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From Immunotherapy

Hymenoptera Venom Immunotherapy

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Abstract and Introduction

Abstract

Subcutaneous venom immunotherapy is the only effective treatment for patients who experience severe hymenoptera sting-induced allergic reactions, and the treatment also improves health-related quality of life. This article examines advances in various areas of this treatment, which include the immunological mechanisms of early and long-term efficacy, indications and contraindications, selection of venom, treatment protocols, duration, risk factors for systemic reactions in untreated and treated patients as well as for relapse following cessation of treatment. Current and future strategies for improving safety and efficacy are also examined. However, although progress in the past few years has been fruitful, much remains to be accomplished.

Introduction

Hymenoptera stings can induce allergic systemic and occasionally fatal reactions. The offending hymenoptera belong to the suborder Aculeate, which are made up of the Apoidea (*Apis mellifera* and *Bombus* species) and Vespidae (Vespinae and Polistinae subfamilies) superfamilies,[1,2] although several of the European vespid species differ from those found in the USA where popular names for vespids are different, which may lead to confusion.[3] In the USA, stinging hymenoptera also include the fire ant (*Solenopsis invicta*), and in Australia, the jack jumper ant (*Myrmecia pilosula*).[4,5] In Europe, systemic reactions (SRs) from ant stings are extremely rare and are therefore not considered within the scope of this article. Hymenoptera venoms are mixtures of various low-molecular-weight substances, peptides and proteins, which often have enzymatic properties. The major allergens in bee venom are phospholipase A_2 (Api m 1), hyaluronidase (Api m 2), and presumably acid phosphatase (Api m 3) and serine protease, while in vespids venoms they are phospholipase A1 (Ves v 1), hyaluronidase (Ves v 2) and antigen 5 (Ves v 5).[1]

Detailed clinical features of insect sting reactions and their pathogenesis are described elsewhere.[1,2] The most frequent clinical patterns of hymenoptera venom allergy (HVA) are large local reactions (LLRs) and SRs; in the latter, the skin, gastrointestinal, respiratory and cardiovascular systems can be involved. Various classifications of the degree of severity of SRs have been proposed, of which the classification of Mueller and Ring are the most frequently used (Box 1). However, Mueller's classification does not take into account the possible absence of cutaneous symptoms and that an isolated cardiovascular shock might be the only allergic sting-induced manifestation, while Ring's system focuses almost entirely on cardiovascular collapse, considering it as more severe than respiratory compromise.[6] A universal definition of anaphylaxis, such as that recently proposed by the National Institute of Allergy and Infectious Disease (NIAID) and the Food Allergy and Anaphylaxis Network (FAAN) symposia,[7] would lead to a more uniform reporting of cases and a more accurate picture of the severity of insect sting reactions after validation by means of prospective multicenter trials.

Box 1. Classifications of systemic reactions to insect stings.

Classification by HL Mueller

- Grade I – Generalized urticaria, itching, malaise and anxiety

- Grade II – Any of the above plus two or more of the following: angioedema, chest constriction, nausea, vomiting, diarrhea, abdominal pain and dizziness
- Grade III – Any of the above plus two or more of the following: dyspnea, wheezing, stridor, dysarthria, hoarseness, weakness, confusion and feeling of impending disaster
- Grade IV – Any of the above plus two or more of the following: fall in blood pressure, collapse, loss of consciousness, incontinence and cyanosis

Classification by J Ring

- Grade I – Generalized skin symptoms (e.g., flush, generalized urticaria and angioedema)
- Grade II – Mild to moderate pulmonary, cardiovascular and/or gastrointestinal symptoms
- Grade III – Anaphylactic shock and loss of consciousness
- Grade IV – Cardiac arrest and apnea

Adapted from [1,130,131].

In the USA, the prevalence of SRs to hymenoptera sting in the general population ranges from 0.5 to 3.3%. European epidemiological studies from the last decade report a prevalence of 0.3–8.9% for SRs, with anaphylaxis reported in 0.3–42.8% of cases.[3,6] The results yielded by a small number of studies indicate that the prevalence rates of SRs are lower in children, ranging from 0.15 to 0.8%, with a recent exception of 4.4% reported in a study from Israel.[8] Although low, mortality rates are not negligible, with the incidence of insect sting mortality due to early anaphylaxis figures ranging from 0.03 to 0.48 fatalities per 1,000,000 individuals per year.[3,6] Underestimates are common worldwide with many sting fatalities going unrecognized and misinterpreted as unexplained.

To date, there is no existing parameter that enables clinicians to predict who will have a future reaction and whether it will be a LLR or generalized anaphylaxis. Several concomitant factors, which include the environment, genetics and individual elements, may account for the occurrence of a SR in any one patient.[6]

An anaphylactic reaction after a hymenoptera sting is an extremely traumatic event for the patient and their family. It has been demonstrated that patients who experience anaphylactic reactions following yellow jacket wasp stings reported impaired quality of life related mainly to the emotional strain of having to remain vigilant while performing everyday routine activities.[9]

Subcutaneous venom immunotherapy (VIT) is a highly effective treatment,[2,4,10] which is designed to reduce the risk of a subsequent SR, prevent morbidity and mortality and also improve health-related quality of life (HRQL), as demonstrated in yellow jacket allergic patients.[11]

Mechanisms of Action of VIT

The underlying immunological mechanisms of VIT are continuously being elucidated. Different mechanisms involving specific cell populations, play a role in the different phases of VIT,[12] even though some described events may represent epiphenomena rather than causative events. Peripheral T-cell tolerance represents an essential step in successful VIT, and it is mainly characterized by generation of allergen-specific Treg cells, with suppressed proliferative and cytokine responses against the major allergen.[13] This is initiated by autocrine action of IL-10 and TGF-β that are increasingly produced by the antigen-specific Tr1 cells,[14–16] which express CD4 and CD25. This finding raises the question as to whether these are inducible Tr1 cells, which have upregulated CD25 or naturally occurring $CD4^+CD25^+$ Treg cells that produce suppressive cytokines.[17] There is some evidence in adults that circulating $CD4^+CD25^+$ Treg cells and IL-10- and TGF-β-secreting Tr1 cells represent overlapping populations, and that $CD4^+CD25^+$ Treg cells from atopic donors have a reduced capability to suppress the proliferation of $CD4^+CD25^-$ T cells following allergen immunotherapy.[17,18] It has been suggested that upregulation of $CD4^+CD25^+$ Treg cells plays a role in allergen immunotherapy. Accordingly, using an ultrarush protocol in vespid allergic patients, an increase in $CD4^+CD25^+$ Treg cells and IL-10-producing T cells has been demonstrated in the hours following the commencement of VIT.[19]

Although peripheral tolerance has been demonstrated in specific T cells, the ability of B cells to produce specific IgE antibodies is not eliminated during allergen immunotherapy.[13] The effects of VIT on venom-specific IgE and IgG antibodies are well documented,[2] although neither concentration (or a change in concentration) of IgG nor the IgE:IgG ratio correlate closely with the clinical response to immunotherapy.[10] The proposal of blocking antibodies has been

re-evaluated on the basis of observations of altered specificity and affinity during immunotherapy and effects on memory B cells and antigen-presenting cells.[20] IL-10, produced and progressively secreted during allergen immunotherapy, appears to counter-regulate synthesis of antigen-specific IgE and IgG_4 antibodies. IL-10 potently suppresses both total and allergen-specific IgE while simultaneously increasing IgG_4 production.[15,21] Therefore, IL-10 not only generates T-cell tolerance but it also regulates specific isotype formation and skews the specific IgE response towards an IgG_4-dominated phenotype.

