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Honeybee venom immunotherapy: certainties and pitfalls

The honeybee is an interesting insect because of the fundamental agricultural role it plays, together with the composition of its venom, which presents new diagnostic and immunotherapeutic challenges. This article examines various aspects of honeybee venom allergy from epidemiology to diagnosis and treatment, with special emphasis on venom immunotherapy (VIT). Honeybee venom allergy represents a risk factor for severe systemic reaction in challenged allergic patients, for the diminished effectiveness of VIT, for more frequent side effects during VIT and relapse after cessation of treatment. Some strategies are available for reducing the risk of honeybee VIT-induced side effects; however, there is considerable room for further improvement in these all-important areas. At the same time, sensitized and allergic beekeepers represent unique populations for epidemiological, venom allergy immunopathogenesis and VIT mechanism studies.

KEYWORDS: beekeeper ■ build-up phase protocol ■ efficacy ■ future strategies ■ honeybee ■ mechanisms ■ recombinant venom allergens ■ relapse ■ risk factors ■ safety ■ venom immunotherapy

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Hymenoptera stings can cause severe systemic allergic reactions and occasionally fatal anaphylaxis. The offending Hymenoptera belong to the Vespidae (Vespininae and Polistinae sub-families) and Apoidea superfamilies. The latter superfamily includes honeybees (HBs; *Apis mellifera*), which are brown in color and moderately hairy, and bumblebees (genus *Bombus*), which are larger than honeybees, much hairier and characterized by distinct alternate yellow and white bands on their abdomen [1].

This article aims to provide a focused understanding of the uniqueness of HB venom allergy, with special emphasis on venom immunotherapy (VIT).

Available epidemiological data on HB stings are thin on the ground. Up to 94.5% of interviewees recalled receiving one Hymenoptera sting, which was climate-related, one-third of which were from HBs, at least as far as Europe is concerned [2].

The estimated prevalence of sensitization is between 9.3 and 28.7% in adults [2]. HB-specific IgE was found in 3.7 to 16% in all adults [3]. One study which revealed a much higher sensitivity to HB venom (HBV) was based on a pediatric population in which only a skin prick test was done [4]. The prevalence of large local reactions (LLRs) in the general population ranges from 2.4 to 26.4% [2]. Although this may be, there are no separate data for HBs.

According to European and American population-based studies, the lifetime prevalence of a

systemic reaction (SR) to Hymenoptera ranges from 0.3 to 7.5% in adults, which is lower in children, and related to factors of geography, the study population, the venoms used for diagnosis and the subject's profession and hobbies. In a recent Spanish epidemiological study on a general population, the HB caused 45.5% of all reactions [5].

Hymenoptera stings were responsible for about 20% of cases of biphasic anaphylaxis where it was not possible to determine the exact role of HB stings [2].

HB-sting fatalities have never been studied as a separate entity, though extrapolation of the literature clearly shows the diverse role of HB stings. Data from the UK and Florida (USA) yielded a lower percentage of HB-sting fatalities than vespid-sting deaths [2], while a Costa Rican study reported that HB stings were mainly responsible [6].

Beekeepers & family members

Due to high-risk exposure beekeepers (BKs) and their family members (FM) are at an especially high risk of becoming allergic, making them unique populations for epidemiological, venom allergy immunopathogenesis and VIT mechanism studies (Box 1).

Depending on the degree of exposure, the prevalence of LLR in BKs ranges from 9 to 31%, while the prevalence of SR ranges from 14 to 38% [7], even though more recent studies document a lower percentage of SRs [8]. None of those

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Box 1. Some features of honeybee venom-allergic beekeepers.

- Prevalence of large local reactions: 9–31%
- Prevalence of systemic reactions: 14–38%
- Protection correlates with receiving more than 200 stings per year
- Sting reactions occur most frequently following the first stings of the year
- Risk of systemic reaction is greater in atopic than in nonatopic beekeepers
- Risk of systemic reaction is greater in beekeepers with allergic rhino conjunctivitis or asthma when managing their hives

Data taken from [8].

BKs receiving more than 200 stings yearly had a SR, whereas 45% of those receiving only up to 25 stings did, the first reaction usually occurring after the winter break [7].

Risk of SR is greater in atopic than in non-atopic BKs. A Turkish study demonstrates an 11-fold increase in the risk of a SR in BKs when there are two or more concurrent atopic diseases [8]. It has been suggested that while they work in their hive, atopic BKs with allergic rhinoconjunctivitis or asthma are more prone to sensitization than nonatopic BKs, which is most likely due to either the inhalation of bee dust or multiple stings.

A recent study of British BKs revealed predisposing factors to SRs to be female sex, having a FM with bee venom allergy, more than 2 years of beekeeping before a SR and antihistamine premedication before attending to the hives [9]. However, the online survey, questionnaire-based study and self-reported history of bee venom allergy are some of the study's limitations. It is interesting to note that only 44% of BKs with SRs attended the emergency department because of their symptoms, 66% of whom were reviewed by an allergist, and only 18% carried an adrenaline autoinjector, suggesting that there is a need to educate this high-risk group [9].

