

24 Immunotherapy for Hymenoptera Venom Hypersensitivity

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INTRODUCTION

Insect stings, especially by Hymenoptera of the families *Apidae* (the honeybee and the bumblebee), *Vespidae* (with the species *Vespula*, *Dolichovespula*, *Vespa* and *Polistes*), and in some regions also *Formicidae* (the ants), are one of the major causes for severe, generalized, immunoglobulin E (IgE)-mediated hypersensitivity reactions that may be fatal. According to the registered data of the Swiss Statistical Department, 132 individuals died from Hymenoptera stings in Switzerland between 1962 and 2004, an average of three per year (Fig. 1). Extrapolation of the Swiss data (population 7.5 million) to the European Union (population 485 million), indicates about 200 yearly fatalities from Hymenoptera stings in the European Union. Government statistics in the United States show at least 40 deaths each year from insect stings, although it is likely that many others are not reported.

The first attempts at immunotherapy for Hymenoptera sting allergic patients were made at the end of the 1920s. Insect venom or venom sac vaccines were first used. The high frequency of side effects with these vaccines and the report of the successful treatment of a beekeeper with whole body vaccine of bees led to their worldwide use. The results of immunotherapy with these preparations were favorable in uncontrolled studies (1). It was only in the late sixties and seventies of the last century that venoms were shown to be superior to whole body extracts for diagnosis and controlled studies documented the superiority of venoms over whole body vaccines for immunotherapy of Hymenoptera sting allergic individuals (2,3). Venoms obtained by electrostimulation or by venom sac extraction were commercially introduced in 1979 and have since been used worldwide successfully for immunotherapy of patients allergic to stings by *Apidae* and *Vespidae*. Venom preparations are as yet not commercially available for *Formicidae*. From the section titled "Indications" to the section "Special Aspects of Ant Hypersensitivity," various aspects of immunotherapy for Hymenoptera sting hypersensitivity are dealt with. The last section considers new approaches to venom immunotherapy (VIT).

INDICATIONS

History

The indications for VIT include only two factors: a history of systemic allergic reaction to a sting and positive diagnostic tests (4,5). The history is especially important because diagnostic tests with venoms are positive in 10% to 20% of asymptomatic individuals. There is an absolute need to correlate the history with the test results (6,7).

Systemic reactions to stings consist of any one or more of the signs and symptoms of anaphylaxis or may be limited to cutaneous manifestations, which is more common in children

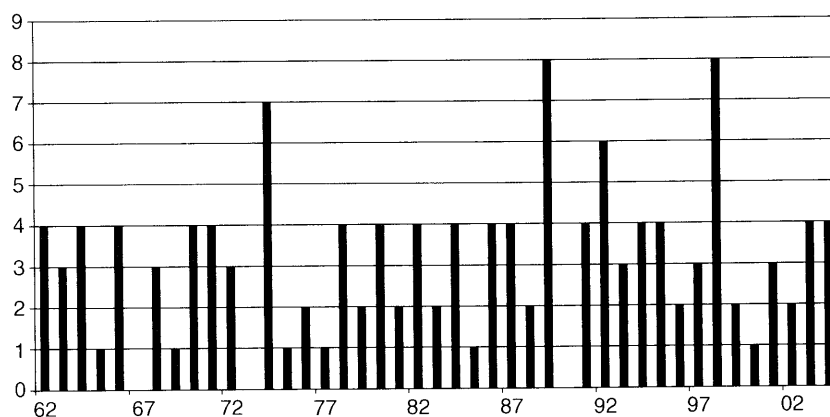


Figure 1 132 fatalities from Hymenoptera sting reactions in Switzerland from 1962 to 2004.

