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EAACI POSITION PAPER

PREVENTION AND TREATMENT OF HYMENOPTERA VENOM ALLERGY

Bonifazi F [1], Jutel M [2], , Bilò MB [1], Birnbaum J [3], Muller U [4]

Bucher C *, Forster J *, Hemmer W *, Incorvaia C *, Mosbech H *, Oude Elberink JNG *, Rueff F *, Fernandez J *, Senna GE *, Jarish R *, Wuthrich B *

[1] Allergy Unit, Department of Internal Medicine, Immunology, Allergy and Respiratory Diseases, University Hospital, Ancona, Italy. Email: f.bonifazi@ao-umbertoprimo. marche.it

[2] Wroclaw Medical University, Department of Internal Medicine and Allergology, Wroclaw, Poland Email:mjutel@dilnet.wroc.pl

3 Service de Pneumo-allergologie, Hopital Sainte Marguerite, Marseille, France. Email : joelle.birnbaum@medecine.univ-mrs.fr

[4] Spital Bern Ziegler, Bern, Switzerland. Email: Ulrich.Mueller@spitalbern.ch

[1,2,3,4] Chairpersons

* Panel of Experts of EAACI Interest Group on Insect Venom Hypersensitivity

SUMMARY

After a systemic allergic reaction, the use of an emergency medical kit should clearly explained to the patient. I.m. injected epinephrine is the treatment of choice for acute anaphylaxis. Venom immunotherapy (VIT) is indicated both in children and adults with a history of severe systemic reactions and documented sensitisation to the respective insect. As for systemic, non life-threatening reactions other factors may influence the decision to initiate VIT. The efficacy of VIT was demonstrated in three prospective controlled and a number of prospective uncontrolled studies. Most patients with mild to moderate anaphylactic symptoms and positive skin tests remain protected even years after VIT lasting three to five years has been discontinued. Longer term or lifelong treatment should be considered in high-risk patients.

Keywords: immunotherapy, hypersensitivity, hymenoptera venom, prevention

1. INTRODUCTION

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 second part of the Position Paper of the Interest group on Insect Venom Hypersensitivity of the EAACI is a revision of previous editions (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).

2. 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.

Table 1

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

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

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 general, patients should exercise caution when taking part in outdoor activities during the insect season, as it is not uncommon to find yellow jackets and bees on the ground or on lawns. Walking barefoot is therefore not advisable. Allergic persons should wear closed footwear when walking around nature spots and gloves when gardening. Extra care should be taken around picnic areas, orchards, and bins, where vespids forage on discarded or rotting fruit. Insect repellents are

of no use against Hymenoptera. As a further precaution bedroom windows should be kept closed or insect screens fitted to prevent any unwelcome guests from getting in. The presence of Hymenoptera nests in buildings, gardens or in the near vicinity, increases exposure to these insects and should therefore be removed by professionals. 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 stinger remains embedded in the skin that determines the degree of envenomisation (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 if non-beta-blocking agents can obtain an equivalent therapeutic effect, they should be used instead.

3. EMERGENCY TREATMENT

3.1 Large local reactions

Large local reactions induced by hymenoptera stings (see Position Paper on the diagnosis of Hymenoptera venom allergy) may persist for several days or more and may be accompanied by itching, pain or both (8, 9). The lymphangitis and lymphoadenopathy which sometimes accompany these reactions are not, as a rule, signs of an infection but rather of an allergic inflammation. Cold compresses may help to reduce local pain and swelling; oral antihistamines and topical corticosteroids will relieve the pain and itching. In cases of very severe and protracted swelling oral corticosteroids equivalent to 50 mg prednisone daily for a few days may be indicated. In general antibiotics are neither helpful nor required. If a large local reaction occurs in the mouth, the patient must be kept under close observation for possible upper airway obstruction (8).

3.2 Systemic reactions

The most effective drugs for dealing with systemic allergic reactions are sympathomimetics, antihistamines and corticosteroids. Injected epinephrine is regarded as the treatment of choice for cases of acute anaphylaxis (1, 10-12).

3.2.1 Sympathomimetics: Epinephrine

Based on its effects in experimental models including peripheral vasoconstriction, decrease of vascular permeability, positive inotropic effect on the heart, bronchodilatation and inhibition of release of mediators from effector cells, as well as uncontrolled observations in human anaphylaxis, there is good evidence for the use of epinephrine as the medication of first choice in anaphylaxis (10-13). Prospective, placebo-controlled studies in patients with anaphylaxis are however not feasible for ethical reasons.
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).
Side effects of epinephrine include arrhythmia, coronary artery spasm, myocardial infarction, respiratory distress, pulmonary oedema and cerebral haemorrhage: these are mainly observed after rapid intravenous injections of high doses (15). 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 (16).

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.

3.2.2 Antihistamines

The effect of H1-antihistamines on cutaneous symptoms like urticaria and itching is well documented, whereas their effect on cardiovascular or respiratory symptoms during anaphylaxis is only marginal (17). Therefore they should never be used as the sole treatment for severe systemic allergic reactions with respiratory or cardiovascular symptoms (10).

3.2.3 Corticosteroids

The efficacy of corticosteroids in allergic diseases, such as rhinitis and asthma, is well documented (18). Although their exact role in the management of anaphylaxis has yet to be fully elucidated, they may inhibit prostaglandin release, vasoactive kinin synthesis, and IgEmediated release of mediators from effector cells, as well as enhancing the effects of endogenous and exogenous catecholamines (18). Systemic corticosteroids may have no effect for between 4 to 6 hours, even when administered intravenously, but they may prevent persistent or biphasic anaphylaxis (19). As with antihistamines, corticosteroids should not be used alone for treatment of anaphylaxis.

3.2.4 Treatment of systemic reactions

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

The most important principle in the management of an anaphylactic shock is its rapid recognition and the prompt initiation of the therapy (10-13).

Important general measures are transfer to supine position and oxygen supply. Next, a weight-adapted dose of epinephrine must be given intramuscularly, i.v. access established and plasma expanders started, followed by the i.v. administration of H1-antihistamines and corticosteroids.

Some false assumptions in the management of anaphylaxis are that anaphylaxis is always preceded by mild symptoms, that a mild reaction will not progress, and that epinephrine is always effective, even if its administration is not immediate. However, the signs and symptoms of anaphylaxis are unpredictable and may vary considerably from patient to patient. In fact there are no clues which enable physicians to predict how the anaphylactic reaction is going to progress; all organ systems may not be involved simultaneously; the absence of cutaneous signs does not rule out anaphylaxis and may contribute to the delay in the administration of epinephrine.

The superiority of i.m. versus 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, randomised, blinded study in patients at risk of anaphylaxis (20,21) and consequently the i.m. route is recommended in international guidelines (12, 22).

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 also be discussed.

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, angioedema	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. and/or	All patients with protracted respiratory symptoms must be hospitalised; 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 Glucagone: 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.

3.3 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 pre-loaded 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

(23,24). In addition, patients should receive a tablet set containing a rapidly effective oral H1-antihistamine (e.g. cetirizine 2 x 10 mg) and corticosteroids (e.g. prednisone 2 x 50 mg).
In case of a re-exposure the patient should take the tablets immediately, prepare the epinephrine for self administration use with the first symptoms of a systemic allergic reaction and seek out medical care (1).

After a systemic sting reaction, patients should be referred to an allergy specialist for evaluation of their allergy and if necessary VIT.

I.m. injected epinephrine 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.

Untreated patients with a history of a systemic reaction are strongly advised to carry emergency kits containing injectable epinephrine for self administration.