Very early effects of VIT are also associated with an early decreased mediator release from mast cells and basophils, although the mechanism of this desensitization effect is, as yet, unknown.[12,22] Ultrarush protocols significantly increase the release of mediators of anaphylaxis into circulation without inducing a systemic anaphylactic response in the majority of cases. This piecemeal release of mediators may decrease the granule content of mediators and affect the threshold of mast-cell and basophil activation.[12,23] An increase in intracellular cAMP levels immediately after vespid rush VIT, parallel to a decrease of allergen-induced release of histamine and sulfidoleukotrienes from basophils, was demonstrated;[24] this intracellular modification might account for the decreased reactivity of basophils to allergen following 1 week of VIT, although this seems not to be a long-term effect as values returned to baseline after 6 months.[24]

Indications & Contraindications

Indications for VIT are based on the history of a SR, a positive venom skin or specific IgE, knowledge of the natural history and established risk factors for a severe outcome.[1,6,25] Nevertheless, the risk of a future allergic reaction can only be expressed statistically since none of the available tests 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.

Patient History

The history is especially important as diagnostic tests with venoms are positive in 10–20% of asymptomatic individuals. The patient is asked to describe his/her symptoms and course of the sting reaction, number of stings, cues to the type of insect involved and individual risk factors for anaphylaxis. In general, cutaneous manifestations are more common in children than in adults, respiratory symptoms occur with equal frequency in approximately 40% of children and adults, while cardiovascular signs and symptoms are common in adults, whereas these are infrequent in children.[2,5] In patients with equivocal clinical histories, the treating physician's report and any records of the reaction and related treatment should be requested and reviewed. Positively identifying the stinging insect as a bee or wasp is a common confusing issue, except for beekeepers.

Diagnostic Testing

The decision to commence VIT requires confirmation of allergic sensitivity to venom allergens by positive venom skin tests and/or detection of venom-specific IgE antibodies in the serum.

The European Academy of Allergology and Clinical Immunology (EAACI)[1] and the American Academy of Allergy, Asthma and Immunology (AAAAI)[4] have recently published clinical practice parameters on diagnosis. Only those patients with a history of a previous SR are suitable candidates for diagnostic testing,[1,4] even though physicians in certain countries perform diagnostic tests in patients who have a history of LLRs despite being rated as having a reduced risk of a future SR.[6] Skin tests are the methods of choice and should be performed at least 2 weeks after a reaction to a sting in order to rule out a false negative during the refractory period.[1] Because the duration of refractoriness may be longer, they should, if negative in the presence of a definite history of a SR, be repeated after 1–2 months.[1] However, some patients reported having only manifest sensitization in the first week after being stung.[26] Guidelines recommend stepwise incremental venom skin tests, starting with a skin-prick test (from a concentration of 0.01 up to 100 µg/ml).

Even at venom concentrations of 100 µg/ml, the sensitivity of the skin prick test is lower than that of the intradermal test, which has to be used in order to confirm the negative result (from a concentration of 0.001 up to 1 µg/ml).[1] Skin tests with hymenoptera venoms are generally safe; nevertheless, SRs do occur, although these are very rare.[2]

Hymenoptera venom products, such as lyophilized protein extract for honey bee, bumble bee, yellow jacket and *Polistes* wasp venoms, are commercially available in many countries, the latter two being mixtures of the clinically relevant species. Due to incomplete cross-reactivity between venoms of the European and American species of *Polistes*,[27] commercial preparations of European *Polistes dominulus* venom have become available.[28] *In vitro* tests, such as the dosage of specific IgE to hymenoptera venom, can be applied to detect sensitization. The most recently developed tests are usually

the most sensitive.[1,29] However, their sensitivity in patients with a history of systemic sting reactions is lower than that of intradermal skin tests, especially when more than 1 year has elapsed after the reaction.[1]

If *in vitro* tests are negative in approximately 20% of positive skin tests,[30] the converse is also true, since approximately 10% of negative skin tests yield a positive *in vitro* result. Therefore, European allergists recommend skin testing and evaluation of venom-specific IgE in all patients with a history of SRs.[31]

There is no positive correlation between the severity of previous sting reactions and skin-test reactivity, or the concentration of venom sIgE.[1] Indeed, the most reactive skin tests often occur in patients with only LLRs, while almost 25% of patients who are referred for evaluation of a sting SR are intradermal skin-test positive only at the 1 µg/ml concentration, thus demonstrating the importance of testing with the full diagnostic range of venoms.[31] In a study that measured IgE antibody levels in sera from 51 venom anaphylaxis fatalities, IgE antibodies were not detected in 10% of the sera, indicating that fatal sting anaphylaxis is a potential occurrence in the presence of widely varying amounts of specific IgE antibody.[32]

Double positivity to both bee and vespid venoms in diagnostic tests has been observed in 25–40% of HVA patients, the majority of whom have a single-positive history and are unable to identify the culprit insect. Double positivity may arise from double sensitization, cross-reactivity between epitopes on hyaluronidase in the two venoms or to cross-reactivity between cross-reactive carbohydrate determinants (CCDs) of venoms and common allergens.[1,33] These widespread pan-allergens are not necessarily clinically irrelevant and are found on bromelain or horseradish peroxidase, which can be used in screening for such antibodies, as well as MUFX (neo-glycoprotein fucosylated/xylosylated *N*-glycans from bromelain). The IgE-inhibition test is of use when distinguishing between cross-reactivity and double sensitization. However, it is costly and results are sometimes difficult to interpret. In a recent study of 200 patients with a history of reaction to honey bee or *Vespula* sting, 59% had double positivity for specific IgE using the ImmunoCAP test, and 32% had double positivity using ADVIA Centaur® (Siemens).[34] Specific IgE to the recombinant nonglycosylated major allergen, Api m 1, was detected in 99% of cases of whole bee venom-positive allergy, while sIgE to Ves v 5 was present in 96% of whole *Vespula* venom-positive allergic patients when tested by ADVIA Centaur, thus reducing the double positivity to 17%.[34] Similarly, a combination of nonglycosylated recombinant bee and wasp venom major allergens (rApi m 1, rApi m 2 and rVes v 5) enabled clinicians to diagnose patients with HVA, which should facilitate the accurate prescription of VIT.[35] According to other authors, the recombinant availability of Ves v 1 from yellow jacket could also contribute to a more detailed 'component resolved diagnosis' of HVA and subsequently to better VIT prescription.[36]