(60%) than in adults (15%) (8,9). Respiratory symptoms occur with equal frequency in about 40% of children and adults. Cardiovascular signs and symptoms are common in adults (30%) but infrequent in children (10%). It is sometimes difficult to be certain whether symptoms are truly anaphylactic because they may result from anxiety, pain, or toxic effects from the stings, especially when many stings occur. It is most helpful when objective signs of anaphylaxis are noted, e.g., generalized urticaria, angioedema, documented hypotension, wheezing, reduced airflow, or oxygen desaturation. The severity of the reaction is one of the most important factors determining the need for VIT, the duration of treatment, and the risk of adverse reactions to injections (10). Although identification of the stinging insect by patients and physicians is unreliable, the identity of the culprit insect is important because honeybee allergy is associated with greater risks and less reliable treatment efficacy (11). In the absence of a history of a sting-induced allergic reaction, sensitization by an asymptomatic sting has been reported to be associated with a 17% chance of a systemic reaction to a future sting, but is not considered an indication for VIT, especially since asymptomatic sensitivity is transient in many cases (6). For this reason, venom allergy testing and treatment is not recommended when requested by some individuals, out of fear alone, such as a family member of someone who had a fatal reaction to a sting.

Contraindications for VIT are the same as for immunotherapy for inhalant allergy, but are relative in nature because of the life-saving potential of therapy. The relative contraindications include severe immunodeficiency, autoimmune and neoplastic diseases, and chronic infections. Treatment with β -blockers may increase the severity of allergic reactions when patients are restung. Elderly patients with cardiovascular disease are at increased risk of developing severe or even fatal sting reactions. Many are on a β -blocker and have a significantly increased survival rate on this treatment. Therefore, VIT on β -blockers may be indicated even in such patients (12).

VIT should not be started during pregnancy, but well-tolerated maintenance VIT may be continued during pregnancy (4). Sting reactions such as Henoch-Schoenlein syndrome, vacuities, acute disseminated encephalomyelitis, or interstitial nephritis should not be treated by VIT, while patients who develop cerebrovascular or myocardial infarction during anaphylaxis and have positive tests are candidates for such therapy (13,14).

Diagnostic Testing: Skin Tests and In Vitro Serum IgE

The decision to begin VIT requires confirmation of allergic sensitivity to venom allergens by positive venom skin tests or detection of venom-specific IgE antibodies in the serum (Table 1).

The standard skin test uses the intradermal test technique with commercially available Hymenoptera venom preparations. For Hymenoptera venom testing, prick tests at 0.1 to 1 $\mu\text{g/mL}$ (6,15) may be used initially for patients with a history of very severe reactions. Aqueous venom preparations are used for intradermal tests beginning with concentrations of 0.001 to 0.01 $\mu\text{g/mL}$ and increasing, if necessary, to 1.0 $\mu\text{g/mL}$ to find the minimum concentration giving a positive reaction. Honeybee venom is somewhat more irritating and can

Table 1 Clinical Recommendations Based on History of Sting Reactions and Results of Venom Skin Test or RAST

Reaction to previous sting	Skin test or RAST	Risk of systemic reaction	Clinical advice
No reaction	Positive	10–15%	Avoidance
Large local	Positive	5–10%	Avoidance
Cutaneous systemic	Positive—Child	10%	No VIT
	Adult	15–20%	VIT
Anaphylaxis	Positive	50–75 ^a %	VIT
	Negative	2–5%	Repeat skin test/RAST

^aAccording to controlled studies.**Table 2** Results of Evaluation of 309 Patients with a Positive History of Systemic Reaction

Skin test positive	68%
ST negative with RAST positive	14%
ST negative and RAST negative with	
sting challenge negative	17%
sting challenge positive	1%

induce weak positive reactions in nonallergic individuals. Yellow jacket (*Vespula*) venom causes false-positive reactions primarily at the 10 µg/mL concentration in 10% of nonallergic subjects (15).

Most patients with a convincing history of insect allergy have positive venom tests but some have negative skin tests (16) (Table 2). Negative skin tests can be due to loss of sensitivity and can also occur in up to 50% during the refractory period of four to six weeks following a sting reaction. They are also caused by antihistamines, certain neuroleptic, and antidepressant drugs. When there is a history of severe anaphylaxis and venom skin tests are negative, in vitro tests for venom-specific IgE antibodies should be obtained, and the patient should continue avoidance precautions. In many cases like this, the in vitro test may be positive. Some cases of apparent sting anaphylaxis are thought to be non-IgE mediated, which would account for the negative in vivo and in vitro test results.