4. VENOM IMMUNOTHERAPY

4.1 Mechanisms

Though it is a well-documented fact that tolerance to insect stings can be achieved through VIT, the mechanism involved is still unclear.
Evidence demonstrates that VIT influences the deviated immune response in allergic individuals in a specific manner and possibly redirects the immune system towards normal immunity. 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 (25-28).
Separately, specific immunotherapy (SIT) with aeroallergens 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 (29-36).
However, the mechanism of repolarisation of specific T-cell activity from dominating Th2-type towards Th1-type is controversial. It appears that the induction of an anergic state in peripheral T cells and their reactivation by cytokines from the tissue microenvironment are essential steps in the mechanism of SIT (29, 30, 32).
Changes in the immune response to bee venom have been extensively investigated during VIT, PLA-peptide immunotherapy (29-33, 37-39) and during high natural allergen exposure in healthy bee keepers (30).
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 (30).
The anergic state of specific cells results from increased IL-10 secretion (32). 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 (30).
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 specialize in the suppression of immune response are pivotal in maintaining peripheral tolerance (40-43).

Treg cells are enriched within the $CD4_+CD25_+$ cells (44-47). 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 (48). However, unlike in mucosal allergies this mechanism is not active in venom allergy.
Differences in the control mechanisms 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 (49). 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 (49). The implications of these observations in terms of clinical efficacy call for further investigation. IL-10 also plays an inhibitory role on IgE and effector cells of allergic inflammation. VIT induced IL-10 inversely regulates antigen-specific IgE and IgG4 antibody synthesis, thus skewing the specific response from an IgE to an IgG4-dominated phenotype (30, 32). 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 (28, 50-52). 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 (53, 54), recent evidence suggests that their use as premedication may enhance the clinical efficacy of VIT (55).
It is well established that histamine released from effector cells influences T cells (56). 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 (56). 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 pre-treatment in all VIT patients.

4.2 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 whole body extract treated groups in controlled studies on VIT (57-59) up to 75% of the patients with a history of systemic anaphylactic sting reaction develop systemic symptoms once again when restung.
The risk factors involved are reported in the Position Paper on the diagnosis of Hymenoptera venom allergy and may be summarized as follows:
1) Time interval between stings: a short interval of only weeks to months between two stings from the same species is associated with a high risk.

2) Number of stings: less than 25 stings per year are associated with an increased risk in beekeepers.
3) Severity of the preceding reaction: higher risk of systemic reactions in patients with history of systemic than large local reactions and in those with severe than only mild systemic reactions.
4) Age: lower risk for children than adults.
5) Concomitant cardiovascular diseases or treatment with beta-blockers: associated with particularly severe sting reactions.
6) Responsible insect: higher risk for honeybees and hornets than for *Vespula.*
7) Mastocytosis or elevated basal serum tryptase.
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 (60, 61) 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.

4.2.1 Indications for 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 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).

VIT is not recommended for large local reactions in either children (62, 63) or adults (64).

As for systemic, non life-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**.

Table 3

Indications for venom immunotherapy

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

4.2.2 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 (65).

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.

4.2.3 Selection of venom to be used in immunotherapy

This is based on:

1) Identification of the species of Hymenoptera involved: with the exception of beekeepersand their families, patients are often unable to identify the offending insect. To overcomethis problem, patients should be asked to describe the insect and to identify it from photographs of the various local species.

2) cross-reactivity between venoms (see Position Paper on the Diagnosis of Hymenoptera venom allergy):

A) Honey bee and bumblebee venoms show marked cross-reactivity.

VIT with honeybee venom alone will be sufficient in non-professionally exposed bumblebeeallergic patients who most likely react on the basis of a crossreactivity in the presence of primary sensitization to bee venom (66, 67). In heavily exposed greenhouse workers who are frequently stung by bumble bees, it is recommended to use bumblebee venom for VIT (68).

B) 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 (see Position Paper on the diagnosis of Hymenoptera venom allergy. In central and northern Europe, *Vespula* is responsible for most allergic reactions. In the Mediterranean basin, *Polistes* may be equally or in some regions even more significant. 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 crossreactivity can be identified by RASTinhibition. Since it can be assumed that most patients with allergic reactions to *Vespa crabro* were first sensitised by *Vespula* stings, VIT with *Vespula* venom alone will be sufficient in patients who reacted to a sting by *Vespa crabro*.
C) Crossreactivity 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 (69, 70). Treatment with both venoms is only indicated in documented double sensitization.

4.3 Treatment protocol and safety

Since the first immunotherapy with pure venom extract was carried out in 1974 (71), protocols of various duration have been devised in an effort to maximise protection, minimise side-effects and optimise patient convenience (**Table 4**). The time required to reach the generally adequate maintenance dose of 100µg with slow protocols is several weeks to months (72-74), whilst rush (75-80) and ultra-rapid (ultra-rush) protocols (81-85) take several days or only a few hours respectively.
VIT aims to induce tolerance to Hymenoptera venom but can be complicated by systemic reactions (SR) (86, 87). The risk for SR to VIT is more related to the nature of the venom than to the regimen used (88).
VIT with bee venom causes more SR than VIT with *Vespula* venom: one explanation may be differences in the quality of the extracts (89). In commercial venom extracts, vespid venom allergens are diluted by non-allergenic venom-sac proteins, whereas honeybee venom is a purified venom with a lower concentration of non-allergenic proteins (90,91). Reports in the literature reveal a high variation (0-46%) in the incidence of side-effects attributable to VIT (8, 53, 78, 83, 86, 88, 92). 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).
An EAACI multicenter study (87) collected data from 840 patients, totaling 26,601 injections with a variety of treatment regimens. Twenty per cent of patients had SR, corresponding to 1.9% of injections during the dose-increase phase and 0.5% during the maintenance phase. The vast majority of the 280 reported reactions were mild and only one-third required medical treatment. Rapid dose increase (rush) regimens were associated with an increased risk of side effects (87).
Some studies using rush protocols have suggested that they are at least as safe as slower protocols (78, 81, 82, 83). An ultra-rush VIT protocol, which used a cumulative venom dose of 101.1µg of venom over 3.5h (210 minutes), resulted in fewer systemic reactions compared to 6-hour and 4-day protocols, which attained cumulative doses of 226.6µg and 527.6µg, respectively (84).
After comparison with several protocols, from 2 days to 7-9 days, other authors concluded that the incidence and severity of adverse reactions are minimised if the VIT dose increase protocol is shortened to 2 days (93).
Some studies of rush and ultrarush VIT included children (80) and even two-year-old toddlers (93). 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 (87).
Immunotherapy with bumblebee venom is as safe and effective as it is with the other venoms (68, 94, 95).

Table 4

Different protocols of venom immunotherapy

	Bernstein (83)			Birnbaum (84)	Van der Zwan (82)	Müller (8)	Tarhini (74)	Brehler (93)
Day	**Time, min**	**Dose µg**	**Time, min**	**Dose, µg**	**Dose, µg**	**Dose, µg**	**Dose, µg**	**Dose, µg**
1	0 10 20 30 40 50 60 70 80 90	0.05 0.1 0.2 0.4 0.8 2 5 10 20 20	0 30 60 90 120 150 180 210 240 270 300 330 360	10^{-1} 1 10 20 30 40	10^{-5} 10^{-4} 10^{-3} 10^{-2} 10^{-1} 0.5 1 5 10 20 30 60 100	10^{-4} 10^{-3} 10^{-2} 0.1 0.2 0.4 0.8 1	0.1 1 5 10 20	0.01 0.1 1 10 20 40 80
2			0 30 60 90 120 150 180 210 240			0,1 1 2 4 8 10 20 40 50		100 100
3		70	0 30 60 90			10 50 80 100		
4						100		
7		80	0 30 60			100	30 30 30	100
10						100		
14		90					100	100
15			0 30	50 50	50 50	100		
20								
21		100						
28								100
42								100
45				100	100	100		
Monthly		100					100	100

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 pre-treatment with antihistamines (53-55, 96, 97), by administering beekeeper gamma globulin (98), or through the use of chemically modified honeybee venom or recombinant Hymenoptera venom allergens, which proved successful to varying degrees (99-105). Pre-treatment with antihistamines, which reduces the number/severity of large local reactions and mild systemic reactions such as urticaria/angioedema, should be prescribed one or two days before VIT and be continued until the maintenance dose has been well tolerated at least 3 times.

