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Review article

Prevention and treatment of hymenoptera venom allergy: guidelines for clinical practice

Based on the knowledge of the living conditions and habitat of social *Aculeatae* a series of recommendations have been formulated which can potentially greatly minimize the risk of field re-sting. After a systemic sting reaction, patients should be referred to an allergy specialist for evaluation of their allergy, and if necessary venom immunotherapy (VIT). An emergency medical kit should be supplied, its use clearly demonstrated and repeatably practised until perfected. This should be done under the supervision of a doctor or a trained nurse. Epinephrine by intramuscolar injection is regarded as the treatment of choice for acute anaphylaxis. H1-antihistamines alone or in combination with corticosteroids may be effective in mild to moderate reactions confined to the skin and may support the value of treatment with epinephrine in full-blown anaphylaxis. Up to 75% of the patients with a history of systemic anaphylactic sting reaction develop systemic symptoms once again when re-stung. Venom immunotherapy is a highly effective treatment for individuals with a history of systemic reaction and who have specific IgE to venom allergens. The efficacy of VIT in yellow jacket venom allergic patients has been demonstrated also by assessing health-related quality of life. If both skin tests and serum venom specific IgE turn negative, VIT may be stopped after 3 years. After VIT lasting 3–5 years, most patients with mild to moderate anaphylactic symptoms remain protected following discontinuation of VIT even with positive skin tests. Longer term or lifelong treatment should be considered in high-risk patients. Because of the small but relevant risk of re-sting reactions, in these patients, emergency kits, including epinephrine auto-injectors, should be discussed with every patient when stopping VIT.

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Through sensible precautions it is possible to lower the risk of receiving a new sting considerably. Detailed written information describing how to avoid stings in future should be provided and explained to bee and vespid sting allergic patients. Additionally, an emergency medical kit should be supplied, its use clearly demonstrated and repeatedly practised until perfected, under the supervision of a doctor or a trained nurse (1). Finally, physicians should inform patients of the possibility of undergoing specific venom immunotherapy (VIT).

This review article is a revision of previous editions of Position Papers (2, 3), the last one dating back to 1993. It considers relevant more recent publications on prevention and treatment of Hymenoptera venom allergy, as well as the evidence of their conclusions graded according to new guidelines (4).

Preventive measures

Based on the knowledge of the living conditions and habitat of social *Aculeatae* a series of recommendations have been formulated which can potentially greatly minimise the risk of field re-sting (Table 1), although there is no hard evidence to support this from controlled studies.

Patients should be made aware that Hymenoptera only sting in self-defence and that anything which is perceived as a potential threat might result in a sting. Detailed information should be provided to subjects at risk, about where the culprit insect builds its nest, as well as the types of food, which attract it. In the case of honeybees, the stinger should be quickly removed regardless of how, since it has been demonstrated that it is the time the

Table 1. Examples of activities implying special risk for insect stings during warm season

Activities
Outdoor eating and drinking
Walking barefoot
Gardening (especially cutting hedges, flowers)
Picking fruit
Outdoor sport (especially with scanty outfit or open mouth)
Staying close to beehives when honey is collected
Removing vespid nests from attic or windows

stinger remains embedded in the skin that determines the degree of envenomization (5).

Sting reactions seem to be more severe and are more difficult to treat if the victim is on beta-blockers (6, 7). Consequently, if patients have a condition for which, beta-blockers have been prescribed and nonbeta-blocking agents can obtain an equivalent therapeutic effect, they should be used instead.

Emergency treatment

Treatment of systemic reactions

The treatment of systemic reactions (SR) (urticaria, angioedema, laryngeal oedema, bronchial asthma, anaphylactic shock) is shown in Table 2.

The most effective drugs for dealing with systemic allergic reactions are sympathomimetics, antihistamines and corticosteroids. H1-antihistamines and corticosteroids should never be used as the sole treatment for severe systemic allergic reactions with respiratory or cardiovascular symptoms (8–10).

Although prospective, placebo-controlled studies in patients with anaphylaxis are not feasible for ethical reasons, injected epinephrine is regarded as the treatment of choice for cases of acute anaphylaxis (1, 10–13).