On the other hand, there are those patients with a clear history of a SR but negative skin and *in vitro* radioallergosorbent test (RAST) results.[30] Negative test results can be due to the involvement of a different pathogenetic mechanism, mastocytosis, the time interval between sting reactions, poor test sensitivity or use of the wrong venom for the diagnostic test.[1,37]

In this respect, a modified assay (ImmunoCAP) to detect very low concentrations of venom-specific IgE,[38] as well as a dialyzed yellow jacket venom extract for skin testing,[39] have been suggested. Additional *in vitro* tests, such as 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.[40–43] However, their use should be restricted to laboratories with expert technicians, bearing in mind that a positive result may also point to a non-IgE mediated mast-cell activation mechanism.[44]

Finally, live insect 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 a SR to a future sting.[45]

Recently, the first comprehensive audit of the diagnosis and management of HVA patients in the UK revealed a wide variation in practice and suggested the need for the development of better educational programmes for specialists and trainees involved in the management of the patients in question.[46]

Natural History & Risk Factors

Reaction severity, confirmation of venom IgE sensitivity, current knowledge of the natural history of HVA and risk factors form the selection criteria for suitable VIT candidates, when deciding whether to start VIT and for how long to treat them for. In general, the risk of recurrence of SRs is linked to the severity of the previous reaction: the more serious the initial reaction, the greater the risk of recurrence. Linking the estimated risk of a future SR with reaction severity, age and sting interval, adults and children with LLRs are a low-risk category for a SR (5–15%) when re-stung.[3,6] Adults with a recent

history of severe anaphylaxis have a 40–60% chance of reacting to a re-sting, whereas those with mild SRs have a 20% chance of a subsequent SR.[47] The prognosis for children is better than that of adults with respect to the risk of SRs to re-stings. However, those who experience a moderate-to-severe SR continue to have a high risk of reactions even 15–20 years later.[48] According to recent data provided by an EAACI multicenter study, preceding less severe SRs would appear to produce a booster effect and predispose the patient to subsequent severe reactions.[25]

In the absence of a history of a sting-induced allergic reaction, sensitization by an asymptomatic sting has been reported by Golden *et al.* to be associated with a 17% chance of a SR to a future sting,[49] while Fernandez *et al.* suggests that this condition is not a clear risk for a future SR, at least in the case of vespids.[50]

Other risk factors associated with the occurrence of a severe field-sting SRs in untreated patients are represented by an increased baseline tryptase concentration,[25] cutaneous and/or systemic mastocytosis[51,52] and even clonal mast-cell disorders,[53] at least in adults. Baseline serum tryptase levels should, therefore, be measured in all patients with SRs as well as a dermatological examination for cutaneous mastocytosis.

Contrary to previous findings,[2,6] vespid stings seem to be associated with more severe reactions than do bee stings.[25,54] The relative risk for life-threatening sting reactions in the Mediterranean area is approximately three-times higher for hornet (*Vespa crabro*) stings than for honeybee or yellow jacket stings.[55]

Recommendations for the use of VIT vary from country to country. While there is a strong consensus that VIT is indicated for severe systemic sting reactions, there is less agreement on whether adults, and especially children, with mild (cutaneous) reactions are suitable candidates, as the prognosis in dermal reactors is usually considered to be good.[2,4] Although current opinion on providing patients with a history of a mild reaction with autoinjectable epinephrine differs among experts,[4,5,31] its negative effect on HRQL could mean that treatment with autojectable epinephrine alone is probably inappropriate.[56] Since the occurrence and severity of anaphylaxis depends on several factors, the advantages and disadvantages of not administering VIT should be thoroughly discussed on an individual patient basis. Furthermore, even in children with mild SRs, management should also take the child's behavioral risks into consideration. Indeed, the updated European Guidelines state that in both children and adults with a history of a systemic, non-life-threatening reactions (e.g., urticaria, erythema and pruritus) other factors may come to bear on the decision to commence VIT, which include occupation and/or hobbies with a high risk of exposure, the culprit insect itself, concomitant cardiovascular diseases, others pathologies, such as mastocytosis, and psychological factors arising from anxiety that can seriously impair patient HRQL (Table 1).[10]

Table 1. Indications for venom immunotherapy.

Type of reaction in adults/children	Diagnostic tests: skin and/or IgE test	Decision regarding venom immunotherapy
Respiratory and cardiovascular symptoms	Positive Negative	Yes No
Urticaria if risk factors or quality-of-life impairment are present	Positive Negative	Yes No
Large local	Positive or negative	No
Unusual	Positive or negative	No

Data taken from [10].

Venom immunotherapy is indicated in HVA patients with mast-cell diseases as the treatment can reduce SRs, albeit to a lesser extent than in otherwise healthy subjects with HVA;[52] moreover, an elevated level of baseline serum tryptase should be included in the decision of which patients should be offered the treatment.[25]

Venom immunotherapy is able to reduce the severity and duration of LLRs and its efficacy improves over a period of 2–4 years.[57] Although VIT is not usually recommended in patients with such reactions, as the risk of a subsequent systemic sting reaction is low, physicians may consider it as a treatment option when confronted with extremely anxious patients with HRQL impairment or highly exposed subjects who require repeated per annum corticosteroid shots.

Contraindications

In Europe, the standards for practical allergen-specific immunotherapy[58] underlines that serious immunological diseases, cancer and chronic infections are absolute contraindications for immunotherapy in general; major cardiovascular diseases are contraindications, except in the case of serious insect venom allergies. Untreated, elderly, cardiopathic patients are at increased risk of developing severe, or even fatal, sting reactions[59] and severe cardiovascular or respiratory disorders are almost practically always an urgent indication for VIT.[45] Untreated patients with anaphylaxis should not be given β-blockers, except under circumstances where the administration of these drugs is urgently required as in the case of certain heart rhythm disorders.

Administration of VIT to patients receiving β-blockers (even in eye drops) is contraindicated as they can aggravate anaphylactic reactions and also interfere with treatment. However, if the cardiac risk in venom allergic patients outweighs the risk of a systemic reaction during VIT, then VIT is appropriate for use in subjects receiving β-blockers but must be performed in an emergency care setting.[60]

Severe allergic reactions, including anaphylaxis, have been described in patients on angiotensin-converting enzyme (ACE)-inhibitors subsequent to being stung or receiving immunotherapy.[10] According to the results of a recent EAACI multicenter study, ACE-inhibitors should be withdrawn in untreated patients.[25]

Currently, there are no data to support or exclude the potentially harmful role of angiotensin-receptor blockers in patients with anaphylaxis in general, or in HVA-untreated patients.[61]

As regards the other contraindications, such as serious immunological diseases and cancer, the European guidelines need to be reviewed in order to cater for cases of severe sting allergic reactions, particularly where there is a high risk of sting exposure, a history of a near-fatal sting reaction and perhaps for mast-cell diseases given the treatment's life-saving potential. Indeed, in some selected cases the advantages of VIT might outweigh the potential negative effects.[45,62] Nevertheless, multicenter studies are required to better evaluate the safety of VIT in these patients, as an acute worsening of these diseases cannot be ruled out, particularly if the diagnosis was made prior to commencing VIT, and the possible reduced effectiveness of VIT in patients with autoimmune diseases, or in immunosuppressant therapy, should be taken into consideration.