The only study to compare allergic BKs with their allergic FMs and normally exposed HB-allergic controls was published some years ago [10]. The prevalence of SRs in allergic FMs was 10%. No difference in the severity of allergic sting reactions emerged. Specific IgG was significantly higher in allergic BK than in the two other groups. Skin sensitivity and serum bee venom-specific IgE antibodies to HB venom were significantly lower in atopic BKs than in the two other groups, suggesting that specific IgG antibodies might interfere directly with the allergen–IgE antibodies interaction during skin testing or in the radioallergosorbent test assay. Suppression of specific IgE production in B cells and increased specific IgG4 formation owing to the stimulation of Tregs by the high

number of stings may be another nonalternative explanation [10].

HB venom allergens

Although their sacs may hold up to more than 300 µg of venom the amount of HB venom protein released by honeybees per sting ranges from 50 to 140 µg [1].

HB venom is a mixture of proteins and other pharmacologically active molecules that diffuse into the tissue after a sting. While it is the biogenic amines and peptides that primarily cause local pain and inflammation at the sting site, allergic sensitization is most often due to proteins [11].

It is of interest that a recent study was able to map the diffusion and distribution of HB venom allergens and toxins in different layers of the skin of a rat from a live HB sting [12].

Knowledge of venom composition and the structure of allergens is a prerequisite for the accurate diagnosis and treatment of insect venom allergy (TABLE 1). The sequences and structures of the majority of major venom allergens have been cloned and sequenced; several major allergens have been expressed in recombinant form.

Currently, a total of 12 HBV allergens are known [101]. The most important allergen in HB venom is phospholipase A2 (PLA2), also referred to as Api m 1, which is a glycoprotein with 134 amino acid residues. In addition to the IgE response it elicits, its actions on the human immune system may enhance an immediate immune response, by increasing leukotriene production [13]. Another well-known HB venom allergen, hyaluronidase (HYA; also called Api m 2) [14], a 43-kd glycosylated enzyme, is able to enhance the penetration of venom components through the tissue fluid. Api m 2 shares a 50% sequence identity with vespid venom HYA, although a recent paper demonstrated that much of the cross-reactivity between yellow jacket and HB HYAs is not due to protein cross-reactivity but rather to cross-reactive carbohydrate determinants (CCDs) [15]. It seems reasonable to assume that antibody binding to HYA from other types of Hymenoptera like the HB may be due to CCDs in many cases, making it important to reinvestigate the role of hyaluronidase in HB venom allergy, in which Api m 2 is considered the second most important allergen after PLA2 (TABLE 1).

Acid phosphatase (Api m 3), a 45-kd, glycosylated enzyme is probably a minor allergen [16]. Melittin (Api m 4), a peptide, with a molecular weight of 2.84 kd, is the most important and abundant peptide in HB venom, even though only 28% of patients have specific IgE antibodies

Table 1. Allergens of honeybee venom.

Allergen	Biochemical name	Molecular weight (kDa)	Major/minor
Api m 1	Phospholipase A2	16	Major
Api m 2	Hyaluronidase	39	Major (?)
Api m 3	Acid phosphatase	43	Minor
Api m 4	Melittin	2.8	Minor
Api m 5	Low dipeptidyl peptidase IV	102	?
Api m 6	Cysteine-rich trypsin inhibitor	8	Minor
Api m 7	CUB serine protease	39	?
Api m 8	Carboxylesterase	70	Minor
Api m 9	Serine carboxylesterase	60	?
Api m 10	Icarapin	50–55	?
Api m 11	Major royal jelly protein	50	?
Api m 12	Vitellogenin	200	?

'?' indicates that it is not yet known whether the allergen is major or minor.

against this peptide [11]. Melittin can trigger lysis of a wide range of cells, such as mast cells, which can lead to the release of histamine and other intracellular components into surrounding tissue. Interestingly, cell lysis is further enhanced when it is presented together with PLA2 (Api m 1) [17].

Other HB venom allergens have also been identified, and include dipeptidylpeptidase (Api m 5), cysteine-rich trypsin inhibitor (Api m 6), CUB serine protease (Api m 7), carboxylesterase (Api m 8), serine carboxylesterase (Api m 9) and icarapin (Api m 10) (TABLE 1) [18–20]. A recent study showed that Api m 10 seems to be a genuine HB venom allergen with IgE-sensitizing potential in a significant percentage of allergic patients, and that three therapeutic HB venom extracts lacked detectable amounts of this component [20].

Other low-molecular weight components of HB venom are histamine, dopamine, noradrenalin, free amino acids, lipids, pheromones and the peptides apamine, mast cell degranulating (MCD) peptide, tertiapin and secapin [11].

Mechanisms of HB VIT

HB VIT represents the best model for the study of the cellular and molecular mechanisms of immune tolerance development in sensitized individuals. At the same time, high-dose bee venom exposure in BKs through natural bee stings enables understanding of the mechanisms of T-cell tolerance to allergens in nonatopic individuals [21].

Different mechanisms involving specific cell populations, play a role in the different phases

of HB VIT [22], which can be outlined as the following below.

Peripheral T-cell tolerance represents an essential step in successful VIT, and is mainly characterized by the generation of allergen-specific Treg cells [23]. In patients on HB VIT, as well as in heavily exposed BKs, the *in vitro* immune response in lymphocyte cultures following stimulation with venom proteins reveals the presence of CD4⁺ Tregs, which secrete IL-10, consequently inhibiting the secretion of cytokines IL-4, IL-5 and IL-13 from TH2 cells in turn impeding specific IgE production, while venom-specific IgG4 increases [22]. It is worth noting that during bee VIT, IL-10 serum levels rise from the second day of the build-up phase [24]. Also, Treg cells directly or indirectly suppress effector cells of allergic inflammation such as mast cells, basophils and eosinophils, and inhibit maturation of dendritic cells [21].