The most important principle in the management of an anaphylactic shock is its rapid recognition and the prompt initiation of the therapy (10–13). Epinephrine should be given promptly in the event of an anaphylactic shock, as rapidly achieving high plasma and tissue concentrations of the drug are crucial for the patient's survival. In an animal model, it was recently confirmed that epinephrine given at *the nadir of shock* fails to produce haemodynamic recovery, despite an elevation in plasma epinephrine concentrations (14).

The superiority of i.m. *vs* s.c. administration of epinephrine with regard to a rapid increase in plasma concentration and start of pharmacological effects has been documented in both an animal model and a prospective, randomized, blinded study in patients at risk of anaphylaxis (15, 16) and consequently the i.m. route is recommended in international guidelines (12, 17).

Table 2. Treatment of systemic reactions to Hymenoptera stings

Type of reaction	Drug and dose	Notes
Mild urticaria	Antihistamines, oral or parenteral	Observe for at least 60 min
Urticaria, angio-oedema	Check blood pressure and pulse rate Establish an i.v. line with saline Antihistamines oral or parenteral Corticosteroids oral or parenteral In case of severe or progressive symptoms: Epinephrine (1 mg/ml) Adults 0.30–0.50 mg i.m. Children 0.01 ml/kg i.m.	Patient must be kept under observation until symptoms completely disappear
Laryngeal oedema	Epinephrine by inhalation and i.m.	Intubation, thacheotomy or cricothyrotomy may be needed in cases of more severe laryngeal oedema
Bronchial obstruction	Mild to moderate: β_2–agonist by inhalation Severe: epinephrine by inhalation β_2–Agonists (0.5 mg/ml) 1 year: 0.05–0.1 mg; 7 years: 0.2–0.4 mg; adults 0.25–0.5 mg i.v.	All patients with protracted respiratory symptoms must be hospitalized; those with laryngeal oedema must be given intensive medical care as soon as possible
Anaphylactic shock	Epinephrine (1 mg/ml) Adults 0.30–0.50 mg i.m. Children 0.01 ml/kg i.m. May be repeated after 5–15 min Exceptionally i.v. Place patient in supine position Oxygen 5–10 l/min Check blood pressure and pulse rate i.v. Access, volume replacement Antihistamines i.v., corticosteroids i.v. Dopamine or norepinephrine infusion Glucagons: 0.1 mg/kg i.v. (nausea, vomiting)	Hospitalization necessary because of the risk of delayed anaphylaxis If epinephrine injections with or without antihistamines and volume expansion fail to alleviate hypotension For refractory hypotension and bronchospasm in patients on β-blockers

Side effects of epinephrine are mainly observed after rapid intravenous injections of high doses (18). In recently reviewed data from 164 cases of fatal anaphylaxis (including sting anaphylaxis) in the UK from 1992 to 1998 epinephrine overdose was considered to be the most likely cause of death in three of the fatalities (19).

Some patients, such as those with cardiovascular or cerebrovascular disease, are at increased risk for adverse effects; however, even in these the benefits of epinephrine treatment in anaphylaxis generally outweigh its risks.

After a systemic sting reaction, patients must be referred to an allergist for diagnostic evaluation, and instruction about preventive measures. Emergency kits and venom immunotherapy should be discussed.

Emergency kits

Patients allergic to hymenoptera venoms should carry an emergency kit for self-administration, especially during the insect season. The aspiration of adrenaline from a vial is time consuming and may delay the effects of the drug, which is of paramount importance in the event of an anaphylactic reaction. Several epinephrine-preloaded preparations for immediate self application are commercially available (1).

Patients, caregivers and health care providers alike benefit from focused instruction and regular review of the optimal use of epinephrine in the first aid treatment of anaphylaxis (20, 21). In addition, patients should receive a tablet set containing a rapidly effective oral H1-antihistamine (e.g. cetirizine 2×10 mg) and corticosteroids (e.g. prednisone 2×50 mg).