The detection in vitro of allergen-specific IgE antibodies is useful. A high level of venom-specific IgE is usually diagnostic but must be correlated with the history. A low level of venom-IgE is more difficult to interpret. Even a very low level of venom-IgE can be associated with near-fatal anaphylaxis. The venom skin test and in vitro test results may not correlate as the in vitro test results are negative in approximately 20% of skin test positive subjects (16–19). Skin tests are preferred clinically for their greater sensitivity. The converse is also true: approximately 10% of skin test negative patients have a positive in vitro test. Therefore, European allergists recommend skin testing and estimation of venom-specific IgE in all individuals with a history of systemic allergic reactions. If both tests are negative in the presence of a strong history, cellular tests like CAST (cellular antigen stimulation test) or BAT (basophil activation test), although more expensive, may be helpful (20,21). A positive result with these tests may also point to a non-IgE mediated mast cell activation mechanism.

Neither the degree of skin test sensitivity nor the titer of specific IgE correlate reliably with the degree of clinical sting reaction. Patients who have had only large local reactions may have very high levels of sensitivity of both skin and in vitro tests but have a very low risk of anaphylaxis, whereas some patients who have had an abrupt and near-fatal anaphylactic reaction have only weak skin test or serologic positivity. In fact, almost 25% of patients presenting for evaluation of systemic allergic reactions to stings are skin test positive only at the 1.0 µg/mL concentration, demonstrating the importance of testing with the full diagnostic range of venoms. Again, these points emphasize the importance of the history in making the correct diagnosis, assessing prognosis, and instituting appropriate treatment.

Another diagnostic option is a supervised live sting challenge. The history and test results help identify patients at high risk, but even patients with previous severe reactions only

have a 50% to 75% chance of reacting to a future sting. The sting challenge helps select those patients who will most likely have another systemic reaction to a field sting (22). However, even a negative sting challenge does not rule out future reactions, because 20% of patients who did not react to one challenge sting do react to a repeat challenge sting (23). Others have considered the diagnostic sting challenge to be unethical and recommend it only as a test to evaluate the efficacy of immunotherapy (24).

Some patients with positive venom skin tests were at low risk of anaphylaxis because their systemic reactions were mild. Subsequent stings usually cause no systemic reaction or a reaction that tends to be equal or less severe than previous reactions. However, there are patients who have reactions of increasing severity to subsequent stings. As currently practiced in North America, adults with sting-induced generalized urticaria and angioedema, as well as patients of all ages with more severe reactions, including pharyngeal symptoms, dyspnea, dizziness, or hypotension are advised to undergo VIT. The majority of children (≤ 15 years) have reactions confined to the skin (generalized erythema, itching, and urticaria and angioedema) but no involvement of the pharynx or respiratory or circulatory systems. In 15 to 20 year follow-up studies, stings caused no systemic reactions in 87%; 7% had another mild cutaneous and 6% a moderate systemic reaction, but none a severe systemic reaction (8,9). Therefore VIT is not generally recommended in this situation. However, it is sometimes given to highly exposed children with repeated reactions to improve their quality of life. The same recommendation for VIT is also used in Europe in adults with reactions limited to the skin because prospective studies indicate a low risk (15–20%) to develop a generalized reaction at reexposure (4).

Some patients with positive venom tests are at a relatively low risk of anaphylaxis because they never had anaphylaxis to a previous sting. Those children and adults with a large local, but no systemic reaction, seem to have a 4% to 10% chance of a subsequent systemic reaction (8,25). Therapy is not usually recommended for test positive patients with a history of a large local reaction, but may be helpful in heavily exposed individuals like farmers or gardeners. There is limited evidence that VIT prevents these reactions (26).

Patients with systemic mastocytosis (27,28) are at a high risk of developing severe or even fatal cardiovascular sting reactions. Two studies (29,30) indicate that severe cardiovascular reactions are associated with an elevated baseline serum tryptase (>11.4 ng/mL) in up to 25% of individuals, indicating an increased whole body mast cell load as a risk factor for life-threatening sting reactions. A routine baseline serum tryptase is, therefore, recommended in all patients with systemic sting reactions.