The very early effects of VIT are also associated with an early reduction in effector cell releasability. Immediately after day 1 of the ultrarush protocol of HB, VIT histamine and leukotriene release from venom-stimulated basophils is already significantly down [25], thus explaining the extremely precocious protection seen in HB venom-allergic patients who have already reached the maintenance dose during rush or ultrarush VIT [26]. This effect during very early effective VIT may be due to the piecemeal release of mediators through the high allergen doses applied over a short period, which may decrease the granule content of mediators and affect the threshold of mast cell and basophil activation.

During VIT, specific IgG, especially IgG4 antibodies, greatly increase within 2 weeks and stay elevated throughout treatment, whereas skin sensitivity and specific IgE antibodies gradually fall during prolonged VIT (after an initial increase). The result is a rapid decrease in the ratio of specific IgE to specific IgG4, and consequently the inhibition of mast cell activation by interaction of venom allergens with effector cell-bound specific IgE. Blocking antibodies also inhibits IgE-facilitated allergen presentation to T cells and prevents an allergen-induced boost of memory IgE production. However, there is no close correlation between the IgG titer and the clinical efficacy of VIT [11,27], while one does exist between IgG4 concentrations and the number of annual stings and years spent in beekeeping [1,11].

The role of histamine and its receptors in VIT is a new field of research [21].

Indications for HB VIT

Indications for HB VIT are the same as for vespid allergy [27]. The history of an SR, the demonstration of an IgE-mediated mechanism and knowing the risk factors for a severe outcome are prerequisites for prescribing VIT [2]. Nevertheless, the risk of a future allergic reaction can only be assessed statistically as none of the tests available are able to accurately predict the outcome of the next sting. This circumstance makes it difficult to specify what level of statistical risk justifies VIT [28].

Diagnostic venom tests are positive in 10–20% of asymptomatic individuals, underlining the importance of patient history as a factor. The patient is asked to describe his/her symptoms and course of the sting reaction. In patients who experience unclear or psychovegetative reactions, the treating doctor's report and any records of the reaction and related treatment should be requested and reviewed. Individual risk factors for anaphylaxis must be investigated. The number of stings and the culprit insect are also requested. Besides BKs, the vast majority of patients are unable to positively identify the stinging Hymenoptera. For normal HB exposed subjects, a single HB is usually unintentionally encountered by direct interaction as HBs are relatively docile [3]. After a sting, the stinger usually remains embedded in the skin because of the multiple small barbs. After shedding its stinger, the HB typically dies within hours or days. Moreover, it is interesting to note that quickly removing the stinger may minimize a reaction as most of the venom is delivered within the first 5–10 s [3].

■ Diagnostic testing

The advantages (in terms of specificity and sensitivity) as well as limitations of the standard diagnostic tests (skin test and detection of venom-specific IgE antibodies) have been discussed extensively elsewhere [1,29]. Even though the diagnosis is often simple, some pitfalls persist, which include the presence of IgE-negative anaphylaxis and of double positivity to both bee and vespid venoms in diagnostic tests with a single-positive history, which has been observed in 25–40% of hymenoptera venom allergy patients, thus making the choice of venom immunotherapy prescription a difficult one [1]. Double positivity may arise from double sensitization, cross-reactivity between epitopes on hyaluronidase in the two venoms or more frequently to CCDs of venoms and common allergens [15]. The IgE-inhibition test is of use when distinguishing between cross-reactivity and double sensitization. However, this is costly and results are sometimes difficult to interpret.

Since several major venom allergens have been expressed in recombinant form, some of which are now commercially available for diagnostic purposes, the so-called molecular diagnosis or component resolved diagnosis (CRD) should also be applicable in the field of HB venom allergy. In the first study published on this topic double IgE positivity to whole venoms was documented in 59% of 200 venom-allergic patients by ImmunoCAP, but only 17% were double positive to rApi m 1 and rVes v 5 (species-specific major allergens without CCDs of the HB and *Vespula*). The author concluded that double positivity to both Api m 1 and Ves v 5 indicates true double sensitization, thus suggesting immunotherapy with both venoms [30]. More recent studies [31–35] found a lower diagnostic sensitivity of ImmunoCAP rApi m 1 for the detection of HB venom allergy, one of which [34] hypothesized a European north–south difference in Api m1 sensitization. However, different laboratory methods [35], different sources of HB allergens [36] as well as different selection of patients with HBV allergy [32,33] may be responsible for these differences in sensitivity.

The addition of other recombinant species-specific bee venom allergens without CCDs (like Api m 3 or Api m10) would further increase diagnostic accuracy [33,35]. In addition, the detection of CCD-specific IgE is also possible, using different methods and carbohydrate antigens (bromelain, MUXF3, horseradish peroxidase and ascorbic acid oxidase) [37,38]. In conclusion, provided that the majority of relevant venom allergens are

available in recombinant form, CRD enables physicians to discriminate between genuine double sensitization and cross-reactivity to CCDs in patients with double-positive IgE results to conventional venom extracts, optimizing patient selection for VIT. At the moment it does not seem that CRD is able to distinguish between more and less severe systemic sting-induced reactions.