Venom immunotherapy

Mechanisms

Though it is a well-documented fact that tolerance to insect stings can be achieved through VIT, the mechanism involved is still unclear. A rise in allergen-blocking IgG antibodies particularly of the IgG4 class, the generation of IgE-modulating $CD8^+$ T cells and a decrease in the release of mediators have been shown to be sometimes associated with successful immunotherapy (22–25). Later on, specific immunotherapy (SIT) was found to be associated with a decrease in IL-4 and IL-5 production by $CD4^+$ T cells, and a shift towards increased IFN-γ production (26–33).

However, the mechanism of repolarization of specific T-cell activity from dominating Th2 type towards Th1 type is controversial (26, 27, 29).

Changes in the immune response to bee venom have been extensively investigated during VIT, PLA-peptide immunotherapy (26–30, 34–36) and during high natural allergen exposure in healthy bee keepers (27). Successfully treated patients develop specific T-cell unresponsiveness against the entire PLA allergen as well as T-cell epitope-containing peptides. These decreased proliferative responses do not arise from deletion as they are restored by the addition of IL-2 and IL-15. The same anergic state of specific T cells has been observed in protected hyperimmune individuals such as bee keepers (27).

The anergic state of specific cells results from increased IL-10 secretion (29). The cellular origin of IL-10 was demonstrated as being the antigen-specific T-cell population and activated $CD4^+CD25^+$ T cells as well as monocytes and B cells (27).

Apparently, T cells observed during SIT and natural antigen exposure represent the so-called T regulatory (Treg) 1 cells in humans. $CD4^+$ Treg cells that specialise in the suppression of immune response are pivotal in maintaining peripheral tolerance (37–40). T regulatory cells are enriched within the $CD4^+CD25^+$ cells (41–44). They include Tr1 cells, which produce high levels of IL-10 and are generated by chronic activation of $CD4^+$ T cells in the presence of IL-10 as well as Th3 cells, which are induced following oral administration of the antigen and secrete predominantly TGF-β. It has been shown that tolerance to aeroallergens is associated with the increased secretion of TGF-β (45). However, unlike in mucosal allergies this mechanism is not active in venom allergy.

Differences in the control mechanism, which regulate immune responses to venoms and to aeroallergens, might be due to different routes of natural allergen exposure.

Some differences in effect on T-cell reactivity were observed when VIT was administered using rapid or conventional protocols. Although rapid immunotherapy, similarly to conventional immunotherapy, is associated with a shift from Th2 to Th1 type cytokine production by peripheral blood lymphocytes, the modulation of T-cell cytokines during conventional VIT takes much longer to develop (46). Moreover, in contrast to ultra-rush VIT inducing rapid T-cell anergy, conventional VIT involves a transient increase in T-cell proliferation in response to the allergen during the incremental phase of allergen administration followed by specific T-cell tolerance (46). The implications of these observations in terms of clinical efficacy call for further investigation.

Most patients are already protected against bee stings at an early stage of VIT, which is not paralleled by changes in antibody formation. It has been shown that lower amounts of mediators of anaphylaxis (e.g. histamine or sulphidoleukotrienes) are released *in vitro* from samples taken during SIT (25, 47–49). These effects may be attributed to the direct suppressive effect of IL-10 on effector cells (mast cells, basophils). Moreover, anergic T cells do not secrete the cytokines, which are required for the priming, survival and activity of the effector cells.

Besides the efficacy of antihistamines in alleviating certain side effects during VIT (50, 51), recent evidence suggests that their use as premedication may enhance the clinical efficacy of VIT (52).

It is well established that histamine released from effector cells influences T cells (53). Histamine enhances Th1 type responses by triggering the histamine receptor type 1 (H1R) whereas both Th1 and Th2 type responses are negatively regulated by H2R. Human $CD4^+$ Th1 cells predominantly express H1R and $CD4^+$ Th2 cells H2R, which results in their differential regulation by histamine (53). Since mast cells and basophils are VIT targets, histamine released by high allergen doses during SIT may redirect the immune response from a dominating Th2-type towards a Th1-type pattern. Administration of antihistamines decreases the H1R/H2R expression ratio, which may enhance the suppressive effect of histamine on T cells.

Further studies are required to substantiate these promising findings supporting the use of antihistamine pretreatment in all VIT patients.