Depot extracts seem to be associated with somewhat fewer side effects than aqueous preparations; a recent paper has documented comparable efficacy of depot versus aqueous extracts (106). 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 multicenter study (87), 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, ultra-rush VIT was routinely performed in a large number of patients, allowing a retrospective analysis on clinical data with and without SRs to VIT in order to detect a patient profile for those at risk for SR to VIT (107). The higher incidence of adverse reactions in patients receiving bee VIT (30%) compared with those receiving *Vespula* VIT (4.6%) is consistent with findings in other reports. The majority of SRs were very mild. 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. The safety of VIT was comparable in children and adults on the same ultra-rush VIT (107).

In patients with underlying mast cell disease (elevated baseline serum tryptase and/or mastocytosis) VIT is well tolerated by the majority of affected patients (108-110). Only a few patients with mastocytosis had repeated severe reactions during immunotherapy necessitating the early suspension of treatment (111,112).

The recommended maintenance dose of Hymenoptera venom is 100µg (113), equivalent to approximately two bee stings and a much higher number of *Vespula* stings. This dose gives better protection than a 50µg dose (114). A dose of 200µg is recommended when a SR follows a maintenance injection or an insect sting in spite of VIT with 100µg (112). A maintenance dose of 200µg is also advised in exposed populations such as beekeepers (115).

The generally recommended interval for maintenance VIT with 100µg venom is four weeks (116). Extended maintenance intervals of six and eight weeks have been investigated for safety, efficacy, and effect on the immune response. Extending the maintenance interval between injections in the first year of treatment from four to six weeks continued to give good clinical protection and maintained the immune response. When the maintenance interval was extended to eight 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 (117). These studies have helped to shape the consensus that the maintenance interval should be kept at four weeks for the first year, then extended to six weeks in the second year, and then to eight weeks if VIT was continued over five 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 (118-121). 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 on 166 patients including 111 honey-bee-venom allergic patients, failure to reach a 3-month interval was observed in 3.8% of patients (122). SRs 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. This single study does not justify administering maintenance VIT at 3-month intervals.

4.4 Efficacy of venom immunotherapy

The efficacy of VIT was analysed in three prospective controlled (Level of evidence: Ib) (57-59) (**Table 5**) and a number of prospective uncontrolled studies with sting provocation tests during immunotherapy (88, 114, 123-126) (**Table 6**).

Table 5

Controlled studies of venom immunotherapy

Author	Immunotherapy	No pts	Systemic reaction at re-exposure (%)	p
Hunt 1978 [57]	venom	19	1 (5.3)	
	wholebody extract	11	7 (63.6)	< 0.01
	Placebo	12	7 (58.3)	< 0.01
Müller 1979 [58]	venom	12	3 (25)	
	wholebody extract	12	9 (25)	< 0.03
Brown 2003 [59]	venom	23	0 (0)	
	Placebo	29	21(72)	< 0.001

In the first controlled trial 60 patients with a history of systemic allergic reactions to Hymenoptera stings were treated in a single blind study with either the venom, the wholebody extract or placebo over 6 weeks and then submitted to a sting challenge with the culprit insect (57). Only 1 out of 18 venom-treated patients, but 7 out of 11 on whole body 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 (127); for this reason the study was stopped.

Table 6

Efficacy of venom immunotherapy in prospective studies with sting challenge during immunotherapy.

Author	Immunotherapy with venom of	No pts	Systemic reaction after challenge (%)
Chipps 1980 [123]	mostly Vespula children	42	1 (2)
Hoffman 1981 [124]	honey bee	25	5 (20)
Golden 1981 [114]	mostly Vespula	147	4 (3)
Mosbech 1984 [125]	Vespula	19	0
Urbanek 1985 [126]	honey bee Children	66	4 (6.1)
Müller 1992 [88]	honey bee	148	34 (23)
Müller 1992 [88]	Vespula	57	5 (9)

In the second controlled study 53 patients were treated in an open trial with either honeybee venom or wholebody extract (58). Twelve patients from each group were reexposed to bee stings during immunotherapy: three of the venom-treated patients developed mild systemic allergic reactions, while nine of those treated with whole body extract manifested mild to severe allergic symptoms.
Recently a placebo-controlled double-blind study on immunotherapy with jack-jumper ant (*Myrmecia pilosula)* venom 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 (Level of evidence: Ib) (59).
In prospective uncontrolled studies with sting provocation tests during immunotherapy (table 6) only 0 to 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 (128, 129), but more recently a figure of 9% per patient, similar to that observed in adults, has been reported (130).
The repeatedly observed difference in the success rates in honey bee and vespid venomallergic patients is not completely clear. The fact that the amount of venom delivered by a honey bee sting is much larger and more consistent (89) may explain this difference in the reaction rate to sting challenges, which has also been observed in untreated patients (131- 133).
Mast cell disease is a risk factor for the failure of VIT (109, 112). 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 (112). In 7 of these 9 patients treatment failed, protection to a further sting challenge could be achieved by increase of the maintenance dose (112). In another study (110) significantly higher reaction rate to a challenge during VIT was observed only in Vespula, but not in honey bee 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 (134). A randomised prospective study compared the effects of VIT versus Epipen as an emergency medication on HRQL (135). Patients were educated using standardised material and procedures. HRQL was measured before and after one year of either measure. The group randomised to VIT showed a statistically significant improvement in their HRQL scores, while in those randomised to the Epipen HQRL scores were unchanged or even deteriorated.
Even though mortality and severe morbidity are low in Hymenoptera venom allergy, this does not mean that treatment is unnecessary. 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 (135).
It is furthermore of importance to underline that the products available for venom specific immunotherapy 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 beregistered in all European countries.

4.5 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 (59, 134).

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) one to three years after stopping VIT of at least three years duration. The results yielded by these studies are summarised in **Table 7** (126, 136-140) and showed continued protection in the vast majority (83 to 100%) of cases with a relatively short period after stopping successful VIT of at least three years duration. Results were somewhat more favourable in *Vespula* than in bee-venom-allergic individuals, and in children as opposed to adults.

Table 7

Prospective studies with sting provocation test after stopping venom immunotherapy

Author	no pts	insect	sting challenge after years	sting challenge no with SR (%)
Urbanek 1985[126]	29	honey bee	1	1 (3)
	14		2	2 (14)
Golden 1989[136]	29	m Vespula	1	0
Müller 1991[137]	86	honey bee	1	15 (17)
Haugaard 1991[138]	25	Vespula	2	0
Keating 1991[139]	51	m Vespula	1	2 (4)
Van Halteren 1997[140]	75	Vespula	1-3	6 (8)

m *Vespula* = mostly *Vespula*

Four studies (130,141-143) analysed long-term protection up to 7 years after discontinuing VIT (**Table 8**).

Reisman (141) encountered relapses following a field sting up to more than 5 years after stopping in 10 out of 113 (9%) cases, most of which were *Vespula*-venom-allergic. Golden (142) followed 74 predominantly *Vespula*-venom-allergic patients for 5 years after stopping VIT of at least five years duration with a CH every year (29 pts), every second year (25 pts) or only after 2 years (20 pts). Seven (9.5%) developed at least one allergic systemic reaction but these were always mild. Interestingly, the same group observed SRs to a field sting in 5 out of 26 (19%) patients out of 125 who were followed up to 7 years after stopping VIT and some of these reactions were severe (143). Finally Lerch reported on 358 patients who were followed up to 7 years after stopping successful VIT: 200 were reexposed to either a field sting or a CH and 25 (12.5%) developed SR, which were mostly mild (130). Taken together, these studies with a prolonged observation period after stopping VIT, 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.