Additional *in vitro* tests, like the cellular antigen stimulation test (CAST) or basophil activation test (BAT), although more expensive, can be used to determine immunological sensitization in difficult-to-diagnose patients in laboratories with an expert technician [39]; however, their predictive value in relation to a sting challenge has not been analyzed in large patient populations yet.

Finally, even if evidence suggests that HB yield more reliable sting challenge results than vespids, sting challenges should not be used as a diagnostic tool in untreated patients, as the absence of systemic symptoms does not rule out the possibility of an SR reaction to a future sting [40].

■ Natural history & risk factors

In many cases the natural history of venom allergy is self-limiting, as many patients spontaneously lose their sensitivity [2]. After a LLR, about 10% of HB venom-allergic patients will develop a SR when next stung by a sting challenge [41]. According to sting challenge studies non-VIT treated bee venom-allergic patients are at a greater risk of a SR on re-sting (44–52%) than those who are vespid venom allergic (24–25%) [42,43]. However, the well-known association between bee allergy and more severe SRs [2] could be untrue for field stings, as demonstrated by a European prospective multicenter study in which vespid venom allergy was significantly associated with severe reactions after a field sting [44]. It is possible that less HB venom is delivered at a field sting as most sting victims attempt to rapidly remove the stinger, thereby reducing the amount of bee venom delivered.

Other risk factors associated with the occurrence of a severe sting SR in untreated patients are cutaneous and/or systemic mastocytosis [45], clonal mast cell disorders [46], at least in adults, and even an increased baseline serum tryptase concentration (BSTC) [44]. BSTC should therefore be measured in all patients with SRs [29,44,47] who should also undergo a dermatological examination for cutaneous mastocytosis [40].

Even if in general the risk of recurrence of SRs is linked to the severity of the previous reaction, more recently it seems that preceding less severe SRs would produce a booster effect

and predispose the patient to subsequent severe reactions [44].

Elderly patients and patients with pre-existing cardiovascular diseases develop particularly severe, even fatal, SRs more often [48]. A recent study confirmed that treatment with angiotensin-converting enzyme inhibitors is a risk factor for SRs [44].

■ Indications

VIT is indicated in patients who have a history of a severe Hymenoptera sting-induced SR and detection of IgE-mediated sensitization to the venom that triggered the reaction [27,29,47]. The 2005 European guidelines state that in both children and adults with a history of a systemic, nonlife-threatening reaction (urticaria, erythema, pruritus), other factors may influence the decision to commence VIT, which include occupations or hobbies where the risk of exposure is high, the culprit insect itself, concomitant cardiovascular diseases, other pathologies, like mastocytosis, and psychological factors due to anxiety, which can seriously impair patient health-related quality of life [27].

Very recent English guidelines prescribe more restricted additional risk factors for VIT in less severe SRs, which include a raised baseline tryptase, a high likelihood of future stings, or effect on quality of life [47].

VIT may benefit venom-allergic patients with mast cell diseases, albeit to a lesser extent than patients without [45].

Even though subcutaneous VIT is effective even in LLRs [49], it is not usually recommended. However, the specialist may consider it as treatment in highly exposed subjects who need repeated corticosteroid shots yearly, like BKs [29].

■ Contraindications

In Europe, serious immunological diseases, cancer and chronic infections are absolute contraindications for inhalant immunotherapy as well as for VIT. Indeed, in some selected venom-allergic patients the advantages of VIT might outweigh the potential side effects, particularly where risk of sting exposure is high and there is a history of a near-fatal sting reaction, and perhaps in the presence of mast cell diseases [28,47]. Nevertheless, multicenter studies are required to better evaluate the effectiveness and safety of VIT in these patients.

In contrast to inhalant immunotherapy guidelines, major cardiovascular diseases are not contraindications for serious insect venom allergies. Untreated elderly cardiopathic patients

are at increased risk of developing severe or even fatal sting reactions. Also, severe cardiovascular or respiratory disorders are almost always an urgent indication for VIT. Again, patients who developed myocardial infarction during sting anaphylaxis and had positive diagnostic tests are candidates for VIT [48]. VIT in patients on β -blockers is not a contraindication if the cardiac risk in venom-allergic patients is greater than the risk of a SR during VIT, however, VIT must be performed in an appropriate hospital setting [48].

In women of childbearing age, VIT should be started before pregnancy. However, a well-tolerated maintenance VIT may be continued to prevent the risk of further SRs, even for the fetus [27].

Efficacy

The efficacy of subcutaneous VIT has been confirmed by both sting challenge and in-field stinging in prospective controlled and uncontrolled studies, in one meta-analysis and in one systematic review demonstrating that the protection rate of HB VIT is lower than that offered by vespid VIT [50–52].

The recommended maintenance dose of 100 μ g of venom, both in children and adults, may be equivalent to one or two HB stings. This standard dose prevents SRs in approximately 75–85% of HB-allergic patients who are re-stung [2,27,29,50]. If a SR occurs, it is usually much less severe than before VIT. According to a recent study, a 50- μ g HB maintenance dose in children may be adequate for protection from future HB stings [53], almost as if the bees were able to tell the difference between an adult and a child and in consideration of such, injected the child with less venom. This study showed a SR rate of 13.6% 1–18 years after completion of 2–7 years of immunotherapy using a 50- μ g maintenance dose, which was considered similar to the protection afforded by using a 100- μ g maintenance dose of bee venom in adults. However, the retrospective nature of this study could be considered a limitation, as the young participants' accounts of the reaction type were subjective [53].