Selection of patients requiring venom immunotherapy

Selecting patients who need VIT is mainly based on the patient's natural history of insect sting allergy. According to the results of re-exposures of placebo or wholebody extract treated groups in controlled studies on VIT (54–56) up to 75% of the patients with a history of systemic anaphylactic sting reaction develop systemic symptoms once again when re-stung. The risk factors involved are reported in the Review Article on the diagnosis of Hymenoptera venom allergy (57).

Higher risk subjects are those who are likely to receive frequent stings and/or to develop particularly severe sting reactions. These patients require treatment for their venom allergy urgently. It is vitally important to take the following specific points into consideration when starting VIT: concomitant internal diseases should be treated before starting VIT; substitution of drugs like beta-blockers (6, 7) or ACE-inhibitors (58, 59) should be discussed; activities where the risk of re-stings is high should be stopped until the maintenance dose of VIT is reached; professional activities like beekeeping should be avoided until a sting challenge is tolerated; in patients who risk a very severe sting reaction (e.g. older age, history of very severe previous sting reactions, mastocytosis, use of beta-blockers) a long-term or lifelong treatment should be considered.

Indications for venom immunotherapy. Venom immunotherapy is indicated both in children and adults with a history of severe SR including respiratory and cardiovascular symptoms and documented sensitization to the respective insect with either skin tests and/or specific serum IgE tests.

Venom immunotherapy is not indicated when neither skin testing nor serum specific IgE antibodies indicate Hymenoptera venom sensitivity, or for unusual reactions, such as vasculitis, nephrosis, fever, thrombocytopenia, etc. (8).

Venom immunotherapy is not recommended for large local reactions in either children (60, 61) or adults (62).

Table 3. Indication for venom immunotherapy

Type reaction	Diagnostic tests (ST and/or IgE)	Decision regarding venom immunotherapy
Adults/children		
Respiratory and cardiovascular symptoms	Positive	Yes
	Negative	No
Urticaria if risk factors or quality of life impairment present	Positive	Yes
	Negative	No
Large local	Positive or negative	No
Unusual	Positive or negative	No

As for systemic, nonlife-threatening reactions (urticaria, erythema, pruritus) other factors may influence the decision to initiate VIT. These include occupations and/or hobbies where the risk of exposure is high, the culprit insect itself, concomitant cardiovascular diseases, other pathologies (like mastocytosis), or psychological factors arising from anxiety, which can seriously impair patient quality of life. The indications for VIT are summarized in Table 3.

Contraindications. Pregnancy is usually not considered a reason for stopping an established and well tolerated VIT, but the treatment should not be started during pregnancy (63).

General contra-indications for VIT are the same as for immunotherapy with other allergens. In relation to the use of beta-blockers, the decision must always consider the risk of cardiac disease if the beta-blocker treatment is stopped and the risk of a systemic reaction during VIT. If the cardiac risk is higher, VIT should either not be started or – in patients at high risk of anaphylaxis – be carried out without taking the patient off beta-blockers, but under careful supervision, including monitoring of blood pressure and electrocardiogram during the dose-increase phase.

Selection of venom to be used in immunotherapy. This is based on the identification of the species of Hymenoptera involved and cross-reactivity between venoms (3):

1. Honey bee and bumblebee venoms show marked cross-reactivity. Venom immunotherapy with honeybee venom alone will be sufficient in nonprofessionally exposed bumblebee-allergic patients who most likely react on the basis of a cross-reactivity in the presence of primary sensitization to bee venom (64, 65). In heavily exposed green house workers who are frequently stung by bumble bees, it is recommended to use bumblebee venom for VIT (66).
2. Pronounced cross-reactivity exists between the major venom components of several vespids, particularly between *Vespula*, *Dolichovespula* and *Vespa* venoms, but less so between *Vespula* and *Polistes* venoms (57). In view of the relatively limited clinical importance of *Polistes* in temperate European climates, treatment with *Vespula* venom alone is usually sufficient in these

areas. In the Mediterranean area, due to the difficulty in distinguishing among *Vespula* and *Polistes*, patients with positive diagnostic tests to both venoms would seem to warrant treatment with both venoms, unless cross-reactivity can be identified by RAST-inhibition. Since it can be assumed that most patients with allergic reactions to *Vespa crabro* were first sensitized by, *Vespula* stings, VIT with *Vespula* venom alone will be sufficient in patients who reacted to a sting by *Vespa crabro*.