Selection of Venoms

The selection of venom vaccines for immunotherapy is dependent on the venom skin test reaction and presence of serum specific IgE antibodies. North American allergists/immunologists recommend that all venoms resulting in positive tests be included for VIT because preventing future sting reactions is not possible without specific therapy. Some investigators recommend treatment only with the venom of the suspected insect culprit (31). When vespids are involved, the most common practice is to treat with *Vespula* venom alone or, in North America, with the mixed vespid venom preparation. It contains equal parts of yellow jacket (*Vespula* spp), yellow hornet (*Dolichovespula arenaria*) and white-faced hornet (*Dolichovespula maculata*) venoms (32), which are not available in Europe. Although *Dolichovespula* are by no means rare, they are responsible for only a small percentage of vespid stings. These insects do not forage on human food and therefore, almost exclusively sting in the proximity of their nests. The same is true for the European hornet, *Vespa crabro*. Moreover, European *Dolichovespula*, in contrast to American *D. maculata*, can be distinguished from *Vespula* only by those trained to do so, with a magnifying glass. Finally, in vitro studies demonstrate ample crossreactivity among venoms of *Vespula*, *Dolichovespula*, and *Vespa*. Therefore, vespid allergic patients in Europe are treated by *Vespula* venom alone, which is effective in more than 95% (1,4) of treated patients.

The skin test is also positive to wasp (*Polistes*) venoms in at least 50% of vespid allergic patients. When positive, it is usually included in therapy as a separate injection, at least in areas where *Polistes* is important, as in the Gulf states of the United States and the Mediterranean countries in Europe. Therapy with *Vespula* or mixed vespid venoms protects against *Polistes* stings, but this has only been established for patients whose *Polistes*-specific

IgE antibodies completely cross-react with *Vespula* venom as assessed by radioallergosorbent test (RAST) inhibition (32).

Dual positivity of diagnostic tests with *Vespula* and honeybee venom in up to 50% of patients is observed, especially in countries where bee venom allergy is more frequent than vespid venom allergy (1). The history sometimes helps to identify the culprit insect, since vespids do not usually sting in spring and do not, in contrast to the honeybee, usually leave the stinger in the skin. However, in the United States, *V. maculifrons* leaves the stinger in 30% to 50% of cases. The limited cross-reactivity on peptide basis between protein allergens of *Vespula* and honeybee venoms is largely confined to hyaluronidase. Double positivity is often due to so-called CCDs (crossreacting carbohydrate determinants), which are probably of no clinical relevance. When tests are definitely positive with the two venoms, both should be included for VIT, unless complete cross-reactivity can be demonstrated by RAST inhibition with whole venoms and CCDs, like horseradish peroxidase or bromelain (33,34).

EFFICACY, SAFETY, AND MONITORING OF VIT

The recommended maintenance dose is 100 µg of venom, both in children and adults. This dose was originally suggested because it was believed to be equivalent to two stings. This is true of honeybee, but may be closer to 10 *Vespula* stings. Doses lower than 100 µg are not reliably effective in adults (35). VIT with honeybee venom gives full protection in 80% to 90% of cases, whereas therapy with *Vespula* venoms is 95% to 98% effective (11,24). When treatment with 100 µg is not fully effective, patients may be protected by using a higher dose (36).

VIT is safer than originally thought. Systemic reactions were expected to be more frequent or severe because of the underlying anaphylactic syndrome, but that has not been confirmed. The incidence of adverse reactions to venom is similar to that reported for inhalant allergen immunotherapy (37). For unexplained reasons, systemic allergic reactions are considerably more frequent during immunotherapy with honeybee venom (11). Systemic symptoms occur in 5% to 15% of patients on vespid venoms and 20% to 40% on honeybee venom, most often during the first weeks of treatment, regardless of the regimen used. Most reactions are mild. In the unusual case of recurrent systemic reactions to injections, therapy may be streamlined to a single venom and given in divided doses, 30 minutes apart. Large local reactions, which may be larger (8–10 cm) than are generally accepted during inhalant allergen immunotherapy, occur in up to 50% of patients, especially during up dosing in the dose ranges of 20 to 50 µg. Large local reactions, however, are not predictive of systemic reactions to subsequent injections and do not prevent attaining the maintenance target dose of VIT.