Venom immunotherapy should not be begun during pregnancy, but well-tolerated maintenance VIT may be continued in order to prevent the risk of further SRs in the mother as well as in the fetus.[10] Patients who develop sting reactions such as Henoch–Schoenlein syndrome, vasculitis, acute disseminated encephalomyelitis or interstitial nephritis should not be treated with VIT.[2,10] By contrast, those who developed cerebrovascular or myocardial infarction during sting anaphylaxis and have positive diagnostic tests are candidates for VIT.[59]

Selection of Venoms

Selection is based on the identification of the hymenoptera species that is involved, on the results of the diagnostic tests and on venom cross-reactivity.[1,4,10,63] It is often difficult to distinguish between vespids and bees, even though vespids do not usually sting in spring and, unlike the honeybee, do not usually leave the stinger embedded in the skin, though exceptionally in the USA, in 30–50% of cases[31] *Vespula maculifronts* does just that.

In North America, allergists and immunologists believe it prudent to prescribe VIT with any venom, which gives a positive skin test, or sIgE result, since there have been cases where VIT was tailored towards the primary culprit insect but the patients subsequently reacted to an insect to which they had previously been sensitized.[47] Other investigators recommend treatment only with the venom of the suspected culprit insect.[63] When vespids are involved, the common practice is to treat with *Vespula* venom alone or, in North America, with a mixed vespid venom preparation, the latter containing equal parts (in general 100 µg) of each venom of yellow jacket (*Vespula* species), yellow hornet (*Dolichovespula arenaria*) and white-faced hornet (*Dolichovespula maculata*),[64] and that are unavailable in Europe.

In Europe, the geographical distribution of each species and the ample cross-reactivity among venoms of *Vespula*, *Dolichovespula* and *Vespa*, usually makes treatment with *Vespula* venom alone sufficient in the temperate European climate.[2,10] The extracts are typically a mix of five or six species of *Vespula* (*vulgaris, germanica, maculifrons, flavopilosa, pensylvanica* and *squamosa*).

Double diagnostic positivities to wasp (*Polistes*) and yellow jacket (*Vespula* species) venoms have been observed in

more than 50% of vespid allergic patients. Owing to the incomplete cross-reactivity between vespinae and paper wasps (*Polistes*) in the Mediterranean area, patients who test positive to both venoms should be treated with both, unless RAST-inhibition reveals cross-reactivity.[10] The same approach is used in the Gulf states of the USA, where *Polistes* is common as a species.[31]

A weaker cross-reactivity between European and American paper wasps was recently demonstrated.[27] The species *P. dominulus* and *Polistes gallicus* are European paper wasps; *P. dominulus* has spread to northeastern USA and has also been reported in Australia. The species *Polistes exclamans*, *Polistes annularis* and *Polistes fuscatus* are indigenous to North America and are not present in Europe. All these findings raise the need to introduce, at least in Europe, the *P. gallicus* or *P. dominulus* extract (the latter only recently being available in some European countries) into clinical practice for diagnostic and therapeutic purposes to replace the American *Polistes* species mixture presently being used.[27,37,65]

Allergic reactions to the European hornet (*V. crabro*) are rare, except in Mediterranean countries where its sting is a risk factor for severe SRs.[55] *V. crabro* venom has some antigens in common with *Vespula* venom; however, a third of *V. crabro*-allergic individuals have positive skin and sIgE tests restricted to only *V. crabro* venom.[66,67] As a consequence, in Italy, it is a common practice to use *V. crabro* extract for both diagnostic and therapeutic purposes.

Dual positivity of diagnostic tests with *Vespula* and honeybee is also frequent, especially in some European countries where bee venom allergy is more frequent than vespid venom allergy.[2] In the case of an uncertain culprit insect, both venoms should be used for VIT,[4,10] unless complete cross-reactivity can be demonstrated by RAST inhibition with whole venoms and CCDS, such as horseradish peroxidase or bromelain.[33]

Honeybee and bumblebee venoms show high cross-reactivity.[1] Immunotherapy with honeybee venom alone may be sufficient in nonprofessionally exposed bumblebee-allergic patients with bee venom primary sensitization, whose reaction is most likely due to cross-reactivity. In occupationally exposed patients, who are frequently stung by bumblebees, purified bumblebee venom for immunotherapy, when available, is recommended owing to the low or absent cross-reactivity with honeybee venom.[68] In the USA, no approved diagnostic or therapeutic extracts for the bumblebee or Mediterranean wasp (*P. dominulus*) are commercially available.[47]

In the USA, whole-body fire ant extract immunotherapy, which contain sufficient venom allergens to provide reasonable clinical protection is used; fire ant venoms are available in Australia (Jack Jumper) where a very successful controlled trial was performed.[69] By contrast, allergic reactions to ants are rare in Europe,[70] and a venom extract is not commercially available.

Efficacy

Subcutaneous VIT is probably the most effective allergen treatment currently available to physicians. The efficacy of VIT has been confirmed in prospective controlled and uncontrolled studies[2,10] and in one meta-analysis (level of evidence Ia),[71] demonstrating that the protection rate of vespid VIT is higher than that offered by honeybee VIT.[72]

The recommended maintenance dose is 100 µg of venom, both in children and adults. This dose was originally proposed because it was believed to be equivalent to two stings. Indeed, between 50 and 140 µg venom are delivered by a bee sting compared with up to 3 µg by that of a *Vespula* sting and up to 17 µg by that of a *Polistes* sting.[1] This standard dose prevents SRs in 75–95% of patients who are re-stung[10] and provides better protection than a 50-µg dose in adults.[4] For the first time, a recent paper demonstrated the efficacy of a *P. dominulus* extract after a field sting.[65]

A dose of 200 µg is recommended when a SR follows an insect sting in spite of VIT with 100 µg and in highly exposed populations, such as beekeepers.[2,10] According to some authors, an elevated maintenance dose (usually 200 µg) from the start of VIT should be administered to honeybee allergic patients with concomitant mastocytosis and also considered in vespid venom allergic subjects with additional risk factors.[51]

Most American patients receive mixed vespid venoms, with an injectable maintenance dose of 300–400 µg, which provides approximately 98% protection.[31,47]

The protection rate of bumblebee VIT, demonstrated by sting challenge and in-field stings, is estimated to be higher than 90%.[68]

Some authors have demonstrated that VIT efficacy in mastocytosis sufferers may be reduced.[52,73] Two female patients

died following a yellow jacket re-sting despite VIT, which nevertheless occurred several years after discontinuing immunotherapy.[74] Contrarily, this indicates that patients with mastocytosis should continue lifelong VIT, increasing the dose, where possible.[52] Moreover, there is a venom-specific effect of baseline tryptase concentration. Higher tryptase levels do not seem to play a role in patients with honeybee allergy but may have an effect in patients with vespid venom allergy.[75]

Box 2 presents risk factors currently known to predispose a patient to a SR at sting challenge or an in-field sting during VIT.