Table 8

Long-term protection after discontinuation of venom immunotherapy

Author	no pts	insect	observation years after stop	reexposure	no with SR (%)
Reisman 1993[141]	113	mV	1 - >5	FS	10 (9)
Golden 1996[142]	74	mV	5	CH	7 (9.5)
Golden 1998[143]	26	mV	3 - 7	FS	5 (19)
Lerch 1998[130]	120 80	B V	3 - 7 3 – 7	FS/CH FS/CH	19 (15.8) 6 (7.5)

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

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 9**.

Age: Children generally have a more favourable prognosis than adults, even after discontinuing VIT: Urbanek (126) noted relapses in only 3% of bee venom allergic children, while Müller (137) observed 17% in 86 individuals who were mostly adult patients after bee VIT, and Lerch (130) recorded 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.
Insect: Analysis of the results presented in table 7 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 (130). The reason for this difference is not entirely clear, but has been discussed extensively elsewhere (88, 133).

Table 9

Risk of relapse after stopping venom immunotherapy

Elevated in	not influenced by
- Adults vs children	- sex
- Honey bee vs Vespula allergic pts	- atopy
- Pts with severe pretreatment GR	- venom specific IgE at stop
- Pts with GR during VIT to treatment injections or restings	- venom specific IgG at stop
- VIT duration 3 years vs ≥ 5 years	diminished if
- Elevated basal serum tryptase	- i.c. skin tests and venom specific IgE negative at stop
- Mastocytosis	
- High skin sensitivity at stop	

Severity of pre-treatment 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 pre treatment SRs (136, 138, 141, 143) ($p < 0.01$).

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 (139).

Duration of VIT: Prolonged VIT seems to reduce the risk of a relapse. Thus in one study, SRs 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 to 49 months (130).

Elevated basal serum tryptase & 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 (108). Two female patients with urticaria pigmentosa and Vespula venom allergy died as the result of a re-sting three and nine years after stopping venom immunotherapy (111). 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 (144), 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: According to Lerch (130) about half of the relapses occur after the first, the other half after multiple re-stings.

High sensitivity according to diagnostic tests: Golden found an association of re-sting reactions after stopping VIT with a persistent high sensitivity in intradermal skin testing (136, 142-143). Others were unable to confirm this observation (8, 145). 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 1mcg/ml and the absence of venom specific serum IgE-antibodies is associated with a strongly diminished risk of relapse (8, 145). Gender and a history of atopic disease do not seem to influence the risk of a relapse after stopping VIT (145).

If both skin tests and serum venom-specific IgE turn negative, VIT may be stopped after 3 years.

After VIT lasting three to five years, most patients with mild to moderate anaphylactic symptoms and positive skin tests remain protected even after VIT has been discontinued.

Longer term or lifelong treatment should be considered in high risk patients:

1. a higher risk of very severe sting reactions (e.g., older age, history of very severe previous sting reactions, elevated basal serum tryptase or mastocytosis, use of beta-blockers);
2. systemic allergic reactions to immunotherapy injections or stings during VIT;
3. highly exposed patients such as beekeepers and their immediate family members.

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.

5. FUTURE STRATEGIES

Potentially there is still much that can be done to improve both the diagnosis and treatment of Hymenoptera venom allergy. Various new strategies are currently being studied in order to achieve this goal.

5.1 Future strategies in diagnosis

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 (**Table 10**) (105, 146).

Table 10

Recombinant Hymenoptera venom allergens (146)

Species	allergen		MW kD
Apis mellifera	Api m 1	phospholipase A2	16-20
	Api m 2	hyaluronidase	43
	Api m 3	melittin	2.9
	Api m 4	acid phosphatase	49
Vespula vulgaris	Ves v 1	phospholipase A1	35
	Ves v 2	hyaluronidase	45
	Ves v 5	antigen 5	25
Dolichovespula	Dol m 1	phospholipase A1	35
maculata	Dol m 2	hyaluronidase	45
	Dol m 5	antigen 5	25
Polistes annularis	Pol a 5	antigen 5	25

Recombinant venom allergens will certainly improve the diagnosis of venom allergy in the near future. There is a very close correlation with regard to their IgE-binding capacity (comparison with the respective natural purified preparations) (147). Some disparities have, however, been disclosed by RAST-inhibition and western blot studies, which revealed that all natural preparations were contaminated with trace amounts of other venom allergens. Recombinant allergens will therefore be superior to highly purified natural preparations when it comes to the determination of the true clinical relevance of an individual allergen. Recombinant technology has also been very helpful in clarifying crossreactivities between venom allergens from different species, genera or even families of hymenoptera. Finally, the use of recombinant cocktails for diagnosis is promising (148).

5.2 Future strategies for treatment

Recombinant venom allergens could also be used for immunotherapy (146). Once all the relevant allergens of a venom are available in recombinant form, the sensitisation 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 (148, 149).

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 cat allergy (150), and also for bee venom allergy (151). Three linear short peptides of phospholipase A2 were identified which were unable to bind to IgE-antibodies in sera from bee venom allergic patients, but induced strong proliferation of their T-lymphocytes in vitro (151). Immunotherapy with these major T-cell epitope peptides in bee-venom-allergic patients resulted in complete protection in three and partial protection in two of the five patients who were challenged by a live bee sting. In vitro studies on lymphocyte cultures of the patients suggested the induction of phospholipase-A2-specific tolerance by this form of peptide immunotherapy (33).

Another fascinating experimental strategy for immunotherapy is DNA vaccination, which consists in the injection of DNA plasmids encoding the relevant allergens. In contrast to environmental allergen exposure and classical immunotherapy this kind of vaccination induces a TH1 response. The successful DNA vaccination of sensitised mice has amongst other allergens been reported with plasmids from bee venom phospholipase A2 (152) Protection to subcutaneously applied phospholipase A2 was complete if the vaccination was performed before sensitisation, whereas only 65% of the animals survived, which were given a therapeutic vaccination after intraperitoneal sensitisation to phospholipase A2.

As mentioned above, many Hymenoptera venom allergic patients are sensitised 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 (153), 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. This chimeric protein indeed induced strong proliferation and cytokine secretion in lymphocyte cultures from bee-venom-allergic patients, but it did not react with specific IgE against either of the three allergens, nor did it induce mediator release fromblood basophils or immediate wheal and flare reaction in i.c. skin testing of allergic patients.