Annual visits with the allergist serve to review the treatment plan and to assure that the patient does not have a new medication or medical condition which influences therapy. There is no need for annual skin tests or blood tests, although repeating the skin tests every two to three years is recommended to identify patients who possibly can stop treatment because their skin tests become negative. The venom-specific IgE level and skin test sensitivity usually increase in the first months of therapy, return to baseline after 12 months, and then decline steadily during maintenance treatment. This decline continues even after therapy is stopped or after a sting (38) (Fig. 2). Even after three to five years of treatment, these tests turn negative only in a minority of patients. Less than 20% of patients are skin test negative after five years, but 50% to 60% become negative after 7 to 10 years (39). Specific IgE may decrease more rapidly than skin sensitivity but also may persist at very low levels even when venom skin tests become negative (40).

Venom-specific IgG antibodies, especially IgG4, are high in beekeepers, and passive immunotherapy with beekeeper gamma globulin protects bee venom-allergic individuals (41). Assays for venom-specific IgG correlate with clinical protection but cannot accurately predict the outcome of stings in every individual. The test may be used to confirm protective levels after initiating therapy and then to verify that the venom-IgG level is adequately maintained at the longer intervals used for maintenance treatment. In one study, the IgG level was considered protective with serum concentrations of >3 µg/mL during the first four years of maintenance therapy, but protection was independent of the IgG after four years of treatment, probably because of other mechanisms of action (42). Profound changes in the T-cell reactivity to allergen stimulation of venom allergic patients with a shift from a TH2- to a TH1- or

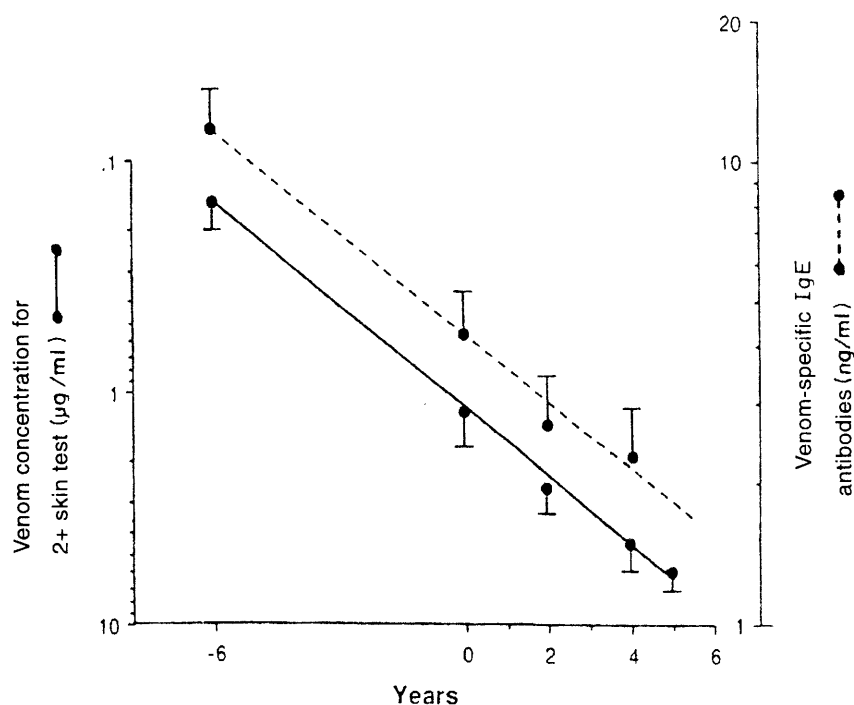


Figure 2 Mean venom skin test sensitivity (concentration in $\mu\text{g/mL}$ for 2+ reaction) and venom-specific IgE antibody level (in ng/mL) shown before venom immunotherapy (time, -6 years), after a mean of six years of treatment (time 0), and 2, 4, and 5 years after stopping therapy. Source: From Ref. 38.

TH0- pattern and induction of IL-10-producing T regulatory cells are described during VIT (43). Data on the relation of these alterations to the efficacy of the treatment, as indicated by a tolerated sting, are not available.

