T cells of individuals who were allergic to bee and wasp venom were isolated through CD3 MACS bead separation from PBMC (expanded by culturing with feeder cells) before and during immunotherapy and exposed to CD1a-transfected K562 cells in the presence of wasp or bee venom. CD1a reactivity of isolated T cells was examined by ELISpot with K562 or K562-CD1a in the presence or absence of bee venom. K562 cells lack MHC surface expression; thus, in co-culture with polyclonal T cells, the effect of MHC alloreactive responses is abrogated and T cell responses to CD1a can be measured. Lymphocyte response was evaluated by assessment of IFN-γ, GM-CSF, and IL-13 cytokine production. Allergic individuals were shown to have elevated frequencies of venom peptide-specific T cells in circulation. The results showed a higher frequency of IFN-γ, GM-CSF, and IL-13 responding T cells in the presence of K562-CD1a and bee venom in a panel of bee venom allergic as compared with non-allergic individuals.

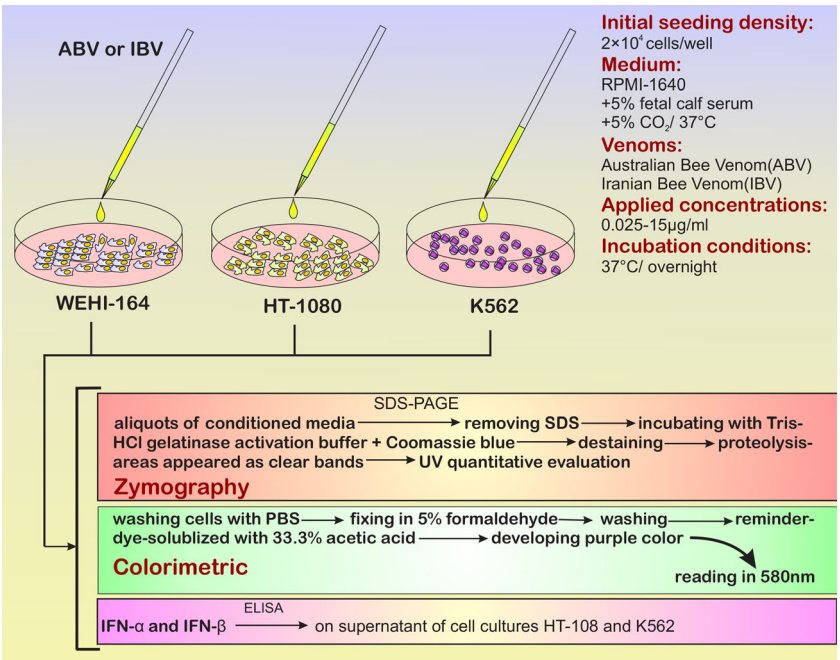

Fig. 4 Bee venom immunosuppressive and immunostimulatory properties can be revealed by cytokine production in venom-treated WEHI-164, HT-1080, and K562 cell lines

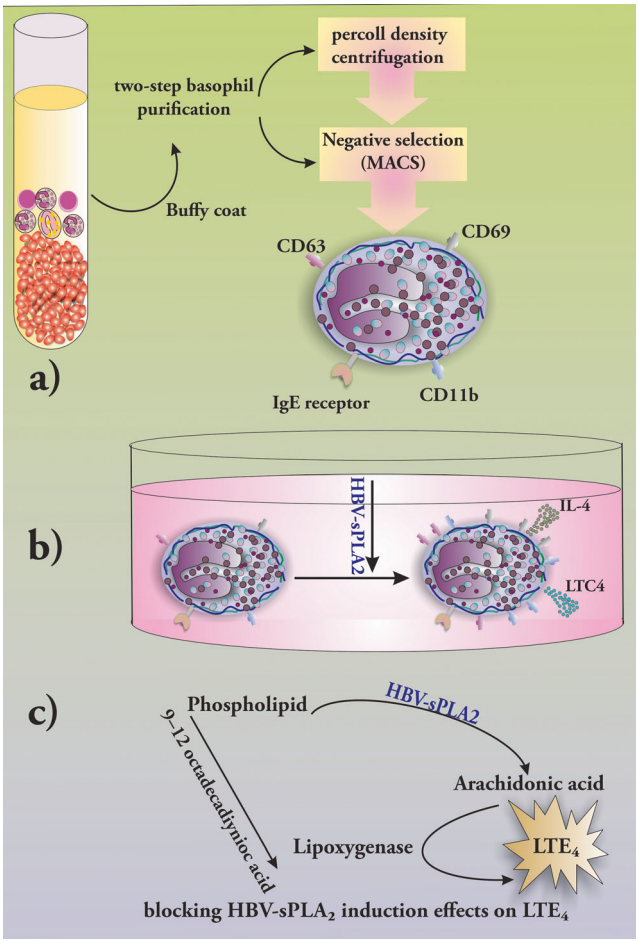

Fig. 5 Released mediators and surface activation markers of basophils under the influence of honeybee venom phospholipase A2 (HBV-sPLA2) in vitro showed a role of basophils in anaphylaxis: **a** protocol used to isolate basophils from buffy coat. **b** Overexpression of surface markers and cytokine release pattern of basophils after introducing HBV-sPLA2 in vitro. **c** HBV-sPLA2-inducing effect on LTC4 could be abrogated by introducing 9–12 octadecadiynioc acid

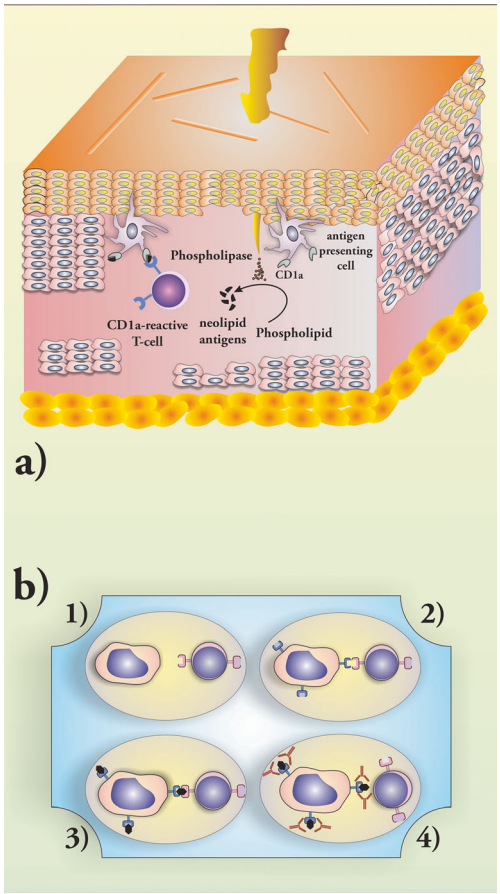

Fig. 6 **a** The mechanism by which neolipid antigens are generated and presented in the presence of bee venom phospholipase. **b** (*1*) Mock transfected K562 cells were used to obtain a detectable background response (IFN-γ, GM-CSF, and IL-13) in the absence of venom in culture. (*2*) K562-CD1a cells and CD1a-reactive T cells showed a higher response in the absence of venom. (*3*) The strongest response could be seen when K562-CD1a cells and CD1a-reactive T cells react in the presence of venom. (*4*) After applying blocking anti-CD1a antibody, the IFN-γ response reduced nearly to background levels

Application of blocking anti-CD1a antibody (OKT6) to CD1a-transfected K562 could significantly reduce the IFN-γ response. The results suggested a role for CD1 in mediating cellular immune responses to bee venom [76] (Fig. 6b).

Venom Immunotherapy

Venom immunotherapy (VIT) is the only treatment that can potentially prevent further severe allergic reactions [77]. Allergen-specific immunotherapy is generally based on the administration of increasing doses of allergen for achieving hyposensitization and reducing early and late responses occurring during the natural exposure to the priming allergen [78]. Generally, starting doses are often around 0.0001 μg per injection and rise to a maintenance does of 100 μg [63]. Achieving the maintenance dose in conventional VIT requires 3–4 months whilst accelerated protocols such as rush, ultrarush, and cluster require shorter time from several hours to a few days [79, 80]. In rush immunotherapy protocols, higher allergen doses are administered at 15- to 60-min intervals over a 1- to 3-day period to achieve maintenance dose; in contrast, cluster immunotherapy protocols recommend receiving several allergen injections (2–4 sequential injections) in a single day on nonconsecutive days to achieve the maintenance dose in 4–8 weeks [81]. After reaching the predetermined maintenance dose of BV, patients may undergo sting challenge to evaluate the efficiency of immunotherapy [82]. Establishing specific anergy state in peripheral T cells requires continuous treatment with high doses of allergen. Immunologic features of this state include suppressed proliferative and T cell-cytokine responses and simultaneous increase in IL-10 production. IL-10 suppresses both specific T cells and specific IgE production while it enhances IgG4