Box 2. Risk factors for treatment failure (or reduced effectiveness) in patients on venom immunotherapy.

- Honeybee venom
- Lower maintenance venom dose
- Repeated side effects during venom immunotherapy
- Mastocytosis
- An elevated baseline serum tryptase concentration (vespid allergic patients)
- Older age (?)
- Treatment with β-blockers (?)/angiotensin-converting enzyme inhibitors (?)

Question marks indicate the existence of discordant data on these topics.

Treatment Protocol

Many treatment protocols for the VIT induction phase have been designed.[10] They vary with respect to the number of injections, venom doses and time needed to reach the final dose (Table 2). A conventional regimen means increasing doses at weekly intervals for outpatients, the induction phase of rush regimens lasts 4–7 days for in-patients, and the ultrarush protocol maintenance dose is reached within 1–2 days or within a few hours. The cluster regimen is a modified rush approach schedule that involves administering several injections at 15–30 min intervals during the first visit, taking approximately 6 weeks to reach the maintenance dose. Rush and ultrarush protocols are particularly efficient in highly exposed subjects (e.g., beekeepers) or in patients referred to the specialist just prior to the start of the insect season, so that the protective dosage can be reached as quickly as possible. In addition, the cost of administering ultrarush VIT is lower than that of the slower protocols. These fast protocols are currently used in most, but not all,[46] European countries and it is not a readily available technique at most American medical centers.[47]

Table 2. Treatment protocols for venom immunotherapy.

Day	Hour	Conventional (µg)	Cluster (µg)	Rush (µg)	Ultrarush (µg)
1	0 0.5 1 1.5 2.5	0.01 0.1	0.001 0.01 0.1	0.01 0.1 1 2	0.1 1 10 20 30 or 40
2	0 1			4 8 10 20	
3	0 1 2			40 60 80	
4	0			100	
8	0 1	1 2	1 5 10	100	

15	0 1	4 8	20 30	100	50 50
22	0 1	10 20	50 50		100
29		40	100	100	
36		60	100		
43		80		100	100
50		100			
57		100			
64			100		
71		100		100	100
85		100			
92			100		
99			100	100	
106			100		

Further injections of the maintenance dose of 100 µg every 4 weeks during the first year, every 6 weeks during the second year and then every 8 weeks if venom immunotherapy is continued for more than 5 years [10].

The starting dose of VIT is between 0.001 and 0.1 µg (Table 2). However, in a recent paper, the authors demonstrated that initiating VIT at the 1 µg dose can be applied safely in rush protocols, both in adults and in children.[76] They performed two different protocols, a rush protocol in 62 inpatients, and a modified rush protocol in 670 outpatients, thereby demonstrating that by starting with 1 µg of venom there was no SR.[76]

Table 2. Treatment protocols for venom immunotherapy.

Day	Hour	Conventional (µg)	Cluster (µg)	Rush (µg)	Ultrarush (µg)
1	0 0.5 1 1.5 2.5	0.01 0.1	0.001 0.01 0.1	0.01 0.1 1 2	0.1 1 10 20 30 or 40
2	0 1			4 8 10 20	
3	0 1 2			40 60 80	
4	0			100	
8	0 1	1 2	1 5 10	100	
15	0 1	4 8	20 30	100	50 50

22	0 1	10 20	50 50		100
29		40	100	100	
36		60	100		
43		80		100	100
50		100			
57		100			
64			100		
71		100		100	100
85		100			
92			100		
99			100	100	
106			100		

Further injections of the maintenance dose of 100 µg every 4 weeks during the first year, every 6 weeks during the second year and then every 8 weeks if venom immunotherapy is continued for more than 5 years [10].

If two or more venoms are required, they should be administered in separate protocols a few days apart. While the build-up phase of VIT should be performed by an allergist, in some countries, maintenance treatment is eventually continued by the general practitioners.

In Europe, VIT may be performed with nonpurified aqueous (NPA), purified aqueous (PA) extracts and purified aluminium hydroxide adsorbed (PAHA) preparations (so-called 'depot' extracts) of yellow jacket and honeybee venoms, administered by subcutaneous injection. Purified venom extracts do not contain vasoactive amines (e.g., dopamine, histamine and serotonin) and have a reduced presence of small peptides (e.g., apamine, kinins and mast-cell degranulating peptide in the final product).[77] *Polistes* species and *P. dominulus* PA and PAHA extracts for diagnostic and therapeutic purposes are not currently commercially available in Europe. The efficacy of PA and depot extracts is supported by studies using both sting challenge and in-field stings, and is comparable to that of nonpurified preparations.[77] In the USA, neither PA nor PAHA preparations are commercially available.[31]

The NPA and PA extracts can be used for ultrarush, rush, clustered and maintenance phases, while PAHA preparations are only administered for the conventional build-up and maintenance schedule. Many European specialists switch to depot preparations following the up-dosing phase.[77]

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 for more than 5 years, provided that the treatment is tolerated.[4,10] It is recommended that the dose of 200 µg venom as a depot extract should not be exceeded over a 4-week period if possible.[45] If a depot extract is used, extending the injection interval to 8 weeks is possible without compromising the effectiveness of the therapy.[77] A longer interval is not recommended for honeybee allergic patients since beekeepers with less than ten stings a year were those who developed SRs most frequently.[78] Moreover, the number of studies assessing the feasibility of extending the maintenance interval for up to 12 weeks included too small a sample of patients with mainly vespid allergy, in which efficacy was evaluated mostly by in-field stings.[10,79] Recently, two studies evaluating the safety and efficacy of a further prolonged interval between injections of 6 months yielded contrasting data that can mainly be accounted for by the different selection criteria that was applied in each of them.[80,81] It is desirable that within the next few years, multicenter clinical trials will produce results that enable clinicians to select patients so that extending the interval between injections becomes risk free.

Side Effects

Although highly effective, patient compliance with VIT may be impaired by LLRs and SRs,[4,10] the latter explaining why, in

some European countries, only selected allergy centers usually opt for treatment. However, some studies demonstrate that the side effects of VITs are less frequent than those caused by subcutaneous immunotherapy for inhalant allergens.[82,83]

Large local reactions to injections are commonplace, especially during the incremental phase, and occur in up to 50% of patients. Although LLRs during VIT are not a risk factor for a SR and do not usually necessitate a reduction in venom dose or prevent the protective dose from being reached, they can be particularly bothersome, sometimes requiring corticosteroid and antihistamine treatment for several days. However, anaphylaxis represents the biggest risk posed by VIT. Other side effects of VIT, such as serum sickness, are extremely rare.[2] The literature reports a large variation (0–46%) in the incidence of VITs side effects,[2,10] with the tolerance of vespid VIT (0–15%) faring better than honeybee VIT (20–40%). Indeed, looking at the results of two multicenter studies[84,85] and one review of eight trials of at least 100 patients,[52] the percentage of patients with VIT-induced SRs is between 2 and 20%, and adrenaline was administered to 0.2–5% of patients who experienced a SR.[52] SRs during VIT pose a serious problem and, at present, there is no reliable test that is 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 needs to be confirmed by further studies.[86] To reduce the incidence of VIT-induced SRs, the risk factors need to be defined. Box 3 summarizes old and new potential risk factors for side effects during VIT.