IMMUNOTHERAPY PROTOCOLS

The starting dose of VIT is between 0.001 and 0.1 μg . The recommended maintenance dose is 100 μg of venom protein, corresponding to one to two bee stings and probably many more *Vespula* stings (4,5,44). Higher maintenance doses (200 μg or more) are recommended for beekeepers (45), who may be stung by several insects at the same time and in treatment failures or incomplete treatment success (4,36). The success rate increases with higher maintenance doses.

A number of protocols are used for the buildup phase, some of which are summarized in Table 3 (conventional, cluster, rush, or ultrarush protocols) (4,46). Many allergists in Europe use aluminium hydroxide-adsorbed venom for conventional protocols, while others use aqueous preparations for the buildup with accelerated protocols and then change to aluminium hydroxide-adsorbed venoms, which have a comparable efficacy to aqueous venoms. They are usually somewhat better tolerated for maintenance immunotherapy (47). In the United States, only aqueous preparations are available. Rush and ultrarush protocols have the advantage of inducing a more rapid protection and are therefore recommended in highly exposed individuals during the Hymenoptera flying season. Moreover, the number of visits during the buildup phase is greatly reduced. However, the incidence of side effects is somewhat higher in these rapid buildup protocols, especially in bee venom allergic individuals (11), and in rush protocols with high cumulative daily doses (46).

Once the maintenance dose is reached, the interval between injections is extended to four weeks in the first year and to six weeks from the second year of immunotherapy, provided that

Table 3 Treatment Protocols for VIT (after 4)

Protocol	Dose in µg venom					
	Day	Hour	Ultrarush	Rush	Cluster	Conventional
	1	0	0.1	0.01	0.001	0.01
		0.5	1	0.1	0.01	0.1
		1	10	1	0.1	
		1.5	20			
		2.5	30	2		
	2		40			
		0		4		
		1		8		
				10		
				20		
	3	0		40		
		1		60		
		2		80		
	4	0		100		
		8		100		
	8	0			1	1
		1			5	2
					10	
	15	0	50	100	20	4
		1	50		30	8
	22	0	100		50	10
		1			50	20
	29			100	100	40
					100	60
	36					80
			100	100		100
	43					100
						100
	50					100
						100
	57					100
						100
	64				100	
						100
	71		100	100		100
						100
	78					100
						100
	85					100
						100
	92				100	
						100
	99		100	100		
						100
	106				100	
						100

Further injections of the maintenance dose of 100 mg every four weeks during the first year, every six weeks during further years of VIT. Abbreviation: VIT, venom immunotherapy

the treatment is tolerated. An even longer interval is not recommended for honeybee allergic patients since beekeepers with less than 10 stings a year, are those who develop systemic allergic reactions most frequently (45). While the buildup phase of VIT should usually be performed by an allergist, injections can be continued by a general practitioner once the maintenance dose is achieved. Some allergists premedicate with antihistamines during the buildup phase because several controlled studies demonstrate reduced side effects (48).

DURATION OF VIT

After introduction in 1979, VIT was—and by some still is today—continued for life or at least until both skin tests and serum venom-specific IgE become negative. However, even after prolonged VIT, only a small proportion of patients develop negative diagnostic tests and compliance with continuation of VIT for many years often decreases (1,4).

For this reason, a number of studies address the protection rate after stopping VIT. In a first series, the reaction to a sting challenge (CH) one to three years after stopping VIT of at least three years duration was analyzed (Table 4) (49–54). All studies report continued

Table 4 Prospective Studies with Sting Provocation Test After Stopping VIT

Author	No. of patients	Insect	Sting challenge (CH)	
			After years	No. with GR (%)
Urbanek (49)	29	Honeybee	1	1 (3)
	14		2	2 (14)
Golden (50)	29	m <i>Vespula</i>	1	0
Müller (51)	86	Honeybee	1	15 (17)
Haugaard (52)	25	<i>Vespula</i>	2	0
Keating (53)	51	m <i>Vespula</i>	1	2 (4)
Van Halteren (54)	75	<i>Vespula</i>	1-3	6 (8)

Abbreviations: CH, sting challenge; GR, generalized allergic reaction; m *Vespula*, mostly *Vespula*.