3. Cross-reactivity is very limited between *Apidae* and *Vespidae*. When present it is mainly due to hyaluronidase. In the case of double-positive tests to honey bee and *Vespula* and where identification of the responsible insect is not possible, RAST-inhibition assays will help to distinguish between cross-reactivity and double sensitization (67, 68). Treatment with both venoms is only indicated in documented double sensitization.

Treatment protocol and safety

Since the first immunotherapy with pure venom extract was carried out in 1974 (69), protocols of various duration have been devised in an effort to maximize protection, minimize side-effects and optimize patient convenience. The time required to reach the generally adequate maintenance dose of 100 μg with slow protocols is several weeks to months (70–72), whilst rush (73–78) and ultra-rapid (ultra-rush) protocols (79–83) take several days or only a few hours respectively.

Venom immunotherapy aims to induce tolerance to Hymenoptera venom but can be complicated by SR (84, 85). The risk for SR to VIT is more related to the nature of the venom than to the regimen used (86). Venom immunotherapy with bee venom causes more SR than VIT with *Vespula* venom; one explanation may be differences in the quality of the extracts (87). In commercial venom extracts, vespid venom allergens are diluted by, nonallergenic venom-sac proteins, whereas honeybee venom is a purified venom with a lower concentration of nonallergenic proteins (88, 89).

Reports in the literature reveal a high variation (0–46%) in the incidence of side effects attributable to VIT (8, 50, 76, 81, 84, 86, 90). It is difficult to compare these reports on incidence of SR with different VIT protocols since the investigators used different classification systems for the severity of adverse reactions (3).

In a recent EAACI-multicentre study (85) 20% of patients had SR corresponding to 1.9% of injections during the dose-increase phase and 0.5% during the maintenance phase. Rapid dose increase (rush) regimens were associated with an increased risk of side effects (85).

However, some other studies using rush protocols have suggested that they are at least as safe as slower protocols (76, 79–82, 91).

Some trials of rush and ultra-rush VIT included children (78) and even 2-year-old toddlers (91). Though their outcome is not mentioned separately, only adults are listed as having suffered severe side effects. Thus childhood does not seem to represent an increased risk with such regimens or, in general, with any stage of VIT (85).

Immunotherapy with bumblebee venom is as safe and effective as it is with the other venoms (66, 92, 93).

The issue of the higher incidence of adverse reactions with honeybee VIT has been addressed using different approaches devised to improve safety by changing protocols, through pretreatment with antihistamines (50–52, 94, 95), by administering beekeeper gammaglobulin (96), or through the use of chemically modified honeybee venom or recombinant Hymenoptera venom allergens, which proved successful to varying degrees (97–103). Pretreatment with antihistamines, which only reduces the number/severity of large local reactions and mild SR such as urticaria/angioedema, should be prescribed 1 or 2 days before VIT and be continued until the maintenance dose has been well tolerated at least three times.

Depot extracts seem to be associated with somewhat fewer side effects than aqueous preparations; a recent paper has documented comparable efficacy of depot *vs* aqueous extracts (104). Depot extracts are of course not recommended for rush or ultra-rush protocols, but many allergists in Europe switch to depot preparations after the updosing phase.

Defining the risk factors for SR to VIT would be helpful in reducing their occurrence. In the previous mentioned EAACI-multicentre study (85), female sex, bee venom extract and rapid dose increase, but not the severity of insect sting reactions, increased the risk of a SR.

In a recent study using ultra-rush VIT in a large number of patients (105), few predictive factors were identified, including bee VIT, dose-increase phase, and severity of the prior sting reaction, whereas the size of positive skin test reactions, and serum IgE concentrations were not risk factors.

In patients with underlying mast cell disease (elevated baseline serum tryptase and/or mastocytosis) VIT is well tolerated by the majority of affected patients (106–108). Only a few patients with mastocytosis had repeated severe reactions during immunotherapy necessitating the early suspension of treatment (109, 110).

The recommended maintenance dose of Hymenoptera venom is 100 μg (111), equivalent to approximately two bee stings and a much higher number of *Vespula* stings. This dose gives better protection than a 50 μg dose (112). A dose of 200 μg is recommended when a SR follows a maintenance injection or an insect sting in spite of VIT with 100 μg (110). A maintenance dose of 200 μg is also advised in exposed populations such as beekeepers (113).

The generally recommended interval for maintenance VIT with 100 μg venom is 4 weeks (114). Extending the maintenance interval between injections in the first year of treatment from 4 to 6 weeks continued to give good

clinical protection and maintained the immune response. When the maintenance interval was extended to 8 weeks immediately upon reaching the full dose, there was no problem initially, but in the second year of this treatment declining levels of venom-specific IgG antibodies and a 20% rate of systemic reaction to challenge stings were found (115). These studies have helped to shape the consensus that the maintenance interval should be kept at 4 weeks for the first year, then extended to 6 weeks in the second year, and then to 8 weeks if VIT was continued over 5 years. Only in the past few years have some studies emerged suggesting that patients who continue therapy might be safely maintained on 12-week maintenance intervals (116–119). The small number of studies assessing the possibility of extending the maintenance interval either included too small a population and patients with mainly vespid allergy, or relied on reaction to field stings only.

In a recent study mainly on honey bee venom allergic patients, SR to maintenance VIT administered at 3-month intervals were observed in 2.6% of patients; 2.8% of patients reacted after a field sting, and 4.5% reacted after a sting challenge (120). This single study does not justify administering maintenance VIT at 3-month interval.

Efficacy of venom immunotherapy

The efficacy of VIT was analysed in three prospective controlled (54–56) (level of evidence: Ib) (Table 4) and a number of prospective uncontrolled studies with sting provocation tests during immunotherapy (86, 111, 121–124).

In the first single blind controlled trial (54), only 1 out of 18 venom-treated patients, but 7 out of 11 on wholebody extract and 7 out of 12 on placebo developed systemic allergic reactions. Some of the reactions in the placebo- and wholebody-extract-treated patients were severe and required intensive care treatment (125).

In the second controlled study (55), 3 out of 12 treated patients who were re-exposed to bee stings developed mild systemic allergic reactions; while 9 of those treated with wholebody extract manifested mild to severe allergic symptoms.

Recently a placebo-controlled double-blind study on immunotherapy with jack-jumper ant (*Myrmecia pilosula*) venom (56) was reported from Australia: of 29 patients on placebo, 21 (72%) developed a systemic reaction following a sting challenge during immunotherapy while all 23 on ant venom were completely protected.

Table 4. Controlled studies of venom immunotherapy

References	Immunotherapy	No. pts	Systemic reaction at re-exposure (%)	*P*
(54)	Venom	18	1 (5.3)	
	Wholebody extract	11	7 (63.6)	<0.01
	Placebo	12	7 (58.3)	<0.01
(55)	Venom	12	3 (25)	
	Wholebody extract	12	9 (25)	<0.03
(56)	Venom	23	0 (0)	
	Placebo	29	21 (72)	<0.001

In prospective uncontrolled studies with sting provocation tests during immunotherapy (10, 86, 121–124) only 0–9% of vespid-allergic individuals but around 20% of bee venom-allergic patients still reacted to the challenge with the culprit insect.

However, even in patients who reacted, the symptoms were usually mild and much less severe than before immunotherapy, indicating at least a partial success of the treatment.

The failure rate for venom-allergic children (mostly Vespula-allergic) was initially reported as lower (1.2% per field sting and 2.8% per patient) than in adults (126, 127), but more recently a figure of 9% per patient, similar to that observed in adults, has been reported (128).

The repeatedly observed difference in the success rates in honeybee and vespid venom allergic patients is not completely clear. The fact that the amount of venom delivered by a honeybee sting is much larger and more consistent (87) may explain this difference in the reaction rate to sting challenges, which has also been observed in untreated patients (129–131).

Mast cell disease is a risk factor for the failure of VIT (107, 110). Indeed, out of 32 patients who had SR to a sting challenges while on maintenance treatment with 100 μg venom, 28.1% had elevated baseline serum tryptase level above 13.5 μg/l (110). In seven of these nine patients treatment failed, protection to a further sting challenge could be achieved by increase of the maintenance dose (110). In another study (108) significantly higher reaction rate to a challenge during VIT was observed only in Vespula, but not in honeybee venom treated patients with elevated basal serum tryptase.