REFERENCES

1. Müller U, Mosbech H, Aberer W, Dreborg S, Ewan P, Kunkel G, Malling H-J, Przybilla B, Vervloet D. EAACI Position Paper. Adrenaline for emergency kits. Allergy 1995;50:783-7.
2. Bousquet J, Müller UR, Dreborg S, Jarish R, Malling HJ, Mosbech H et al. Immunotherapy with Hymenoptera venoms. Allergy 1987;42 :401-13.
3. Müller U, Mosbech H. Position Paper: Immunotherapy with Hymenoptera venoms. EAACI. Allergy 1993;48:36 46.
4. Shekelle PG, Woolf SH, Eccles M, Grimsham J. Clinical guidelines: developing guidelines. BMJ 1999;318:593 6.
5. Visscher PK, Vetter RS, Camazine S. Removing bee stings. Lancet 1996; 348:301-302.
6. Toogood JH. Betablocker therapy and the risk of anaphylaxis. Can Med Assoc J 1987;136:929-933.
7. Hepner MJ, Ownby DR, Anderson JA, Rowe MS, Sears-Ewald D, Brown EB. Risk of systemic reactions in patients taking beta-blocker drugs receiving allergy immunotherapy injections. J Allergy Clin Immunol 1990; 85:407-411.
8. Müller UR. Insect sting allergy. Clinical picture, diagnosis and treatment. Stuttgart-New York; Gustav Fischer Verlag 1990.
9. Portnoy JM, Moffitt JE, Golden DBK, Bernstein IL, Berger WE, Dykewicz MS et al. Stinging insect hypersensitivity: a practice parameter. J Allergy Clin Immunol 1999;103:963-80.
10. Müller U, Mosbech H, Blaauw P, Dreborg S, Mallimg HJ, Przybilla B et al. Emergency treatment of allergic reactions to Hymenoptera stings. Clin Exp Allergy 1991;21:281-8.
11. AAAAI Board of Directors. The use of epinephrine in the treatment of anaphylaxis. Position Statement. J Allergy Clin Immunol 1994;94:666-8.
12. Project Team of the Resuscitation Council (UK). Emergency medical treatment of anaphylactic reactions. J Accid Emerg Med 1999;16:243-247.
13. Simons FER. First aid treatment of anaphylaxis to food: Focus on epinephrine. J Allergy Clin Immunol 2004; 113:837-844.
14. Bautista E, Simons E, Simons K, Becker A, Duke K, Tillet M, Kepron W, Mink SN. Epinephrine fails to hasten hemodynamic recovery in fully developed canine anaphylactic shock. Int Arch Allergy Immunol 2002;128:151 164.
15. Hoffman BB, Lefkowitz RJ. Catecholamines, sympathomimetic drugs, and adrenergic receptor antagonists. In: Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, Gilman AG, eds. Goodman and Gildman's. The Pharmacological Basis of Therapeutics. 9th Ed. New York: McGraw-Hill Companies, Inc. 1996:204-9.
16. Pumphrey RSH. Lessons for management of anaphylaxis from a study of fatal reactions. Clin Exp Allergy 2000;30:1144-50.
17. Simons FER. Antihistamines: In Middleton E Jr, Reed CE, Ellis EF, Adkinson NF Jr, Yunginger JW, Busse WW, eds. Allergy Principles and Practice. 5th Ed. St. Louis: Mosby-Year Book, Inc.,1998;612-637.
18. Schleimer RP. Glucocorticosteroids: In Middleton E Jr, Reed CE, Ellis EF, Adkinson NF Jr, Yunginger JW, Busse WW, eds. Allergy Principles and Practice. 5th Ed. St. Louis: Mosby-Year Book, Inc.,1998;638-660.
19. Stark BJ, Sullivan T. Biphasic and protracted anaphylaxis. J Allergy Clin Immunol 1986; 78:76-83.
20. Gu X, Simons FER, Simons KJ. Epinephrine absorption after different routes of administration in an animal model. Biopharm Drug Dispos 1999;20:401-5.
21. Simons FER, Gu X, Simons KJ. Epinephrine absorption in adults: Intramuscular versus subcutaneous injections. J Allergy Clin Immunol 2001;108:871-3.
22. Cummins RO, Hazinski MR. Guidelines 2000 for cardiopulmonary resuscitation and emergency cardiovascular care: An international consensus on science: American Heart Association in collaboration with the international Liaison Committee on Resuscitation (ILCOR). Part 8: Advanced challenges in resuscitation. Circulation 2000;102(suppl) :I229-I252.
23. Grouhi M, Alshehri M, Hummel D, Roifman CM. Anaphylaxis and epinephrine auto-injector training: who will teach the teachers? J Allergy Clin Immunol 1999;104:190-3.
24. Gold MS, Sainsbury R. First aid anaphylaxis management in children who were prescribed an epinephrine autoinjector device (Epipen). J Allergy Clin Immunol 2000;106:171-6.
25. Creticos PS, Franklin Adkinson Jr. N, Kagey-Sabotka A, Proud D, Meier HL, Naclerio RM, Lichtenstein LM, Norman PS. Nasal challenge with ragweed in hay fever patients: Effect of immunotherapy. J Clin Invest 1985; 76:2247-2253.
26. Wetterwald A, Skvaril F, Müller U, Blaser K. Isotypic and idiotypic characterization of anti-bee venom phospholipase A_2 antibodies. Int Arch Allergy Appl Immunol 1985; 77:195-197.
27. Rak S, Rowhagen O, Venge P. The effect of immunotherapy on bronchial hyper-responsiveness and eosinophil cationic protein in pollen allergic patients. J Allergy Clin Immunol 1988; 82:470-480.
28. Jutel M, Müller UR, Fricker M, Rihs S, Pichler WJ, Dahinden C. Influence of bee venom immunotherapy on degranulation and leukotriene generation in human blood basophils. Clin Exp Allergy 1996; 26:1112-8.

29. Akdis CA, Akdis M, Blesken T, Wymann D, Alkan SS, Müller U, Blaser K. Epitope specific T cell tolerance to phospholipase A_2 in bee venom immunotherapy and recovery by IL-2 and IL-15 *in vitro*. J Clin Invest 1996; 98:1676-1683.
30. Akdis CA, Blesken T, Akdis M, Wüthrich B, Blaser K. Role of IL-10 in specific immunotherapy. J Clin Invest 1998; 102:98-106.
31. Akdis CA, Blesken T, Wymann D, Akdis M, Blaser K. Differential regulation of human T cell cytokine patterns and IgE and IgG4 responses by conformational antigen variants. Eur J Immunol 1998; 28:914-925.
32. Akdis CA, Blaser K. IL-10 induced anergy in peripheral T cell and reactivation by microenvironmental cytokines: two key steps in specific immunotherapy. FASEB J 1999; 13:603-609.
33. Müller UR, Akdis CA, Fricker M, Akdis M, Bettens F, Blesken T, Blaser K. Successful immunotherapy with T cell epitope peptides of bee venom phospholipase A_2 induces specific T cell anergy in bee sting allergic patients. J Allergy Clin Immunol 1998; 101:747-754.
34. Jutel M, Pichler WJ, Skrbic D, Urwyler A, Dahinden C, Müller UR. Bee venom immunotherapy results in decrease of IL-4 and IL-5 and increase of IFN-γ secretion in specific allergen stimulated T cell cultures. J Immunol 1995; 154:4178-4194.
35. Bellinghausen I, Metz G, Enk AH, Christmann S, Knop J, Saloga J. Insect venom immunotherapy induces interleukin-10 production and a Th2-to-Th1 shift, and changes surface marker expression in venom-allergic subjects. Eur J Immunol 1997; 27:1131-1139.
36. Marcotte GV, Braun CM, Norman PS, Nicodemus CF, Kagey-Sabotka A, Lichtenstein LM, Essayan DM. Effects of peptide therapy on ex vivo T cell responses. J Allergy Clin Immunol 1998; 101:506-513.
37. Akdis CA, Blesken T, Akdis M, Alkan SS, Heusser CH, Blaser K. Glucocorticoids inhibit human antigen-specific and enhance total IgE and IgG4 production due to differential effects on T and B cells *in vitro*. Eur J Immunol 1997; 27:2351-2357.
38. Akdis CA, Blesken T, Akdis M, Alkan SS, Wüthrich B, Heusser CH, Blaser K. Induction and differential regulation of bee venom phospholipase A_2-specific human IgE and IgG4 antibodies *in vitro* requires allergen specific and non-specific activation of T and B cells. J Allergy Clin Immunol 1997; 99:345-352.
39. Carballido JM, Carballido-Perrig N, Kägi MK, Meloen RH, Wüthrich B, Heusser CH, Blaser K. T cell epitope specificity in human allergic and non-allergic subjects to bee venom phospholipase A_2. J Immunol 1993; 150:3582-3591.
40. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alfa-chains (CD25). Breakdown of a single mechanism of self tolerance causes various autoimmune diseases. J Immunol 1995; 155:1151-1164.
41. Groux H, O'Garra A, Bigler M, Rouleau M, Antonenko S, de Vries JE, Roncarolo MG. A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. Nature 1997; 389:737-742.
42. Shevach EM. Regulatory T cells in autoimmunity. Annu Rev Immunol 2000; 18:423-49.
43. Read S, Powrie F. CD4(+) regulatory T cells. Curr Opin Immunol 2001;13:644.
44. Suri-Payer E, Amar AZ, Thornton AM, Shevach EM. $CD4_+CD25_+$ T cells inhibit both the induction and effector function of autoreactive T cells and represent a unique lineage of immunoregulatory cells. J Immunol 1998; 160:1212-1218.
45. Thornton AM, Shevach EM. $CD4_+CD25_+$ immunoregulatory T cells suppress polyclonal T cell activation in vitro by inhibiting interleukin 2 production. J Exp Med 1998; 188:287-296.
46. Read S, Mauze S, Asseman C, Bean A, Coffman R, Powrie F. $CD38CD45RB_{low}$ T cells: a population of T cells with immune regulatory activities in vitro. Eur J Immunol 1998; 28:3435-3447.
47. Jonuleit H, Schmitt E, Stassen M, Tuettenberg A, Knop J, ENK AH. Identification and functional characterization of human $CD4_+CD25_+$ T cells with regulatory properties isolated from peripheral blood. J Exp Med 2001;193:1285-129
48. Jutel M, Akdis M, Budak F, Aebischer-Casaulta C, Wrzyszcz M, Blaser K, Akdis CA. IL-10 and TGF-β cooperate in the regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. Eur J Immunol 2003; 33, 233-41.
49. Kammerer R, Chvatchko Y, Kettner A, Dufour N, Corradin G, Spertini F. Modulation of T-cell responses to phospholipase A2 and phospholipase A2-derived peptides by conventional bee venom immunotherapy. J Allergy Clin Immunol 1997; 100: 96-103.
50. Stephan V, Kuhr J, Urbanek R. Relevance of basophil histamine release changes during venom immunotherapy. Allergy 1989; 44:453-9.
51. Bernstein DI, Mittman RJ, Kagen SL, Korbee L, Enrione M, Bernstein IL. Clinical and immunologic studies of rapid immunotherapy in hymenoptera sensitive patients. J Allergy Clin Immunol 1989; 84:951-9.
52. Eberlein-Konig B, Ullmann S, Thomas P, Przybilla B. Tryptase and histamine release due to a sting challenge in bee venom allergic patients treated successfully or unsuccessfully with hyposensitisation. Clin Exp Allergy 1995;25: 704-12.
53. Berchtold E, Maibach R, Müller U. Reduction of side effects from rush-immunotherapy with honey bee venom by pre-treatment with terfenadine. Clin Exp Allergy 1992;22:59-65.