Table 5 Long-Term Protection After Discontinuation of VIT

Author	No. of patients	Insect	Observation years after stop	Re-exposure	No. with GR (%)
Reisman (55)	113	mV	1->5	FS	10 (9)
Golden (38)	74	mV	5	CH	7 (9.5)
Golden (40)	26	mV	3-7	FS	5 (19)
Lerch (44)	120	B	3-7	FS/CH	19 (15.8)
	80	V	3-7	FS/CH	6 (7.5)

Abbreviations: GR, generalized allergic reaction; mV, mostly *Vespula*; B, honey bee; FS, field sting; CH, sting challenge; no, number.

protection in the vast majority (83-100%) of patients. Results are somewhat more favorable in *Vespula* than in bee venom-allergic individuals and in children rather than adults.

Long-term protection for up to seven years after discontinuing VIT (Table 5) is analyzed in four studies (38-40,55,56). Reisman (55) observed relapses following a field sting up to more than five years after stopping VIT in 10 of 113 (9%) mostly *Vespula* venom allergic patients. Golden (38) followed 74 predominantly *Vespula* venom allergic patients for five years after stopping VIT of at least five years duration with a challenge every year (29 patients), every second year (25 patients) or only after two years (20 patients). Seven (9.5%) developed at least one generalized mild allergic reaction (GR). The same group (39) observed a GR to a field sting in 5 of 26 (19%) patients out of 125 who were followed up to seven years after VIT, and some of these reactions were severe. Lerch (56) reported on 358 patients up to seven years after stopping successful VIT. Two-hundred patients were reexposed by either a field sting or a challenge and 25 (12.5%) developed a general reaction: In 14 this was only cutaneous, in eight, there were also respiratory, in three cardiovascular symptoms. In 8 of the 11 patients with respiratory or cardiovascular symptoms, these occurred only after the second sting after stopping VIT. These studies of a prolonged observation after stopping VIT found relapses somewhat more frequently than the earlier shorter follow-up studies. Still, the great majority, 80% or more, remained protected when re-stung up to seven years after VIT was discontinued.

A number of risk factors for the recurrence of GR following Hymenoptera stings can be identified.

Age: Children generally have a more favorable prognosis than adults (8,9), both without VIT and also after discontinuing VIT. Urbanek (49) saw relapses in only 3% of BV allergic children, whereas Müller (51) observed 17% in 86 mostly adult patients after BV-immunotherapy. Lerch (56) recorded 8.3% relapses in 24 children as compared to 13.1% in 176 adults who were reexposed up to seven years after stopping VIT.

Insect: Analysis of the results presented in Table 5 as well as the recurrence rates after VIT reported by Lerch (56) of 7.5% for *Vespula* venom and 15.8% for bee venom treated patients indicate a higher risk of relapse in bee venom than in *Vespula* venom allergic patients. The reason for this difference is not entirely clear but has been discussed elsewhere (4,11,24).

H)
with GR (%)

1 (3)
2 (14)
0
15 (17)
0
2 (4)
6 (8)

with GR (%)

0 (9)
7 (9.5)
5 (19)
9 (15.8)
6 (7.5)

; CH, sting

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Severity of pretreatment reaction: In four prospective studies involving 386 patients, relapses were observed in 5 (4.1%) of 123 with mild but 38 (14.5%) of 263 with severe pretreatment GR (53,55-57) ($X^2 = 9.128$, $p < 0.01$). In addition there is also a higher risk that a recurring reaction after stopping VIT in these patients will be more severe than in those with milder pretreatment reactions.

Safety and efficacy of VIT: Patients who develop generalized allergic side effects to VIT injections have a relapse risk of 38%, whereas those who do not, have a relapse risk of 7% (51). Similarly, incomplete protection with a re-sting during VIT is associated with an increased risk of relapse (38).

Duration of VIT: The risk of a relapse seems to be reduced with more prolonged VIT. 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, developed GRs when restung after discontinuation ($X^2 = 7.382$; $p < 0.01$) (56).