The efficacy of VIT has been demonstrated by yet another approach, namely that of assessing health-related quality of life (HRQL). In a cross-sectional study, about one-third of venom allergic patients held self-imposed debilitating beliefs with impairment of their HRQL (132). A randomized prospective study compared the effects of VIT *vs* Epipen as an emergency medication on HRQL (133). After 1 year the group randomized to VIT showed a statistically significant improvement in their HRQL scores, while in those randomized to the Epipen HQRL scores were unchanged or even deteriorated (133). Awareness that VIT prevents anaphylactic reactions to future stings does improve a patient's HRQL. This is an important reason for offering VIT to insect allergic patients (133).

It is furthermore of importance to underline that the products available for venom SIT respond to the definition of Pharmaceutical Specialty (European Directive 89/342/EEC/explanatory note CPMP/BWP243/96). The products and their manufacturing processes have to be validated so as to guarantee the quality, safety and efficacy of each batch that is produced. It is highly

desirable that products with these properties be registered in all European countries.

Duration of venom immunotherapy

After its introduction in 1979 VIT was initially recommended for life or at least until both skin tests and serum venom-specific IgE turned negative. It soon became evident, however, that even after prolonged VIT only a small number of patients gave negative diagnostic tests. On the other hand, patient compliance for continuation of VIT over many years often decreases (56, 132).

For this reason a number of studies were initiated which addressed the protection rate after giving VIT for a limited period. The first series analysed reactions to a sting challenge (CH) 1–3 years after stopping VIT of at least 3-year duration. The results yielded by these studies (124, 134–138) showed continued protection in the vast majority (83–100%) of cases with a relatively short period after stopping successful VIT of at least 3-year duration. Results were somewhat more favourable in *Vespula* than in bee-venom-allergic individuals, and in children as opposed to adults.

Four studies (128, 139–141) analysed long-term protection up to 7 years after discontinuing VIT (Table 5). Taken together these studies found relapses somewhat more frequently than the earlier studies with a shorter follow up. Still, the vast majority – 80% or more – remained protected when re-stung up to 7 years after VIT (128, 139–141).

By careful analysis of all these prospective studies a number of risk factors for the recurrence of SR following Hymenoptera stings can be identified and are summarized in Table 6.

Age: Children generally have a more favourable prognosis than adults, even after discontinuing VIT: One study (124) reported relapses in only 3% of bee venom allergic children, while others recorded 17% in 86 individuals who were mostly adult patients after bee VIT (135), and 8.3% relapses in 24 children as compared to 13.1% in 176 adults who were re-exposed up to 7 years after stopping VIT (128).

Insect: Analysis of the results of the studies with sting provocation test after stopping VIT (124, 134–138) as well as the recurrence rates of 7.5 and 15.8% for *Vespula*-venom and bee-venom treated patients, respectively, indicate a higher risk of relapse in bee venom than in vespid venom allergic patients (128). The reason for this difference is not entirely clear, but has been discussed extensively elsewhere (86, 131).

Table 5. Long-term protection after discontinuation of venom immunotherapy

References	No. pts	Insect	Observation years after stop	Re-exposure	No. with SR (%)
(138)	113	mV	1–>5	FS	10 (9)
(139)	74	mV	5	CH	7 (9.5)
(140)	26	mV	3–7	FS	5 (19)
(127)	120	B	3–7	FS/CH	19 (15.8)
	80	V	3–7	FS/CH	6 (7.5)

SR, systemic allergic reaction; mV, mostly *Vespula*, B, honey bee; FS, field sting; CH, sting challenge.

Table 6. Risk of relapse after stopping venom immunotherapy

Elevated in
Adults *vs* children
Honey bee *vs Vespula* allergic pts
Pts with severe pretreatment SR
Pts with SR during VIT to treatment injections or restings
VIT duration 3 *vs* ≥5 years
Elevated basal serum tryptase
Mastocytosis
High-skin sensitivity at stop
Not influenced by
Sex
Atopy
Venom specific IgE at stop
Venom specific IgG at stop
Diminished if
i.c. Skin tests and venom Specific IgE negative at stop

VIT, venom immunotherapy.