A dose of 200 μ g or more is recommended when a SR follows an insect sting in spite of VIT with 100 μ g and in highly exposed populations such as BKs [2,10,54]. In one recent study, out of 79 HB venom-allergic patients who agreed to be challenged, nine had mild or moderate SRs, among which four received a higher dose of venom and tolerated a second challenge [26]. It is noteworthy that when VIT is effective, its protection starts immediately after the conventional maintenance

dose has been reached, as demonstrated by sting challenge performed 1 week after reaching the maintenance dose, even after a 3–5 day rush protocol. Some authors believe that an elevated maintenance dose (usually 200 μ g) from commencing VIT should be administered to HB-allergic patients with concomitant mastocytosis and also considered in vespid venom-allergic subjects with other risk factors [40]; however, such recommendations may be difficult to follow in patients with a very severe SR or cardiovascular diseases.

Some authors have shown that VIT efficacy in mastocytosis sufferers may be reduced [45]. However, from the limited available data, it is not possible to confirm whether there is any difference in the reduced VIT effectiveness between HB and vespid mastocytosis-treated patients.

The repeatedly observed difference in the success rates in HB and vespid venom-allergic patients is not completely clear. The fact that the amount of venom delivered by a HB sting during a sting challenge is much larger and more consistent may partially explain this difference in the reaction rate to sting challenges, which has also been observed in untreated patients [27,44]. The composition of HB venom, which is a mixture of proteins and other pharmacologically active molecules, including melittin [11,17], may be another explanation.

Treatment protocol

Slow to ultrarush induction protocols have been designed to maximize protection, minimize side effects and optimize patient convenience. Rush and ultrarush protocols make it possible to reach the maintenance dose in either a few days or hours, respectively, with subsequent boosters before initiating a monthly schedule [28]. These treatment regimens are particularly useful in highly exposed subjects like BKs or in patients referred to the specialist just prior to the start of the insect season, in order that the protective dosage be reached as quickly as possible. Furthermore, the cost of performing ultrarush VIT is lower than that of the slower protocols [55]. Rush VIT is routinely used as standard treatment in most European countries.

TABLE 2 shows the current rush protocol used in our department in HB venom-allergic patients [56].

In general, the starting dose of VIT is between 0.001 and 0.1 μ g. However, it seems that initiating HB VIT at the 1- μ g dose can be applied safely in rush protocols, both in adults and in children, since there was no SR to this starting dose in 428 HB venom-treated patients [57].

In Europe, subcutaneous HB VIT may be done using nonpurified aqueous (NPA), purified aqueous (PA) extracts, and purified aluminum hydroxide adsorbed preparations (so-called 'depot' extracts), though in some countries just one type of extract is available. Purified venom extracts do not contain vasoactive amines (like dopamine, histamine and serotonin) and the presence of small peptides like apamine, kinins and MCD peptide in the final product is reduced [58]. The efficacy of PA and depot HB extracts is supported by studies using both sting challenge and in-field stings and is comparable to that of nonpurified preparations [58].

The general consensus is that the maintenance interval should be kept at 4 weeks for the first year, extended to 6 weeks in the second year, and then to 8 weeks if VIT is continued over 5 years, provided that the treatment is tolerated [27]. Where possible, it is recommended that the dose of 200 µg venom as a depot extract not be exceeded over a 4-week period [40].

In the case of depot extracts, extending the injection interval to 8 weeks is possible without reducing the effectiveness of the therapy [40]. A number of studies showed that extending the maintenance interval up to 12 weeks may be safe and effective [59]. However, they included very few HB venom-allergic patients, in which efficacy was evaluated mostly by in-field stings.

While waiting for the results of future multicenter clinical trials, it is advisable to avoid a maintenance interval that exceeds 8 weeks for HB-allergic patients, especially as BKs who

received fewer than ten stings a year were those who developed SRs most frequently. A further prolonged interval between injections of 6 months did not provide suitable protection in bee venom-allergic patients, as demonstrated by in-field stings and challenge [60].

At the moment no data are available on the best maintenance interval to be used in mastocytosis patients [45], even though it is presumable that prolonging the maintenance interval may be dangerous.

Safety

Patient compliance with VIT can be impaired by LLRs and SRs, among which, anaphylaxis represents the biggest risk posed by VIT. Presently there is no reliable test able to predict the extent of the risk. The finding that increased basophil sensitivity to allergen-specific *in vitro* stimulation is significantly associated with major side effects to VIT even in children needs to be confirmed by further studies [61]. Therefore VIT should only be carried out by allergists with experience and knowledge in this field and in centers that have administered VIT to large numbers of patients and where the team has expertise in treating anaphylaxis [47].

The literature reports a large variation (0–46%) in the incidence of VIT's side effects, with the tolerance of HB VIT (20–40%) faring worse than that of vespid VIT (0–15%) [3,50].

A recent systemic review reported the incidence of SRs during VIT as being 25.1% for HB venom and 5.8% for vespid venom [62]. Again, in

Table 2. Protocol of honeybee venom immunotherapy build-up rush phase.