54. Brockow K, Kiehn M, Riethmuller C, Vieluf D, Berger J, Ring J. Efficacy of antihistamine pretreatment in the prevention of adverse reactions to Hymenoptera immunotherapy: a prospective, randomized placebo-controlled trial. J Allergy Clin Immunol 1997; 100: 458-63.
55. Müller U, Hari Y, Berchtold E. Premedication with antihistamines may enhance efficacy of specific-allergen immunotherapy. J Allergy Clin Immunol 2001;107:81-6.
56. Jutel M, Watanabe T, Klunker S, Akdis M, Thomet OAR, Malolepszy J, Zak-Nejmark T, Koga R, Kobayashi T, Blaser K, Akdis CA. Histamine regulates T-cell and antibody responses by differential expression of H1 and H2 receptors. Nature 2001; 413: 420-5.
57. Hunt KJ, Valentine MD, Sobotka AK, Benton AW, Amodio FJ, Lichtenstein LM. A controlled trial of immunotherapy in insect hypersensitivity. New Engl J Med 1978;299:157-161.
58. Müller U, Thurnheer U, Patrizzi R, Spiess J, Hoigne R. Immunotherapy in bee sting hypersensitivity. Bee venom versus wholebody extract. Allergy 1979;34: 369-378.
59. Brown S, Wiese M, Blackman K, Heddle R. Ant venom immunotherapy: A double blind, placebo-controlled cross-over trial. Lancet 2003;361: 1001-1006.
60. Tunon-de-Lara JM, Villanueva P, Marcos M, Taytard A. Ace inhibitors and anaphylactoid reactions during venom immunotherapy. Lancet 1992;340:908.
61. Ober AI, MacLean JA, Hannaway PJ. Life-threatening anaphylaxis to venom immunotherapy in a patient taking an angiotensin-converting enzyme inhibitor. J Allergy Clin Immunol 2003;112:1008-9.
62. Schuberth KC, Lichtenstein LM, Kagey-Sobotka AK, Szklo M, Kwiterovich KA, Valentine MD. Epidemiologic study of insect allergy in children. II. Effect of accidental stings in allergic children. J Pediatr 1983;102 : 361-365.
63. Graft DF, Schuberth KC, Kagey-Sobotka AK, Kwiterovich KA, Niu Y, Lichtenstein LM et al. A prospective study of the natural history of large local reactions after Hymenoptera stings in children. J Pediatr 1984;104 : 664-668.
64. Mauriello PM, Barde SH, Georgitis JW , Reisman RE. Natural history of large local reactions from stinging insects. J Allergy Clin Immunol 1984;74 : 494-498.
65. Schwartz HJ, Golden DBK, Lockey RF. Venom immunotherapy in the Hymenoptera-allergic pregnant patient. J Allergy Clin Immunol 1990;85 : 709-712.
66. Kochuyt AM, Van Hoeyveld E, Stevens EAM. Occupational allergy to bumble bee venom. Clin Exp Allerg 1993;23:190-195.
67. Bucher C, Korner P, Wüthrich B. Allergy to bumblebee venom. Curr Opin Allergy Immunol 2001;1: 361-365.
68. Stern A, Mullner RG, Wüthrich B. Successful treatment of occupational allergy to bumble-bee venom after failure with honeybee venom extract. Allergy 2000;55:88-91.
69. Reisman RE, Müller UR, Wypych JI, Lazell MI. Studies of coexisting honeybee and vespid-venom sensitivity. J Allergy Clin Immunol 1984;73:246-252.
70. Straumann F, Bucher C, Wüthrich B. Double sensitization to honeybee and wasp venom: immunotherapy with one venom or with both venoms? Int Arch Immunol 2000;123:268-274.
71. Lichtenstein LM, Valentine MD, Sobotka AK. A case for venom treatment in anaphylactic sensitivity to Hymenoptera sting. N Engl J Med 1974 ;290 : 1223-1227.
72. Golden DBK, Valentine MD, Kagey-Sobotka A, Lichtenstein LM. Regimens of Hymenoptera venom immunotherapy. Ann Intern Med 1980 ;92 : 620-624.
73. Ramirez DA, Londono SA, Evans R. Adverse reactions to venom immunotherapy. Ann Allergy 1981; 47:435.
74. Tarhini H, Knani J, Michel FB, Bousquet J. Safety of venom immunotherapy administered by cluster schedule. J Allergy Clin Immunol 1992 ;89 : 1198-1199.
75. Yunginger JW, Paull BR, Jones RT, Santrach PJ. Rush venom immunotherapy program for honeybee sting sensitivity. J Allergy Clin Immunol 1979 ;63 : 340-347.
76. Bousquet J, Fontez A, Aznar R, Robinet-Levy M, Michel FB. Combination of passive and active immunization in honeybee venom immunotherapy. J Allergy Clin Immunol 1987 ;79 : 947-954.
77. Müller U, Morris T, Bischof M, Friedli H, Skarvil F. Combined active and passive immunotherapy in honeybee sting allergy. J Allergy Clin Immunol 1986 ;78 : 115-122.
78. Gillman SA, Cummins LH, Kozak PP, Hoffman DR. Venom immunotherapy: comparison of «rush» vs «conventional» schedules. Ann Allergy 1980 ;45 : 351-354.
79. Golden DB, Valentine MD, Kagey-Sobotka A, Lichtenstein LM. Regimens of Hymenoptera venom immunotherapy. Ann Intern Med 1980;92:620-624.
80. Laurent J, Smiejan JM, Bloch-Morot E, Herman D. Safety of Hymenoptera venom rush immunotherapy. Allergy 1997 ;52 : 94-96.
81. Michils A, Baldassarre S, Ledent C, Mairesse M, Gossart B, Duchateau J. Early effect of ultrarush venom immunotherapy on the IgG antibody response. Allergy 2000; 55: 455-462.
82. Van Der Zwan JC, Flinterman J, Jankowski IG, Kerckhaert JAM. Hyposensitization to wasp venom in six hours. BMJ 1983 ;287 : 1329-1331.
83. Bernstein AJ, Kagen SI, Bernstein DI. Rapid venom immunotherapy is safe routine in the treatment of patients with Hymenoptera anaphylaxis. Ann Allergy 1994 ;73 : 423-442.