Severity of pretreatment reactions: In four prospective studies involving 386 patients, relapses were observed in 4.1% of 123 with mild, but 14.5% of 263 with severe pretreatment SR (134, 136, 139, 141) ($P < 0.01$).

Safety and efficacy of VIT: Patients who developed systemic allergic side effects to VIT injections ran a relapse risk of 38%, while those who did not only ran a 7% risk (8). Similarly, incomplete protection when re-stung during VIT is associated with an increased risk of relapse (137).

Duration of VIT: Prolonged VIT seems to reduce the risk of a relapse. Thus in one study, SR to re-sting discontinuing VIT were reported on only 4.8% of 82 patients with a VIT duration of ≥50 months as opposed to 17.8% of 118 with a VIT duration of 33–49 months (128).

Elevated basal serum tryptase and mastocytosis: For a number of years it has been known that in patients with urticaria pigmentosa insect venom allergy is often associated with severe shock reactions (106). Two female patients with urticaria pigmentosa and Vespula venom allergy died as the result of a re-sting 3 and 9 years after stopping venom immunotherapy (109). More recently it has been observed that up to one quarter of patients with severe shock reactions following Hymenoptera stings have an elevated basal serum tryptase level (142), indicating the presence of an increased whole body mast cell load. It must be assumed that patients like this have an increased risk of developing a severe reaction after stopping VIT.

Repeated re-exposure after stopping VIT: About half of the relapses occur after the first, the other half after subsequent re-stings (128).

High sensitivity according to diagnostic tests: Some studies by one author report an association of re-sting reactions after stopping VIT with a persistent high sensitivity in intradermal skin testing (134, 140, 141). Others were unable to confirm this observation (8, 143). Specific serum IgE and IgG antibodies *per se* have no predictive value with regard to the re-sting risk after stopping VIT. On the whole, currently used diagnostic tests are of limited predictive value with regard to long-term protection after VIT. Only the combination of a negative i.c. skin testing at 1 mcg/ml and the absence of venom specific serum IgE-antibodies is associated with a strongly diminished risk of relapse (8, 143). Gender and a history of atopic disease do not seem to influence the risk of a relapse after stopping VIT (143).

Future strategies

Potentially there is still much that can be done to improve the treatment of Hymenoptera venom allergy. Thanks to modern molecular biology technology, a considerable number of major venom allergens both from the honeybee and various vespids are available today in recombinant form (103, 144).

Once all the relevant allergens of a venom are available in recombinant form, the sensitization pattern of an individual patient can be exactly determined. A patient-tailored cocktail containing all the allergens to which the patient has IgE antibodies could then be prepared for immunotherapy (145, 146).

The mostly conformational B-cell epitopes can be modified in unrefolded or point mutated recombinant allergens. Cocktails of such preparations have a highly reduced reactivity to IgE antibodies fixed on effector cells; they will therefore induce much less mediator release and be better tolerated. On the other hand their capacity to interact with T cells and thus induce protective immunologic effects will be preserved.

Major T-cell epitope peptides can be prepared synthetically or expressed as recombinant fragments. They have been used for immunotherapy in preliminary studies for bee venom allergy in few patients (147).

Another fascinating experimental strategy for immunotherapy is DNA vaccination, which consists in the injection of DNA-plasmids encoding the relevant allergens. The successful DNA-vaccination of sensitized mice has amongst other allergens been reported with plasmids from bee venom phospholipase A2 (148).

Many Hymenoptera venom allergic patients are sensitized to several different venom allergens from Vespids or honeybees. Treatment with one major allergen in recombinant unrefolded or point mutated form, with peptides thereof, or with DNA-plasmids encoding it, may therefore be insufficient. One elegant solution to this problem has recently been presented (149), using a chimeric protein consisting of one to three fragments each belonging to the important bee venom allergens PLA2, hyaluronidase and melittin, produced by genetic engineering via directional fusion-PCR technology. The fragments were designed in a way to preserve all relevant T-cell epitope peptides while conformational B-cell epitopes were destroyed.

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