Elevated basal serum tryptase, mastocytosis: Insect venom allergy in patients with urticaria pigmentosa is most often associated with severe shock reactions (27). Two female patients with urticaria pigmentosa and *Vespula* venom sensitivity died from a re-sting 1.3 and 9 years after stopping VIT (28). One further fatal sting reactions was observed in a patient with systemic mastocytosis: The patient had been successfully treated by VIT for severe anaphylaxis following bee stings, but died when he was stung by a yellow jacket (58). Up to one quarter of patients with severe shock reactions following Hymenoptera stings have an elevated baseline serum tryptase level indicating the presence of an increased whole body mast cell load (29,30). Patients with mastocytosis and/or elevated baseline serum tryptase are at an increased risk to develop very severe or even fatal sting reactions. Since VIT is effective in such patients (27), it should be continued for life.

Repeated reexposure after stopping VIT: About half of the relapses occur after the first, the other half after later re-stings (56). The risk of a severe reaction increases significantly in the presence of repeated re-stings. Golden et al. (40) also described an increasing frequency of generalized reactions four years after stopping VIT compared with the first one to two years. Such was also the case in patients who developed systemic reactions after 7 to 13 years off VIT despite a nonreaction to a previous sting during the first few years after discontinuing VIT. On the basis of these observations, sting challenge after stopping VIT is not recommended.

High sensitivity according to diagnostic tests: An association of re-sting reactions has been observed with a persisting high sensitivity with intradermal skin testing (40). Others (51,56) are unable to confirm this. Specific serum IgE and IgG antibodies, per se, have no predictive value with regard to the re-sting risk after stopping therapy. Diagnostic tests currently utilized are of limited predictive value with regard to long-term protection after VIT. Only the combination of a negative intracutaneous skin test at 1 $\mu\text{g/mL}$ and the absence of venom specific serum IgE-antibodies is associated with a strongly diminished risk of relapse (51,56). Gender and a history of atopic disease do not seem to influence the risk of a relapse after stopping VIT.

Most patients with Hymenoptera venom sensitivity remain protected, following discontinuation of VIT of at least three to five years duration. An even longer treatment duration has to be considered in the high-risk situations discussed above. Because of the small but relevant risk of re-sting reactions, epinephrine for self-administration should be considered for patients stopping VIT.

SPECIAL ASPECTS OF ANT HYPERSENSITIVITY

Classification

There are nearly 10,000 species of ants (order Hymenoptera, family *Formicidae*) recognized worldwide. Some of these sting victims, as do other Hymenoptera, and human reactions span the spectrum from a self-limited local reaction to life-threatening anaphylaxis. In the United States, only members of the genera *Solenopsis* (S.) (Fig. 3), the imported fire ant (IFA), and



Figure 3 Imported fire ant (*Solenopsis invicta*).

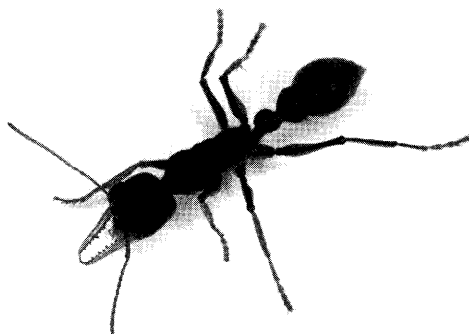


Figure 4 Jack jumper ant (*Myrmecia pilosula*).

Pogonomyrmex, the harvester ant, induce such reactions (59). In Australia, 88 species of the genus *Myrmecia* (bull ants) are of clinical relevance (60). The most well known, the jack jumper ant (*Myrmecia pilosula*) (Fig. 4), is responsible for one quarter of all anaphylaxis treated in some Australian areas. Different species of stinging ants that cause anaphylaxis have also been found in other parts of the world. Details of the taxonomy of stinging ants and their worldwide significance in Hymenoptera venom allergy are outlined in chap. 14.

Reactions

The IFA attaches to the skin by means of a powerful mandible and stings, releasing venom that produces a characteristic "fire-like" pain. If not removed, the IFA will continue to rotate in a pivotal fashion, repeatedly injecting small amounts of venom provoking a sharp pain. An initial local reaction begins as a 25- to 50-mm erythematous flare. This is followed a few minutes later by a larger wheal and, within the next 24 hours, an umbilicated pustule forms that usually remains for 3 to 10 days later rupturing and leaving a residual macule, nodule, or scar.

Stings of the IFA commonly produce large local reactions that are similar to those induced by stings of the other flying Hymenoptera. