

REVIEW

Bee venom allergy

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Introduction

Insects that sting are members of the order Hymenoptera of the class Insecta. In Europe, there are two major subgroups: *Vespidae* includes the wasps, hornet and paper wasp, and *Apidae* include the honeybee and bumblebee [1]. Honeybees and bumblebees sting only when provoked. The honeybee's sting has multiple barbs, which usually cause the venom sac and sting to detach from the insect, leading to its death. In bees, at least 90% of the venom sac contents are delivered within 20 s [2].

Hymenoptera venom is composed of biogenic amines, basic peptides and higher molecular weight proteins, mostly enzymes [1]. The major allergens in honeybee venom are phospholipase A₂ (PLA₂), hyaluronidase, acid phosphatase, and melittin. Antigenic crossreactivity exists between honeybee and bumblebee venom. However, a major part of venom-specific IgE in bumblebee venom-sensitized patients binds to components in bumblebee venom, which are not recognized in honeybee venom [3]. Major allergens in bee and vespid venom do not crossreact [4]. Therefore, patients allergic to wasp venoms are rarely allergic to bee venom.

Sensitization to bee venom

Sensitization is the presence of antigen-specific IgE or positive skin tests to antigen. Sensitization can occur without clinical reactivity, and therefore, sensitization to hymenoptera venom is far more common than a history of hymenoptera allergy. After a bee has injected the venom in the skin, antigen presenting cells (APCs) take up venom allergens and processes them. APCs present peptides to T-helper precursors (Th0) cells via MCH class II (MCH II) and the T-cell receptor (TCR) with obligatory costimulatory signals [5]. Enzymatic activity of bee venom causes release of preformed interleukin (IL)-4 from mast cells [6]. In tissues of atopic individuals Th2 cytokines are released more easily than in nonatopic subjects. Basophils and mast cells are able to induce human IgE synthesis in B cells, and they may orientate Th cells towards a Th2-like

phenotype [7]. Cultured basophils produce biologically active IL-4 and IL-13 and express functional CD40L after cross-linking of the high affinity IgE receptor (FcεRI), thereby contributing to IgE production by B cells [8]. Moreover, basophils are capable of producing large amounts of IL-4 and IL-13 in response to IgE-independent activation [9,10].

Th cells activated in the presence of Th2 cytokines tend to develop into the Th2 phenotype and release IL-4 and IL-13 [11,12]. These cytokines cause IgE isotype selection during the maturation of immature B cells activated by binding B cell epitopes of allergen via surface IgM, and the subsequent production of IgE [5]. The allergen-specific IgE binds to mast cells and basophils, resulting in allergic sensitization. Low doses of major allergen PLA₂ cause IL-10 release from APC, whereas high doses cause IL-12 production. In the latter case Th2 cytokine production are inhibited and interferon-γ (IFNγ) production induced, resulting in a Th1 response and no allergic sensitization [5]. However, the enzymatic activity of PLA₂ does not influence the specific activation and cytokine production by T cells from bee venom sensitized or hyperimmune individuals, or the IgE/IgG4 antibodies synthesis by B cells *in vitro* [13]. Carbadillo *et al.* [14] found that the regulation of IgE and IgG₄ secretion in response to bee venom PLA₂ was governed by the ratio of IL-4 to IFNγ production. It has been demonstrated that the *in vitro* expressed cytokine pattern of PLA₂-specific T-cell clones directly depends on the concentration of allergen in the culture, and the ratios of IL-4 to IFNγ decline with increasing allergen doses [15].

CD40L expressed on the T-cell surface has a pivotal role in B-cell activation through CD40. This interaction is required in T-cell-B-cell collaboration for antibody production to T-cell-dependent antigens and for the generation of memory B cells and germinal centre cells [16]. Moreover, activation of CD40 was shown to increase B-cell responsiveness to IL-4 and the promotion of IgE and IgG₄ formation [16]. Interaction of CD40L with CD40 prevents B-cell apoptosis [17]. When antigen is presented without appropriate costimulation, functional unresponsiveness of antigen-specific T lymphocytes is induced. One such critical costimulatory signal is the CD86/CD80:CD28/CTLA4 ligand-receptor pair expressed on APC and T lymphocytes, respectively [18]. This complex prevents the induction of

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antigen-specific tolerance and its blocking induces *in vitro* antigen-specific hyporesponsiveness [19].

In the general population, the serum venom-specific IgE for bee can be detected in 6–17% and for wasp in 12–21% of subjects [20,21]. In 9–30% of those who have hymenoptera venom-specific IgE in serum have specific IgE for both bee and wasp venoms depending on the method used [21,22]. Bee and wasp venom-specific IgE are more prevalent in men than in women [23]. The male subject predominance suggests that the degree of exposure is significantly greater for male compared to female subjects. The prevalence of venom-specific IgE is somewhat lower in Scandinavia than in southern parts of Europe [23]. Zora *et al.* [24] showed that the prevalence of venom-specific IgE is seasonably variable in general population.

Bees frequently sting beekeepers, and therefore, serum bee venom-specific IgE has been found in 51–79% of beekeepers [25,26]. In addition to bee venom allergy, observations of beekeepers with an inhalant allergy to bee body components have been made [27,28]. Beekeepers and their family members may be sensitized to bee venom through inhalation as well as stings. Thirty-one percent of beekeepers had experienced nasal or eye symptoms while working at beehives and these symptoms correlated with the history of systemic sting reactions [29].

There appears to be a hereditary aspect for Hymenoptera sensitivity. A significant decrease in human leucocyte antigen (HLA) DR4 and DQw3 alleles have been reported in bee venom sensitive patients suggesting that either HLA-DR or HLA-DQ has a protective role in controlling the IgE immune response [30]. Faux *et al.* [31] found that bee venom sensitive subjects had greater prevalence of DRB1*07 alleles than the control population. It is therefore suggested that immune response HLA class II genes may, in part, control IgE responses.

Epidemiology of hymenoptera sting reactions

Retrospective population studies according to case history suggest that the incidence of systemic sting reactions in a general population range from 0.3 to 7.5% depending on the population [20,21,23,32]. Owing to the fact that honeybees frequently sting beekeepers, the prevalence of adverse sting reactions is common among them. Systemic sting reactions have been reported in 22–43% and large local reactions in 31–38% of beekeepers [25,26,29]. The male subject predominance is probably caused by the greater exposure of men to bee stings. In Finland 80% of beekeepers are male. In study of beekeepers from Canary Islands 89% of the subjects were male [26]. The risk of being repeatedly stung by bumblebees is normally very low, because these insects do not sting deliberately. However, the increased use of bumblebees in greenhouses for the pollination of vegetable

flowers has increased the number of anaphylactic reactions to bumblebees [33].

About 25–32% of individuals in general population who have insect sting anaphylaxis are atopic [34,35]. A personal history of atopy has been found in 41–48% of beekeepers that had anaphylactic symptoms [25–29]. Therefore, it is obvious that atopic beekeepers more easily become allergic to honeybee venom than nonatopic ones. De la Torre-Morin and coworkers [26] found that sensitization to bee venom was highest among atopic beekeepers and those with fewest years of working experience in beekeeping.

Mortality from insect stings varies from country to country between 0.09 and 0.45 deaths per million inhabitants per year [1]. Fatalities occur predominantly in men over 40 years of age. However, post mortem studies suggest that these data may underestimate fatal Hymenoptera allergy. Elevated IgE antibodies to at least one insect venom were found on post mortem sera of 23–50% of sudden death victims [36].

Clinical features

The normal reaction of the skin to a bee or wasp sting consists of a painful, sometimes itchy, local weal, developing up to 2 cm diameter, surrounded by a swelling involving subcutaneous tissue several centimetres in diameter [1]. Allergy is a harmful physiological event that is mediated through immunological reactions [37]. Almost all patients who are allergic to bee venom are beekeepers, their family members, or their neighbours. The clinical presentation of allergic reactions to bee stings may be a large local swelling at the sting site or an immediate or delayed systemic reaction.

Large local reactions involve oedema at the sting site with a diameter exceeding 10 cm and lasting over than 24 h. Systemic reactions vary greatly in severity, and they can be classified in four grades [1]. Symptoms of grade I reactions are pruritus, urticaria, malaise and anxiety. Grade II includes angio-oedema, constriction in chest, nausea, vomiting, diarrhoea, abdominal pain and dizziness. Grade III reaction can cause dyspnea, wheezing, stridor, dysphagia, hoarseness, confusion, palpitation and a feeling of impending doom. Grade IV includes hypotension, collapse, or loss of consciousness and incontinence. The onset of systemic reactions is early, usually within 10 min of a sting.

Diagnosis

The diagnosis of bee venom allergy is based on a history of adverse sting reaction and on positive skin tests to venom or the presence of serum venom-specific IgE. It is important to check the basis of patient's assertion about the insect responsible for the sting. The severity of sting reaction has

no significant correlation with the serum levels of venom-specific IgE or skin tests [21,38]. The immediate systemic reactions to insect stings are not always IgE-mediated. Approximately 10% of patients experiencing an anaphylactic reaction to an insect sting have no detectable specific IgE or positive skin test results against the insect concerned [38,39]. A provocation test with a living insect as a diagnostic test seem to be the best predictor of recurrent systemic reactions, and it appears to have the highest negative predictive value [40,41]. The majority of individuals with severe reactions had no warning of their sensitivity. And even worse, most individuals who die of insect sting anaphylaxis have not had prior generalized allergic reactions [42].

Acute management

Systemic allergic reactions to hymenoptera stings are treated according to the guidelines of anaphylactic shock. Medications used are intramuscular adrenaline, corticosteroids and antihistamines. Volume substitution and pressor substances are given when needed. Subjects who have a history of potentially fatal insect hypersensitivity should carry an emergency kit containing aqueous adrenaline in a preloaded automatic syringe. If they are stung they should administer adrenaline intramuscularly and take tablets of corticosteroids and antihistamines. After these medications all patients should seek the assistance of an adult for medical help is required. Instructions on how to avoid insect stings should also be given to these patients. In case of bee venom allergy, a bee sting must be removed within 20 s after autotomization, without concern for the method of removal to avoid venom delivery.

Ice packs applied to the sting site are beneficial in extensive local reactions. Antihistamines and analgesics are useful to relieve the pruritus and painful swelling. Systemic glucocorticosteroids given for 3–5 days are most effective for widespread painful reactions. Where there is severe swelling in the extremities, rest and raising the affected limb bring additional relief from pain.

Venom immunotherapy

Venom immunotherapy (VIT) has been recommended to all patients who have had a life-threatening systemic reaction to bee sting and a positive venoms skin test or serum venom-specific IgE [43]. VIT has been protective in approximately 80% of bee and 95% of wasp venom-allergic patients, and it reduces the risk of re-sting anaphylaxis from 40 to 60% to less than 5% in subjects who have had systemic reaction [1]. The recommended duration of the therapy is 3–5 years [43]. Because VIT is costly and laborious, it is usually only given

to patients at high risk of experiencing potentially fatal symptoms after future stings.

Bee VIT induces a shift from a Th2 cytokine response to a Th1 dominant pattern [44]. The immediate drop in IL-4 production in rush VIT suggests profound down-regulation in T-cell responsiveness to allergen [44]. The mechanism might be due to T-cell anergy or activation induced apoptosis [45,46]. Activation-induced apoptosis may be prevented by CD28 costimulation of T cells [46]. The ability of antigen presenting cells to costimulate T cells through the CD28 molecule is impaired by crosslinking of surface immunoglobulin receptors, and thus, induction of allergen-specific IgG provides a possible mechanism by which immunotherapy might inhibit costimulation of allergen-specific T cells [47]. Immunotherapy may also result in the induction of specific suppressor T cells, which are able to suppress allergen-specific T-cell responses [48]. Changes in cytokine or chemokine production might have an early effect by down regulating mast cell or basophil reactivity [44]. T cells producing IL-10 and IFN γ play a key role for the inhibition of histamine and sulphidoleukotriene release of effector cells [49].

Immunoglobulins and bee venom allergy

Beekeepers that have been stung more frequently have lower serum levels of bee-venom specific IgE than those who have been stung less frequently [50]. High IgE and low IgG venom-specific antibody levels have been shown to possess an unfavourable prognosis in venom-allergic patients [51]. Serum venom-specific IgG has been significantly higher in nonallergic than in allergic beekeepers [50]. It has been suggested that allergen specific IgG₄ might react as allergen neutralizing or blocking antibody in a certain type of allergic disorders. Venom-specific IgG and IgG₄ antibodies become prominent upon chronic bee venom stimulation and are not associated with protection from adverse reactions to stings [39,52]. Akdis and colleagues [53] demonstrated that the allergen dose-dependent regulation of specific IgE and IgG₄ antibodies follows a mechanism different from those of total IgE and IgG₄ production. It has been suggested that antibodies to bee venom could neutralize the toxic and lethal effects of bee venom [54].

Lessof and coworkers [55] first demonstrated that the infusion of beekeepers' gammaglobulin protects patients with a bee venom allergy from a venom-induced reaction. Furthermore, hyperimmune beekeeper plasma reduced the maximum histamine release from peripheral blood leucocytes of strongly bee-venom allergic patients [56]. Müller *et al.* [57] showed with crossed radioimmuno-electrophoresis lack of an IgG response to particular bee venom components to which IgE was present in patients who had allergic side-effects in VIT. In addition, there was no IgG₂ response in

these patients. Jeep *et al.* [58] demonstrated with immunoblot studies of bee venom-allergic patients that the reactors differed from nonreactors in showing intense IgE and less IgG₄ binding to at least one single component of the venom extract. Thus, measurements of the absolute amount of specific IgG and IgE do not reveal the antibody pattern to the individual allergens in the extract.

It is possible that long-term bee venom stimulation induces the production of IgG autoantibodies. These autoantibodies could be as anti-idiotypes reactive with allergen-specific antibodies or specific anti-IgE [59,60]. The fine specificity of IgG antibody changed in 15 days during rush VIT, and the change was attributed to idiotypic antibodies [61]. Autoanti-IgE may modulate allergen-induced basophil histamine release [60]. Passive infusion of beekeepers' plasma rich in anti-idiotypic antibodies to a patient who experienced systemic reactions to honeybee venom decreased the clinical sensitivity to bee venom and sensitivity of both skin mast cells and basophils [59]. During VIT increased levels of anti-idiotypic antibodies in the serum of the patient were associated with diminution of specific antibodies to honeybee venom [59]. Batard *et al.* [62] showed that both allergen-specific IgG and autoanti-IgE inhibited basophil histamine release. It is not necessary that blocking antibodies bind to the same epitopes on the allergen molecule as IgE. Inhibition of IgE crosslinking could occur through steric hindrance as opposed to direct competition for allergen, thus accounting for the lack of effect of immunotherapy on allergen binding by IgE in serum immunoblots [63].

The natural course of bee venom allergy

The natural history of venom allergy is a self-limiting disease in a great number of cases. Many patients lose their sensitivity to future stings without having received any therapy [39,64]. Van Halteren *et al.* [41] found that 29% of those with severe systemic sting reaction in history had a severe reaction in sting challenge with living insects. In subsequent field stings only 1.8% of those with no reaction or mild systemic reaction in sting challenge reported serious complaints. In previous studies of patients having a history of allergic reactions after insect stings the rate of systemic reaction to a sting challenge was 25–30% [40,52]. However, when only bee-sensitive individuals are considered, the rate of systemic reaction has been 44–52% [40,52]. When re-sting reactions occur, the nature of the symptoms tends to be similar to the symptoms of the initial sting reaction [65]. Reisman found that only 11% of patients had more severe reactions to re-sting, and therefore, he suggested that patients with mild to moderate anaphylactic symptoms probably do not require VIT [65]. Among children up to the age of 16 with dermal reactions only

(hives, angioedema) after insect stings the re-sting reaction rate is remarkably low (9.2%) and re-sting reactions milder than the original reactions [66]. The occurrence of a large local insect sting reaction does not increase the risk of subsequent generalized allergic reactions [67]. A follow-up study without immunotherapy showed that venom-specific IgE decreases, and in 33% became negative, while skin tests became negative in 24% of allergic subjects [68]. Asymptomatic venom sensitization in adults is common but transient, disappearing at a rate of 12% per year [69].

Sensitization and desensitization to bee venom has complicated mechanisms. Venom sensitization may disappear when a person avoids bee stings. In beekeepers desensitization may occur through chronic bee venom exposure with similar mechanisms that occurs in VIT. VIT should be given to persons that have increased risk of a life-threatening allergic systemic reaction. The prediction of future sting reactions is difficult. A provocation test with a living insect seems to be the best predictor of recurrent systemic reactions, although it has been criticised and is not widely used. The increasing knowledge of the mechanisms involved in allergic reactions is crucial when developing better diagnostic tests and new effective therapies for bee venom allergy.

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