Box 3. Risk factors for side effects during venom immunotherapy.

- Honeybee venom > vespid venom
- Build-up phase > maintenance phase
- Dose increase schedule (ultrarush > rush > conventional)
- Increasing age
- Treatment with β-blockers (?)/angiotensin-converting enzyme inhibitors (?)
- Cardiovascular disease
- Mastocytosis
- An elevated baseline serum tryptase concentration (during the build-up phase)

Question marks indicate the existence of discordant data on these topics.

In a European, multicenter study, published in 2000, a greater risk of SRs was demonstrated during the incremental phase of VIT in female patients, in subjects receiving bee venom vaccines and in patients undergoing the rapid incremental phase, but not in patients with a history of a severe, original SR.[85] Similar findings for several of these parameters were observed in a previously conducted, larger, retrospective study in the USA, which reported that SRs were most likely to occur at venom doses between 1 and 50 µg and at maintenance dosage.[84] In a recent review, which evaluated eight VIT studies in the general population, honeybee VIT was confirmed to be a risk factor for an SR; side effects occurred in 26.6% of patients treated with honeybee venom and in 11.2% of patients treated with yellow jacket venom.[52] In contrast to the findings of the previously mentioned old, European, multicenter study,[85] several retrospective studies concluded that treatment with rush and ultrarush protocols are tolerated at least as well as, or even better than, treatment with slower protocols.[87] However, there may be a difference between honeybee and vespid venoms with respect to the tolerability of the different protocols.[88–90] The results of a very recent European, multicenter study, collecting data from more than 600 patients, confirmed the rapid dose increase (ultrarush > rush > conventional phase) during the build-up phase of VIT as a risk factor for a SR.[91]

At present, ultrarush protocols should only be used by specialists who are experienced in managing VIT and preferably only in an emergency care setting, particularly in bee venom allergic patients.

Even though there are good theoretical grounds for the contraindication of β-blockers during immunotherapy, this does not seem to apply to VIT.[60] ACE-inhibitors may possibly contribute to the onset of SRs during VIT in some highly selected patients,[92] although further data are required to confirm this.[93]

Side effects during VIT are probably more frequent in mastocytosis patients, especially in those with a yellow jacket venom allergy.[52] However, risk factors for adverse reactions in these patients (e.g., type of mastocytosis and protocol) require further evaluation.[51] Moreover, baseline tryptase concentration correlates significantly with the frequency of severe side effects during the build-up phase of VIT.[91] However, the extent of this effect depends on the type of venom and is more marked in patients treated with vespid VIT.[91]

In general, repeated anaphylactic reactions are rare. In these cases, it should be assumed that VIT will not prevent further SRs following a sting. Moreover, treatment should be continued for approximately 6 months with the highest tolerated dose of insect venom (injection interval 1–2 weeks) and then renewed attempt at dose increase.[45]

In conclusion, risk factors for VIT-induced SRs must be taken into account and patients with one or more risk factor should be treated and monitored with special care.

How can VIT Safety be Improved?

Pretreatment with a H1 antihistamine has been demonstrated to reduce the number and severity of LLRs and mild SRs to VIT, such as urticaria and angioedema.[10] Antihistamine pretreatment medication during the dose-increase phase could increase the long-term efficacy of VIT through enhancement of Treg cell actions by activation of histamine receptor-2-dependent events,[12] as suggested by one study demonstrating that antihistamine premedication with terfenadine improved the clinical efficacy of ultrarush honeybee VIT.[94] However, this finding was not confirmed by another recent study, although levocetirizine was able to affect the expression of histamine receptors and cytokine production by allergen-specific T cells.[95] Further studies are needed in order to elucidate this topic.

Pretreatment with a combination of H1 antihistamine and a corticosteroid have not yet been performed in honeybee and vespid VIT, except in patients with mast-cell diseases.[52] In a prospective, double-blind, randomized, placebo-controlled pilot study the occurrence of local reactions following VIT was significantly delayed by pretreatment with the leukotriene antagonist montelukast.[96]

Pretreatment with anti-IgE monoclonal antibodies may permit more rapid and higher doses of allergen immunotherapy while improving its safety. Moreover, this pretreatment could play an important role in insect-venom allergic patients who are intolerant to VIT. There are several case reports of bee venom allergic patients,[97] including those suffering from indolent systemic mastocytosis,[98] who experienced SRs to VIT but were able to tolerate VIT following pretreatment with omalizumab. However, Soriano Gomis *et al.* did report a case of systemic allergic reaction with bee VIT despite pretreatment with omalizumab and antihistamines.[99]

Until now, the optimal time for its administration during VIT (should it be administered 6 months, 2 weeks, 1 week or 1 h before VIT?), the appropriate dosage (should we use the the recommended dose of 150 or 300 mg?), the long-term effects (should omalizumab be discontinued after first administration or administered before each shot?) and the best incremental protocol of VIT (should the protocol be conventional or rush/ultrarush?) to be used are still unknown. It is important to underline that omalizumab is not approved for the prevention of anaphylaxis and it must be prescribed as off-label. In addition, taking into account its high cost, omalizumab should be limited to patients with repeated severe SAR to VIT injections preventing reaching the maintenance dose.

Although the object of a certain amount of criticism,[100] sublingual immunotherapy (SLIT) is increasingly being used in European countries thanks to its good safety profile.[101] Even though LLRs are not an indication for VIT, in a placebo-controlled, double-blind study on bee venom SLIT in patients with a history of LLRs, the diameter of LLR to a bee sting challenge was reduced by more than 50% in 57% of active-treated patients.[102] However, on the basis of these findings alone (a partial or complete treatment failure in 43% of patients with LLRs) and without experimental data on the pharmacokinetics of venom SLIT, caution should be exercised when considering SLIT as a therapeutic option for patients with severe SRs.[103]

There is a sound theoretical basis for believing that PA and PAHA extracts, whose efficacy is comparable to that of NPA preparations, have the potential to reduce the incidence of VITs side effects. A recent study compared the safety and tolerability of VIT with purified extracts and nonpurified products in yellow jacket and honeybee allergic patients. The induction phase was carried out using a 2–7-day ultrarush protocol and results showed that VIT with purified extracts resulted in a significantly lower number of severe LLRs compared with VIT using nonpurified preparations.[104] At present, compared with the therapy with an aqueous extract (both purified and nonpurified), VIT with a depot extract is superior with respect to the frequency of occurrence of LLRs and SRs, and purified extracts appear to be safer than nonpurified, especially with respect to the frequency of LLRs.[77] 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.[77]