production. IL-10 regulates cytokine profiles in inflammatory responses and suppresses cytokine synthesis in T cells via inhibiting accessory CD28/B7.1 receptor interaction. During successful VIT, a rise in allergen-blocking IgG antibodies (especially IgG4 class) and the generation of IgE-modulating CD8+ T cells decrease in IL-4 and IL-5 production by CD4+ T cells; reduction in the number of mast cells and eosinophils and their mediators can be observed at molecular and cellular levels [83]. The IgG4 antibody production by memory B cells depends on the presence of IFN-γ, while IgE remains IL-4-dependent and suppressed by IFN-γ [84]. In addition, venom immunotherapy induces the generation of IL-10 and TGF-β-secreting Treg cells, which play suppressive roles in proliferative and cytokine responses against the venom allergens. Tregs influence B cells to suppress IgE production and induce the production of IgG4 against venom antigens [71]. Concentration of allergen and affinity of antigenic peptide to MHC-II and TCR molecules have been shown to determine T-cell cytokine profiles. For instance, in PLA-specific human T-cell clones, a 10–50 times lower threshold amount of antigen was required for the induction of IL-4 than for IFN-γ while increasing antigen concentrations could favor IFN-γ production by T cells. Applying chemically modified allergen variants instead of natural allergen is another approach to establish VIT. There are a number of strategies such as mineral oil precipitation and urea denaturation to render allergens to modified allergen variants, with low IgE-binding properties. Native allergens are capable of degranulating mast cells and basophils and utilize an IgE-mediated antigen presentation leading to increased Th2 cytokine and IgE production whereas modified allergens, which lack IgE-binding sites, utilize phagocytic or pinocytic antigen-uptake mechanisms by dendritic cells and monocytes/macrophages. The antigen-presenting properties of these cells lead to the generation of a balanced Th0/Th1-like cytokine pattern by T cells and result in normalized isotype production by memory B cells [83].

Discussion and Conclusion

Bee venom, a conglomeration of allergens, toxins, and other triggers of immune responses, has been known to mediate both healing effects and life-threatening anaphylaxis. However, much remains to be learned. In recent years, application of in vitro cell culture methods in addition to in vivo animal models has taught us surprising immunological events regarding bee venom effects at molecular and cellular levels. These findings shed the light on applying new methods for immunotherapy and allergy diagnosis. There are some aspects of immunologic interactions of bee venom components with immune cells remaining to be discovered. For instance, our knowledge regarding degranulation of mast cells following MCD peptide exposure is limited to the fact that MCD mediates mast cell degranulation by increasing the free cytoplasmic Ca2+ concentration which eventually leads to exocytosis of histamine-containing granules. However, MCD-specific/common cell surface receptors on mast cells are unknown. There are still some challenging open questions regarding the relationship between mastocytosis and hymenoptera venom allergy including the following: why do such patients react to hymenoptera venom or is there a specific mast cell dysfunction which leads to increased risk for anaphylaxis? Advances in Hymenoptera venom immunotherapy such as accelerated protocols including rush, ultrarush, and cluster have successfully served to lessen the regular immunotherapy sessions and also reduced the time needed to establish the immunity. However, most recent investigations revealed the possible immunologic mechanisms involved in VIT by shifting investigations in favor of understanding the venom allergen-immune cells interactions. In this regard, regulatory T cell activity including lymph node homing, tolerance induction, and modulation of TH1 responses has been monitored during wasp venom immunotherapy. Moreover, increased number of myeloid dendritic cells during immunotherapy and changes in function-associated surface molecules including FcgRII and Toll-like receptor 2 provide further fields of investigation associated with venom-immune cells interaction during immunotherapy [85]. Most researches aimed to investigate the role of bee venom components in the treatment of diseases have used animal models mainly mice, and the benefits of bee venom components in treating of human diseases have not proven. Interestingly, the allergen composition of bee venom varies not only among winter or summer workers but even in queens (caste differentiation). For example, queens lack six toxins in their venom in proportion to workers. Also, phospholipase A2 and melittin contents follow a semestral synchronized variation. Such variations cause significant changes in dominant allergens of bee venom [37]. Putting all the abovementioned aspects aside, there are some promising prospects in the field of bee venom immunology. Application of recombinant bee venom allergens in allergy investigations, for example, has dramatically reduced the possible cross-reaction among numerous allergens present in bee venom and also in discrimination between protein- and CCD-based IgE reactivity [58]. Moreover, specific recombinant allergens have been reported to be useful in the detection of cross-reactivity among hymenoptera venoms. This technology can be very helpful in deciding which specific venoms to use in the treatment of patients in which the allergic individual in uncertain about the culprit insect. Finally, there is a need for more sensitive diagnostic tests especially to distinguish individuals who probably will experience severe reactions in future stings from those with minimal risk but with similar levels of venom IgE.

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Conflict of Interest Daniel Elieh Ali Komi, Farzaneh Shafaghat, and Ricardo D. Zwiener declare that they have no conflict of interest.

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