84. Birnbaum J, Charpin D, Vervloet D. Rapid Hymenoptera venom immunotherapy : comparative safety of three protocols. Clin Exp Allergy 1993 ;23 : 226-230.
85. Van der Brempt X, Ledent C, Mairesse M. Accelerated desensitization for hymenoptera venom allergy in 30 hours: efficacy and safety in 150 cases. Rev Med Brux 1997; 18: 120-124.
86. Lockey RF, Turkeltaub PC, Olive ES, Hussard JM, Baird-Warren IA, Bukantz SC. The Hymenoptera venom study. III. Safety of venom immunotherapy. J Allergy Clin Immunol 1990 ;86 : 775-780.
87. Mosbech H, Müller U. Side-effects of insect venom immunotherapy : results from an EAACI multicenter study. Allergy 2000 ;55 : 1005-1010.
88. Müller U, Helbling A, Berchtold E. Immunotherapy with honeybee venom and yellow jacket venom is different regarding efficacy and safety. J Allergy Clin Immunol 1992 ;89 : 529-535.
89. Hoffman DR, Jacobson RS. Allergens in Hymenoptera venoms. XII. How much protein is in a sting ? Ann Allergy 1984 ;52 : 276-278.
90. Sanchez F, Blanca M, Miranda A, Carmona M, Garcia J, Fernandez X et al. Comparison of *Vespula Germanica* venoms obtained from different sources. Int Arch Immunol 1994; 104: 385-389.
91. Wood C, Benson E, Timmons E, Hoffman D. Allergens in Hymenoptera venoms. X. Vespid venoms versus venom extracts: comparison by two-dimensional polyacrylamide gel electrophoresis. Ann Allergy 1983; 51: 441 445.
92. Youlten L, Atkinson B, Lee T. The incidence and the nature of adverse reaction to injection immunotherapy in bee and wasp venom allergy. Clin Exp Allergy 1995; 25:159-165.
93. Brehler R, Wolf H, Kutting B, Schnitker J, Luger T. Safety of a two-day ultrarush insect venom immunotherapy protocol in comparison with protocols of longer duration and involving a larger number of injections. J Allergy Clin Immunol 2000; 105: 1231-1235.
94. De Jong NW, Vermeulen AM, De Groot H. Allergy to bumblebee venom. III. Immunotherapy follow-up study (safety and efficacy) in patients with occupational bumblebee-venom anaphylaxis. Allergy 1999; 54: 980-984.
95. De Groot H, De Graaf-in't Veld C, Van Wijk RG. Allergy to bumblebee venom. I. Occupational anaphylaxis to bumblebee venom: diagnosis and treatment. Allergy 1995; 50: 581-584.
96. Reimers RE, Hari Y, Müller U. Reduction of side-effects from ultrarush immunotherapy with honeybee venom by pretreatment with fexofenadine : a double-blind, placebo-controlled trial. Allergy 2000 ;55 : 484-488.
97. Scribner TA, Bernstein DI. Rapid venom immunotherapy update. Curr Opin Allergy Clin Immunol 2003 Aug;3:295-8.
98. Przybilla B, Ring J, Galosi A, Geursen RG, Stickl HA. Bee venom immunoglobulin for prophylaxis of anaphylactic reactions during bee venom immunotherapy (rush hyposensitization). Immunol Allergy Pract 1986 ;8 : 107-111.
99. Jarisch R. Passive immunotherapy in bee venom allergic patients. Arch Dermatol Res 1981 ;270 :230-235.
100. Malling HJ, Djurup R, Sondergaard I, Weeke B. Clustered immunotherapy with yellow jacket venom. Allergy 1985 ;40 : 373-383.
101. Müller U, Rabson A, Bischof M, Lomnitzer R, Dreborg S, Lanner A. A double-blind study comparing monomethoxy polyethylene glycol modified honeybee venom and unmodified honeybee venom for immunotherapy. I. Clinical results. J Allergy Clin Immunol 1987 ;80 : 252-261.
102. Wyss M, Scheitlin T, Stadler BM, Wüthrich B. Immunotherapy with aluminium hydroxide absorbed insectvenom extracts (Alutard SQ): immunologic and clinical results of a prospective study over 3 years. Allergy 1993;48: 81-86.
103. Quercia O, Rafanelli S, Puccinelli P, Stefanini GF. The safety of cluster immunotherapy with aluminiumhydroxide-absorbed honeybee venom extract. J Investig Allergol Clin Immunol 2001; 11: 27-33.
104. Poli F, Longo G, Parmiani S. The safety and efficacy of immunotherapy with aluminium hydroxide-absorbedvenom extract of Vespula spp. An open, retrospective study. Allergol Immunopathol (Madr) 2001; 29:191-196.
105. Müller UR. Recombinant Hymenoptera venom allergens. Allergy 2002; 57: 570-576.
106. Rueff F, Wolf H, Schnitker J, Ring J, Przybilla B. Specific immunotherapy in honey bee venom allergy: a comparative study using aqueous and aluminium adsorbed preparations. Allergy 2004; 59:589-595.
107. Birnbaum J, Ramadour M, Magnan A, Vervloet D. Hymenoptera ultra-rush venom immunotherapy (210 min): a safety study and risk factors. Clin Exp Allergy 2003 ;33 : 58-64.
108. Fricker M, Helbling A, Schwartz L, Müller U. Hymenoptera sting anaphylaxis and urticaria pigmentosa :Clinical findings and results of venom immunotherapy in ten patients. J Allergy Clin Immunol 1997 ;100 : 11-15.
109. Ruëff F, Ludolph-Hauser D, Przybilla B. Erhöhte basale Serumtryptrase als Risikofaktor derInsektengiftallergie. Allergo J 2003;12 (Sonderheft 1): S32-S38.
110. Haeberli G, Bronnimann M, Hunziker T, Müller U. Elevated basal serum tryptase and hymenoptera venom allergy: relation to severity of sting reactions and to safety and efficacy of venom immunotherapy. Clin Exp Allergy. 2003;33:1216-20.