VIT Monitoring

The current options for monitoring VIT include skin tests, *in vitro* tests and sting challenge tests. The level of venom-

specific IgE level and skin-test sensitivity usually increase in the first months of therapy, return to baseline after 12 months and then decline steadily during maintenance VIT.[31] This decline continues even after cessation of therapy or a sting.[105] Less than 20% of patients are skin-test negative after 5 years of VIT, but 50–60% become negative after 7–10 years.[106] The reduction of skin reactivity may be attributable to a factor present in the patient's serum, since serum of VIT-treated patients is able to neutralize skin reactions to yellow jacket extracts *in vivo* [107] and this ability correlates with VIT duration as well as with the concentration of venom-specific IgG and IgG_4.[107] However, the protective role of VIT at a re-sting, which may correlate with this lower skin test reactivity, was not evaluated by sting challenge test.

Specific IgG antibodies increase and remain elevated at least as long as VIT is continued.[107,108] Initially, these are IgG_1 antibodies, and later on IgG_4 antibodies. The ratio of venom-specific IgE:IgG_4 first increases, then later decreases during VIT,[107] although no difference in the pattern of venom-specific IgE and IgG_4 was observed in patients who tolerated a field sting compared with those who continued to experience SRs to stings despite VIT.[109]

In one study by Ebo *et al.*, no effect of VIT on BAT was observed after a 5-day incremental phase, but a significant decrease in CD63 expression after 6 months of VIT was.[41] Following 3 years of VIT, 60% of the patients had a negative BAT, whereas only 17.9% of them had a reduction of wasp IgE.[41] Moreover, BAT showed a higher reactivity in patients who still reacted to bee or wasp stings compared with those who tolerated field stings.[109] By contrast, there was no change in basophil CD63 expression compared with pretreatment values in 20 vespid-venom allergic patients who received VIT and had already tolerated a sting challenge.[110]

Recently, a small but continuous decrease in baseline tryptase concentration over time was reported, which correlated with VIT duration, suggesting a dampened mast-cell function or decline in mast-cell burden.[111] However, despite the availability of new laboratory techniques, it is not yet possible to measure clinical efficacy based on laboratory parameters,[110] even though the concentration of specific IgG antibodies is used by some specialists to confirm protective levels after starting VIT and then to verify that the IgG level is adequately maintained at the longer intervals used for maintenance treatment.[5]

A sting challenge with a single, live insect can be used in treated patients to identify those who are not protected.[112] This should be performed in an emergency care setting approximately 6–18 months after the maintenance dose has been reached and also immediately after the dose-increase phase in patients at greater risk of exposure.[45] A tolerated sting challenge has a higher predictive value in terms of the results of a single later sting, even though some patients may still experience a reaction to a subsequent sting.

Whether protected patients also tolerate several stings is unknown. In the case of incomplete VIT protection, revealed by sting challenge or in-field stings, increasing the maintenance dose to 200 µg almost invariably bestows complete protection.[113]

Annual appointments with the allergist serve to review the treatment plan, ensure that the patient has not been prescribed any new medication or has a medical condition that could influence VIT or has not tolerated an in-field sting. According to some authors, there is no need for annual skin or blood tests, although repeating the skin tests every 2–3 years is recommended so that patients who can be taken off VIT can be identified.[31] Other specialists believe tests should be performed annually and after any accidental hymenoptera sting.[45]

Duration of VIT

The question of how long VIT should last so that long-term protection can be achieved after discontinuation is a long-standing one.[10,114,115]

On the premise that the duration of VIT should be decided following a thorough patient–physician discussion of the issues involved, the present recommendations for discontinuing VIT take into account all these findings, which are very similar in Europe[10] and America.[4] Although not a common occurrence, conversion to a negative venom skin test is an unequivocal indication that VIT should be stopped immediately. If the patient has had a very severe initial allergic reaction, an *in vitro* test should be carried out to confirm disappearance of venom specific IgE,[10] as only the combination of negative intradermal skin testing at 1 µg/ml with the absence of venom-specific serum IgE antibodies is associated with a strongly diminished risk of relapse.[10,115]

Most patients with mild-to-moderate anaphylactic symptoms and positive skin tests remain protected even years after a 3–5-year course of VIT has been discontinued.[10] In fact, a number of studies have investigated the reliability of short-

and long-term VIT despite the persistence of positive venom skin tests, as a criterion for discontinuing VIT. To sum up, the studies where long-term protection, up to 7 years after discontinuing VIT of at least a 3-year duration, were evaluated on the basis of field stings or sting challenges revealed more frequent relapses than those with a shorter follow-up (protection rate of ~80 vs 83–100%), with a more favorable outcome in *Vespula* than in bee venom allergic patients and in children than in adults.[10,31] There are no data on the outcome after more than 15 years of VIT.

It is likely that short- and long-term results are more favorable with the much higher total dose administered in the USA than that with the 100 µg applied elsewhere. More European data on the discontinuation of VIT in allergic vespid patients with 100 µg maintenance dose are needed.[28]

Owing to the small but relevant risk of a reaction from a re-sting, self-administering emergency kits, including epinephrine autoinjectors, should be discussed with every patient when discontinuing VIT.[116]

A number of risk factors for the recurrence of SRs following hymenoptera stings after discontinuing VIT have since been identified and are summarized in Box 4.[10,31,48,115,117–119] They include insect type, 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.[10,31]

Box 4. Risk factors for the recurrence of a systemic reaction after discontinuing venom immunotherapy.

- Honeybee venom
- Being of adult age
- Severe pretreatment systemic reactions
- Systemic reactions during venom immunotherapy to treatment injection or restings
- Mastocytosis
- Elevated baseline tryptase level

In a recent paper on the discontinuation of VIT, the reaction rate to stings in the subgroup that prematurely stopped VIT was greater, but not statistically significantly different, from that of the group of patients treated for more than 3 years. The risk of SR increases with subsequent stings after discontinuing VIT and all patients reported that symptoms experienced with stings after stopping VIT were milder than those before VIT.[120] However, the study was mostly carried out via email and telephone interviews, patients were not stratified by the severity of their initial reaction, by the venom they were allergic to or by the occurrence of reaction during VIT, but in relation to the duration of VIT alone.

According to EU guidelines, longer-term treatment (or life-long 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, mastocytosis or use of β-blockers);
- Generalized allergic reactions to stings or to immunotherapy injections or during VIT;
- Highly exposed patients, such as beekeepers and their immediate family members.[10]

However, some patients at a low risk of relapse prefer to perform long-term VIT out of a sense of personal safety and improved quality of life. At present, we do not know if an improvement in HRQL remains even after discontinuing VIT.