Day	Dose	Dose administered (ml)	Dose administered (µg)	Dose concentration (µg/ml)
1	1	0.10	0.01	0.1
	2	0.10	0.1	1
	3	0.10	1	10
	4	0.20	2	10
2	5	0.30	3.0	10
	6	0.35	3.5	10
	7	0.35	3.5	10
3	8	0.10	10	100
	9	0.15	15	100
	10	0.15	15	100
4	11	0.20	20	100
	12	0.25	25	100
	13	0.25	25	100
5	14	0.30	30	100
	15	0.35	35	100
	16	0.35	35	100

Data taken from [56].

a recent multicenter European study, one of the most important factors significantly associated with severe reactions during the build-up phase of VIT was bee venom allergy [63]. The reasons why HB VIT induces SRs more frequently than vespid VIT have yet to be fully understood.

However, other risk factors for VIT-induced SRs must be taken into account, which may also be present at the same time in the HB-allergic patient [28].

Discordant data exist on the safety profile of rapid dose increase protocols compared with conventional or cluster up-dosing regimens used mainly in vespid-allergic patients [28,64,65], even though in a large European study rapid dose increase was associated with increased risk of side effects during the build-up phase of VIT [63]. Indeed, the number of HB-allergic patients that have been studied to date is lower than the vespid patient numbers. In a recent paper, 132 HB-allergic subjects were administered a 3-day rush VIT using a NPA extract resulting in a rate of SR of 31% during the maintenance phase [55]. After excluding hyper-reactive subjects (e.g., patients who failed to reach the maintenance dose with conventional VIT because of multiple SRs), in 85% of whom the maintenance was reached, the difference between HB and vespid-treated patients was not significant (23.7 vs 16%, respectively) [55]. A similar rate of SRs (27.5%) occurred using the same HB extract and a 5-day rush protocol in an Italian paper, which was significantly higher than the rate of SRs (2.5%) observed by using the corresponding PA [56]. At present, ultrarush protocols should only be carried out by clinicians who are experienced in managing VIT, and preferably in the hospital setting, especially where HBV-allergic patients are concerned.

Unlike β -blockers [66], angiotensin-converting enzyme inhibitors may, in part, be responsible for the onset of SRs during VIT in some highly selected patients, although more data are required to confirm this [28].

Finally, mastocytosis patients developed side effects during VIT more frequently [45]. Moreover, BSTC correlates significantly with the frequency of severe side effects during the dose-up phase of VIT, even though the extent of this effect seems to be more marked in patients treated with vespid VIT [44].

Box 2 shows the possible current and future strategies for reducing the risk of VIT side effects. Pretreatment with an H1 antihistamine has been shown to reduce the number and severity of LLRs and mild SRs to VIT, such as urticaria and

angioedema [67]. The finding that antihistamine premedication may improve the clinical efficacy of ultrarush HB VIT was not confirmed in HB venom-allergic subjects using the same protocol and a different antihistamine [68].

Pretreatment with anti-IgE monoclonal antibodies has been successful according to several case reports describing HB venom-allergic patients, including patients with indolent systemic mastocytosis, almost all of whom experienced SRs to VIT were able to tolerate VIT after pretreatment with omalizumab [69,70]. However, until now the optimal time for its administration during VIT, the appropriate dosage, the long-term effects, and the best incremental protocol of VIT to be used still remain unknown [28].

Sublingual immunotherapy using HB venom was recently shown to reduce the diameter of LLR to a bee sting challenge by more than 50% in only 57% of active treated patients [41]. On the basis of these findings and without experimental data on the pharmacokinetics of venom sublingual immunotherapy, significant doubt exists as to its clinical relevance at preventing future SRs [71].

There is a sound theoretical basis for believing that PA and purified aluminum hydroxide adsorbed extracts, whose efficacy is comparable to that of NPA preparations, have the potential to reduce the incidence of VIT side effects [58,72,73]. Very recently, it was shown that the majority of low-molecular weight HB venom components of purified extracts are rapidly removed from the site of VIT administration. Furthermore, the purified aluminum hydroxide-adsorbed preparation demonstrated a depot effect, prolonging the availability of bee venom allergens at the site of administration, elucidating for the first time the kinetics of HBV components and the local responses at the site of subcutaneous administration, at least in an animal model [17]. From a clinical point of view, comparing PA extracts with the correspondent purified depot extract and using different incremental phases in HB venom-allergic patients, Rüeff *et al.* revealed that treatment with the PA extract caused significantly more frequent LLRs than those caused by the depot extract during both the rush and maintenance phases, while only a tendency of more frequent SRs during the incremental phase was observed between the groups [72]. For the first time a recent paper compared, in terms of the occurrence of SRs, a PA extract and the corresponding NPA extract under the same 5-day rush protocol in HB venom-allergic patients with a normal BSTC [56]. SRs during the build-up phase occurred significantly more often in patients treated with NPA extracts than

in those treated with the corresponding PA preparations. All patients who developed a SR tolerated the depot extract reaching the maintenance dose [56]. Hence, if protection through VIT is to be achieved rapidly, the use of purified preparations is preferable for rush treatment with the use of depot preparations for maintenance therapy, at least in HB venom-allergic patients [58].