111. Oude Elberink JNG, De Monchy JGR, Kors JW, Van Doormaal JJ, Dubois AEJ. Fatal anaphylaxis after a yellow jacket sting, despite venom immunotherapy, in two patients with mastocytosis. J Allergy Clin Immunol 1997 ;99 :153-154.
112. Rueff F, Wenderoth A, Przybilla B. Patients still reacting to a sting challenge while receiving conventional Hymenoptera venom immunotherapy are protected by increased venom doses. J Allergy Clin Immunol 2001; 108:1027-1032.
113. Golden DBK. Practical considerations in venom immunotherapy. Allergy and Asthma Proc 1997 ;18 : 79-8.
114. Golden D, Kagey-Sobotka A, Valentine M, Lichtenstein L. Dose dependence of Hymenoptera venom immunotherapy. J Allergy Clin Immunol 1981; 67: 370-374.
115. Bousquet J, Menardo JL, Michel FB. Systemic reactions during maintenance immunotherapy in honeybee venom. Ann Allergy 1988;61: 63-68.
116. Golden DBK, Kagey-Sobotka A, Valentine MD, Lichtenstein LM. Prolonged-maintenance interval in Hymenoptera venom immunotherapy. J Allergy Clin Immunol 1981 ;67 : 482-484.
117. Gadde J, Sobotka A, Valentine M, Lichtenstein L, Golden D. Intervals of six and eight weeks in maintenance venom immunotherapy. Ann Allergy 1985 ;54 : 348.
118. Goldberg A, Reisman RE. Prolonged interval maintenance venom immunotherapy.Ann Allergy 1988;61:177 9.
119. Confino-Cohen R, Goldberg A, Mekori YA. Deliberate bee sting challenge of patients receiving maintenance venom immunotherapy at a 3-months interval. J Allergy Clin Immunol 1993 ;91 : 189-194.
120. Goldberg A, Confino-Cohen R, Mekori Y. Deliberate bee sting challenge of patients receiving maintenance venom immunotherapy at 3-months intervals. J Allergy Clin Immunol 1994 ;93 : 997-1001.
121. Kochuyt AM, Stevens EAM. Safety and efficacy of a 12-week maintenance interval in patients treated with Hymenoptera venom immunotherapy. Clin Exp Allergy 1994 ;24 : 35-41.
122. Goldberg A, Confino-Cohen R. Maintenance venom immunotherapy administered at 3-month intervals is both safe and efficacious. J Allergy Clin Immunol 2001; 107: 902-906.
123. Chipps B, Valentine M, Kagey-Sobotka A, Schuberth K, Lichtenstein L. Diagnosis and treatment of anaphylactic reactions to Hymenoptera stings in children. J Allergy Clin Immunol 1980; 97: 177-184.
124. Hoffman D, Gillman S, Cummins L, Kozak P, Oswald A. Correlation of IgG and IgE antibody levels to honey bee venom allergens with protection to sting challenge. Ann Allergy 1981; 46: 17 – 23.
125. Mosbech H, Malling H, Biering I, Böwadt H, Sooborg M, Weeke B, Löwenstein H. Immunotherapy with yellow jacket venom. Allergy 1984; 39: 543-549.
126. Urbanek R, Forster J, Kuhn W, Ziupa J. Discontinuation of bee venom immunotherapy in children and adolescents. J Pediatrics 1985; 107:367-71.
127. Smith P, Kagey-Sobotka A, Bleeker E, Traystman R, Kaplan A, Gralnick H. Physiologic manifestations of human anaphylaxis. J Clin Invest 1980; 66:1072-80.
128. Graft DF, Schubert KC, Kagey-Sobotka A, Kwiterovoch KA, Niv Y, Lichtenstein LM, Valentine MD. Assessment of prolonged venom immunotherapy in children. J Allergy Clin Immunol 1987; 80: 162-169.
129. Valentine MD, Schubert KC, Kagey-Sobotka A, Graft DF, Kwiterovoch KA, Szklo M, Lichtenstein LM. The value of immunotherapy with venom in children with allergy to insect stings. N Engl J Med 1990; 323: 1601 1603.
130. Lerch E, Müller UR. Long-term protection after stopping venom immunotherapy: results of re-stings in 200 patients. J Allergy Clin Immunol. 1998 May;101(5):606-12.
131. Blaauw PJ, Smithuis LOMJ. The evaluation of the common diagnostic methods of hypersensitivity for bee and yellow jacket venom by means of an in-hospital insect sting. J Allergy Clin Immunol 1985; 75:556-62.
132. van der Linden PWG, Hack CE, Struyvenberg A, van der Zwan JK. Insect-sting challenge in 324 subjects with a previous anaphylactic reaction: Current criteria for insect-venom hypersensitivity do not predict the occurrence and the severity of anaphylaxis. J Allergy Clin Immunol 1994; 94:151-9.
133. Rueff F, Przybilla B, Müller U, Mosbech H. The sting challenge test in Hymenoptera venom allergy. Allergy 1996; 51:216-25.
134. Confino-Cohen R, Melamed S, Goldberg A. Debilitating beliefs, emotional distress and quality of life in patients given immunotherapy for insect sting allergy. Clin Exp Allergy 1999; 29:1626-1631.
135. Oude Elberink J, de Monchy J, van der Heide S, Guyatt G, Dubois A. Venom immunotherapy improves health related quality of life in patients allergic to yellow jacket venom. J Allergy Clin Immunol 2002; 110:174 182.
136. Golden DBK, Addison BI, Gadde J, Kagey-Sobotka A, Valentine MD, Lichtenstein LM. Prospective observations on stopping prolonged venom immunotherapy. J Allergy Clin Immunol 1989; 84:162-7.
137. Müller U, Berchtold E, Helbling A. Honeybee venom allergy: Results of a sting challenge 1 year after stopping successful venom immunotherapy in 86 patients. J Allergy Clin Immunol 1991; 87:702-9.
138. Haugaard L, Nörregaard OF, Dahl R. In-hospital sting challenge in insect venom-allergic patients after stopping venom immunotherapy. J Allergy Clin Immunol 1991; 87:699-702.
139. Keating MU, Kagey-Sobotka A, Hamilton RG, Yunginger JW. Clinical and immunologic follow-up of patients who stop venom immunotherapy. J Allergy Clin Immunol 1991; 88:339-48.

140. van Halteren HK, van der Linden PWG, Burgers JA, Bartelink AKM. Discontinuation of yellow jacket venom immunotherapy: Follow-up of 75 patients by means of deliberate sting challenge. J Allergy Clin Immunol 1997; 100:767-70.
141. Reisman RE. Duration of venom immunotherapy: relationship to the severity of symptoms of initial insect sting anaphylaxis. J Allergy Clin Immunol 1993;92:831-6.
142. Golden DBK, Kwiterovich KA, Kagey-Sobotka A, Valentine MD, Lichtenstein LM. Discontinuing venom immunotherapy: Outcome after five years. J Allergy Clin Immunol 1996; 97:579-87.
143. Golden DBK, Kwiterovich KA, Kagey-Sobotka A, Lichtenstein LM. Discontinuing venom immunotherapy: Extended observations. J Allergy Clin Immunol 1998; 101:298-305.
144. Ludolph-Hauser D, Rueff F, Fries C, Schöpf P, Przybilla B. Constitutively raised serum concentration of mastcell tryptase and severe anaphylactic reactions to Hymenoptera stings. Lancet 2001; 357:361-2.
145. Reisman RE, Lantner R. Further observations of stopping venom immunotherapy: Comparison of patients stopped because of a fall in serum venom-specific IgE to insignificant levels with patients stopped by self-choice. J Allergy Clin Immunol 1989; 83:1049-54.
146. Müller U. Recent developments and future strategies for immunotherapy of insect venom allergy. Current Opin Allergy Clin Immunol 2003; 3:299-303.
147. Müller U, Fricker M, Wymann D, Blaser K, Crameri R. Increased specificity of diagnostic tests with recombinant major bee venom allergen phospholipase A_2. Clin Exp Allergy 1997; 27:915-20.
148. Müller U, Soldatova L, Weber M. Bee venom allergy: Comparison of IgE-binding capacity of purified natural and recombinant-synthetic venom allergens. J Allergy Clin Immunol 1998; 101: 33.
149. Valenta R, Lidholm J, Niederberger V, Hajek B, Kraft D, Gronlund H. The recombinant allergen-based concept of component resolved diagnostics and immunotherapy. Clin Exp Allergy 1999; 29: 896-904.
150. Norman PS, Ohman J, Long A, et al. Treatment of cat allergy with T-cell reactive peptides. Am J Respir Crit Care Med 1996; 154:1623-28.
151. Carballido J, Carballido M, Kägi M, Meloen T, Wüthrich B, Heusser C, Blaser K. T celll epitope specificity in human allergic and non allergic subjects to bee venom phospholipase A2. J Immunol 1993; 150: 3582- 91.
152. Jilek S, Barbey C, Spertini F, Corthesy B. Antigen independent suppression of the allergic immune response to bee venom phospholipase A2 by DNA vaccination in CBA/J mice. Immunology 2001;166: 3612-3621.
153. Karamloo F, Kussebi F, Schmid-Grendelmeier P, Blaser K, Crameri R, Akdis C et al. Gene recombination of molecular bee venom allergen fragments as a novel vaccine for allergen specific immunotherapy. Proceedings of the second EAACI Davos Meeting on Basic Allergy and Clinical Immunology. Davos 2003; 60: p 16.