According to some authors,[45] the tests recommended as treatment monitoring should continue to be performed regularly, even after discontinuing VIT, while sting challenge tests are not recommended for routine testing of protection studies as they may have a booster effect.[112]

After ending VIT, patients should continue to avoid being stung. Some authors agree that patients should still carry an emergency kit.[91] If SR occurs after stopping VIT, further allergy tests are required; there is no contraindication to resuming VIT.

Conclusion

Insect sting allergy and VIT remain an excellent model for the study of anaphylaxis and immune tolerance. VIT is probably the most effective form of specific allergen immunotherapy.

Patient knowledge that VIT prevents anaphylactic reactions to future stings improves HRQL, which, in itself, is an important reason for offering immunotherapy to patients with insect allergies.

However, neither the efficacy nor the safety of the treatment are optimal, especially as regards to bee VIT, and so there is considerable room for further improvement in these all-important areas.

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

Future Perspective

Modern molecular biology has provided us with a considerable number of major honeybee and various vespid venom allergens in recombinant form,[121,122] whose use greatly enhances the accuracy of the diagnosis.[34–36] Recombinant venom allergens may pave the way towards novel future therapeutic techniques, even though they have yet to be used for VIT.

Major T-cell epitope peptides can be prepared synthetically or expressed as recombinant fragments. A mixture of three dominant T-cell peptides of phospholipase (PLA) has, so far, only been used for immunotherapy in a preliminary bee venom allergy study in five patients where complete protection was bestowed in three subjects and partial protection in the remaining two after a bee sting challenge with no side effects.[123]

In a double-blind, randomized, placebo-controlled trial in bee venom allergic patients, using three long, synthetic, overlapping peptides (LSPs), mapping the entire PLA2 amino acid sequence, this therapy induced T-cell anergy, immune deviation toward a Th1-type T-cell cytokine response, enhanced IL-10 secretion, and PLA2-specific IgG_4 production. LSP immunotherapy was safe and did not cause any severe systemic reactions. The efficacy of treatment with these long peptides, however, was not verified by a sting challenge.[124]

DNA vaccination is where DNA plasmids encoding the relevant allergens are injected. The successful DNA vaccination of sensitized mice has, among other allergens, been reported with plasmids from bee venom PLA2.[125] To date, DNA vaccination for VIT has not been tested in a human model.

However, many hymenoptera venom-allergic patients are sensitized to more than one vespid or honeybee venom allergen, signifying that treatment with one major allergen in recombinant unrefolded or point mutated form, with peptides or DNA plasmids encoding it, may be insufficient. A fusion protein composed of the two major allergens of bee venom PLA2 and hyaluronidase (HYA) was constructed through genetic engineering and characterized by destroyed conformational B-cell epitopes but intact T-cell epitopes of the two allergens.[126] The fusion protein induced T-cell proliferation and both Th1- and Th2-type cytokine responses; on the other hand, IgE reactivity was abolished and basophil degranulation reduced. The use of the fusion protein for VIT could minimize anaphylactic side effects and increase efficacy compared with the whole-venom-based treatment.[126]

Another model used a recombinant chimeric protein consisting of the whole amino acid sequences of three major bee venom allergens (PLA2, HYA and melittin). The fragments were designed to preserve all relevant T-cell epitope peptides while conformational B-cell epitopes were destroyed.

Use of this chimeric protein in mouse models has led to a significant reduction of specific IgE development towards the native allergen, which has produced a protective vaccine effect *in vivo*.[127]

Finally, the intralymphatic allergen administration for specific immunotherapy has been recently proposed in grass pollen allergic rhinitis. Compared with a 3-year course of conventional subcutaneous immunotherapy, intralymphatic application enhanced safety and efficacy of immunotherapy and reduced treatment time from 3 years to 8 weeks.[128] The therapeutic potential of bee venom intralymphatic immunization was then analyzed in sensitized mice using an anaphylaxis model.[129] Direct injection of the major bee venom allergen phospholipase A2 into inguinal lymph nodes enhanced allergen-specific IgG and T-cell responses when compared with subcutaneous injections. Moreover, only intralymphatic immunization stimulated the production of the Th1-dependent subclass IgG_{2a}, which is associated with improved protection against allergen-induced anaphylaxis. The authors concluded that this approach should improve both the efficacy and safety of VIT.[129] Similar studies in humans, including clinical parameters and sting challenges, are needed to assess the effectiveness of this route of allergen administration.

Sidebar

Executive Summary

Hymenoptera venom allergic reaction

- Hymenoptera stings may cause systemic reactions of varying severity up to fatal anaphylaxis.
- Several new factors influencing the severity of hymenoptera sting reactions in untreated patients have been identified and include an elevated baseline serum tryptase level and treatment with angiotensin-converting enzyme inhibitors. Owing to its clinical implications, serum tryptase should be measured in patients with a history of a severe sting reaction.

Venom immunotherapy indications

- Venom immunotherapy (VIT) is indicated both in children and adults with a history of severe systemic reactions, including respiratory and cardiovascular symptoms, and documented sensitization to the respective insect with either skin tests and/or specific serum IgE tests.
- VIT is not indicated in large, local reactions.
- As for systemic, non-life-threatening reactions (e.g., urticaria, erythema and pruritus), other factors may influence the decision to commence VIT and should include occupation and/or hobbies where the risk of exposure is high, the culprit insect itself, concomitant cardiovascular diseases, other diseases (e.g., mastocytosis) and psychological factors arising from anxiety, which can seriously impair patient health-related quality of life.

Selection of venoms

- Selection is based on identification of the hymenoptera species involved, on the results of the diagnostic tests and on venom cross-reactivity.
- The current availability of recombinant venom allergens may contribute to a 'component resolved diagnosis' and, subsequently, to a more accurate prescription of VIT.

Efficacy

- Subcutaneous VIT is the only effective treatment for hymenoptera venom allergic patients.
- Certain risk factors for treatment failure or reduced effectiveness have been identified such as the type of venom (honeybee), a lower maintenance dose, repeated side effects during VIT, mast-cell diseases and an elevated baseline serum tryptase concentration.

Safety

- VIT may induce large local and systemic reactions.
- Treatment with honeybee venom, the build-up phase, the rapid dose-increase schedule, mastocytosis and an elevated baseline serum tryptase concentration (during the build-up phase) are among the most important risk factors for side effects during VIT.
- Patients with one or more risk factor should be treated and monitored with special care.

VIT duration

- Certain risk factors for relapse after discontinuing VIT have been identified.
- The duration of VIT should be decided after a thorough patient–physician discussion of the individual risk factors and also patient choice.
- Owing to the small but relevant risk of re-sting reactions, self-administering emergency kits should be discussed with every patient when discontinuing VIT.

Future strategies

- A number of new strategies for VIT, mostly based on genetic engineering, have been described, as well as different routes of VIT administration (e.g., intralymphatic). However, the majority of these approaches have not been used for VIT in humans.

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Papers of special note have been highlighted as:
• of interest
•• of considerable interest

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