Duration of VIT

Analysis of available data indicates a higher risk of relapse in HB venom than in vespid venom-allergic patients. The reason for this difference is not entirely clear, but it has been discussed extensively elsewhere [50]. Using 100- μ g maintenance doses of HB venom extract for at least 3 years, SR rates ranged from 15.8 to 23% by administering a sting challenge or in-field stings 0–10 years after cessation of VIT [50,74,75]. There are no data on the outcome after more than 10 years of HB VIT; any definite conclusion can be extrapolated from studies mainly performed in American vespid-allergic patients often treated with high doses of venoms [76].

Other risk factors for the recurrence of SRs following Hymenoptera stings after stopping VIT, which may also be present in the same HB-allergic patient theoretically increasing the risk of relapse, have since been identified and include old age, severity of pretreatment reactions, generalized allergic side effects to VIT injections, incomplete protection upon re-sting during VIT, mast cell diseases and an elevated baseline tryptase level [28].

On the premise that the duration of VIT should be decided after a thorough patient–physician discussion of the issues involved, the current recommendations for stopping VIT from the European and American Academy are very similar [27,29]. According to these guidelines the recommended duration is 3–5 years while a longer-term treatment (or lifelong treatment) should be considered in patients with a higher risk of very severe sting reactions (e.g., older age, history of very severe previous sting reactions, elevated basal serum tryptase or mastocytosis or use of β -blockers); generalized allergic reactions to immunotherapy injections or stings during VIT; highly exposed patients such as BKs and their immediate FMs.

Conversely, in the UK, the usual duration of VIT is 3 years. Longer or even lifelong treatment in patients with mast cell diseases or a raised baseline tryptase is not advocated in the UK since it is not considered as being evidence-based, based on case reports [47]. Out of four

Box 2. Possible current and future strategies for reducing the risk of honeybee venom immunotherapy-induced side effects.

- Premedication: antihistamines, omalizumab
- Purified aqueous and depot extracts
- Route of administration: intralymphatic
- Modified allergens

patients who died because of a yellow jacket sting after stopping VIT, three had mastocytosis, of whom two had received yellow jacket VIT while the other one HB VIT. The fourth patient, who had received HB VIT and died after an insect sting (the type of insect is not known) 9 years after stopping VIT had no history of mastocytosis and no autopsy was done, thus mastocytosis could not be excluded [47]. It is of note that it is only in the UK that a review was ever commissioned to assess the clinical effectiveness and cost–effectiveness of Pharmedgen® (ALK Abelló, West Berkshire, UK; the only marketed extract authorized in UK) in providing VIT to individuals with venom SRs [77].

VIT in BKs

In allergic BKs and allergic FMs who agree to stop beekeeping, the indications of VIT are the same as in other HB venom-allergic patients. However, although prone to severe SRs, many BKs refuse to stop beekeeping not only because of the source of income it represents, but also out of the fondness they harbor for their bees. Some of them use complementary and alternative methods to treat their allergy, which include homeopathic remedies [78].

VIT is strongly recommended in patients with positive diagnostic tests and a history of a moderate-to-severe SR [7,10]. In patients with only cutaneous reactions, VIT is indicated if they remain highly exposed or in the case of repeated reactions. A rush or ultrarush protocol up to 100 μ g provide rapid protection, even though a 200 μ g maintenance dosage is recommended [7,40] since BKs may receive multiple stings at a time, even with protective clothing. Some experts suggest performing a sting challenge in order to verify VIT protection before going back to beekeeping; otherwise, an increased dosage of HB venom is administrated [7,40].

Comparing the side effects of VIT and its efficacy by a sting challenge among allergic BKs, allergic FMs and allergic controls, allergic side effects were observed less frequently in allergic BKs than in the other two groups, even though these allergic BKs received a maintenance dose of 200 μ g, which was 100 μ g for the other two

Box 3. Honeybee venom allergy: unmet needs.

- Epidemiological data specifically addressing honeybee allergy and its natural history
- More studies to evaluate the role of recombinant allergens in improving the sensitivity and specificity of diagnostic testing in honeybee venom allergy
- Multicenter studies on the efficacy, safety and duration of venom immunotherapy in mastocytosis patients and in those with increased baseline serum tryptase concentration
- More prospective controlled studies on the safety of purified extracts
- Safety of rapid build-up phase protocols
- Feasibility of hypoallergenic preparations (modified recombinant allergens, T-cell epitopes and DNA vaccination) and/or of other routes of administration (e.g., intralymphatic)
- Quality-of-life studies in untreated and venom immunotherapy-treated honeybee allergic patients

groups. Side effects occurred in all three groups more frequently in patients who had experienced a severe pretreatment SR compared with those who had experienced a mild-to-moderate reaction [7,10]. As expected, during VIT the re-sting rate was higher in allergic BKs. Efficacy based on a sting challenge was similar, and the severity of pretreatment reaction did not influence the results of a re-sting during VIT [7,10].

In an old study, Müller *et al.* proposed a special protocol to maintain tolerance by self-application of bee stings. Some BKs, once-monthly maintenance injections of 200 µg of venom and field stings having been well-tolerated for 3 years, were instructed to avoid more than two stings when wearing protective clothing and to apply one or two weekly stings during the warm season and during winter to apply at least one sting per month or to restart monthly maintenance injections of 200 µg of HBV. As a result, out of seven BKs only one developed a mild SR each spring, when resuming his beekeeping, during the first years after stopping VIT [10].

In BKs who did beekeeping as a hobby and who underwent VIT for an average of 3.7 years, a recent study showed the complete absence of symptoms after re-exposure to bee stings, since out of 63 patients only one reported a mild allergic reactions [78]. However, the study has several limitations, which include the method used (questionnaire, which was probably answered only by successful VIT-treated BKs) and the missed maintenance dosage [78].

Conclusion

The HB is a unique insect, not only thanks to the important agricultural role it plays, but also because of the composition of its venom, which poses diagnostic and immunotherapeutic challenges. HBV allergy represents a risk factor for severe SR in challenged allergic patients, for poorer effectiveness of VIT, for more frequent side effects during VIT and relapse after stopping the treatment. There is considerable room

for further improvement in these all-important areas. At the same time, sensitized and allergic BKs represent unique populations for epidemiological, venom allergy immunopathogenesis and VIT mechanism studies.

A number of new strategies for bee venom immunotherapy, mostly based on genetic engineering, have been described and we are eagerly awaiting the advent of their application in clinical practice.

Future perspective

The production of recombinant venom allergens will greatly enhance the accuracy of the diagnosis and undoubtedly help pave the way towards novel therapeutic techniques in the future.

As for diagnosis, provided that the majority of relevant HB venom allergens are available in recombinant form, CRD will enable physicians to discriminate between genuine double sensitization and cross-reactivity in patients with double-positive IgE results to conventional venom extracts, allowing optimization of patient selection for VIT.

Concerning treatment, a number of new strategies using sequences of one or more HB allergens, mostly based on genetic engineering, have been studied though only in animal models [79–83].

The only trial performed in humans in whom the efficacy of the treatment was verified by a sting challenge, used a mixture of three dominant T-cell peptides of phospholipase. Out of five patients, complete protection was observed in three and partial protection in the remaining two [84], confirming that treatment with only one major allergen, even in recombinant unrefolded or point mutated form, may be insufficient.

Finally, the therapeutic potential of phospholipase bee venom intralymphatic immunization was analyzed in sensitized mice using an anaphylaxis model, showing an enhanced allergen-specific IgG and T-cell responses and a Th1-dependent subclass IgG2a production when

compared with subcutaneous injections [85,86]. Similar studies in humans, including clinical parameters and sting challenges, are needed to assess the effectiveness of this route of allergen administration. Unmet needs are summarized in Box 3.

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Executive summary

Honeybee venom allergic reaction

- Even though relatively docile, honeybees (HBs) can adopt defensive awareness and induce allergic reaction in sensitized subjects.
- The amount of venom released by one HB sting ranges from 50 to 140 µg of venom protein per sting.
- The rate of a systemic reaction (SR) following a sting challenge with HB is greater than that of vespids.
- Beekeepers are at higher risk for sting-induced SRs, if they receive fewer than 25 stings per year.
- The risk of a SR is greater in atopic than in nonatopic beekeepers.

HB allergy diagnosis

- The standard tolls continue to play a fundamental role in the diagnosis of HB venom allergy.
- Component-resolved diagnosis may greatly enhance the accuracy of the diagnosis, allowing physicians to optimize patient selection for immunotherapy, especially in double-positive patients with a single history.
- Some of the relevant HB venom allergens are not commercially available in recombinant form.

HB venom immunotherapy

- Subcutaneous venom immunotherapy (VIT) is the only effective treatment for Hymenoptera venom-allergic patients.
- HB VIT represents the best model in the study of the cellular and molecular mechanisms of immune tolerance development in sensitized individuals.
- The mechanisms of protection induced by immunotherapy were first demonstrated using HB VIT.
- Indications for HB VIT are the same as for vespid allergy.

Efficacy

- HB venom allergy represents a risk factor for a lesser effectiveness of the standard 100 µg VIT (protection rate of 75–85%).
- Besides the type of venom (HB), other risk factors influencing a reduced effectiveness have been identified, and include a lower maintenance dose, repeated side effects during VIT and mast cell diseases.
- Prospective studies are needed to confirm the preliminary data on the effectiveness of a maintenance dose of 50 µg in children.
- Indeed, some patients may require doses higher than the standard 100 µg to prevent a future SR, as in the case of beekeepers.
- A maintenance dose interval more than eight weeks should be carefully considered in HB venom-allergic patients.

Safety

- HB venom allergy represents a risk factor for more frequent side effects during VIT (SR rates ranged from 20 to 40%).
- Additional risk factors for side effects during VIT have been identified, and include the build-up phase, the rapid dose increase schedule, mastocytosis and an elevated baseline serum tryptase concentration during the build-up phase.
- Patients with one or more risk factors need to be treated and monitored with special care.
- Treatment with purified aqueous and depot extracts can reduce the frequency of large local and systemic side effects, even during a rush build-up phase using the former.
- Although not approved for prevention of anaphylaxis, the anti-IgE antibody omalizumab may be used in selective cases.

VIT duration

- HB venom allergy represents a risk factor for more frequent relapse after cessation of treatment (SR rates ranged between 16 and 23%).
- Certain other risk factors for relapse after the discontinuation of VIT have been identified.
- The duration of VIT should be established on an individual basis taking into account the individual risk factors and also the patient's point of view.

Future perspective

- The majority of the new approaches and routes (e.g., intralymphatic) for HB VIT have not been tested in humans.

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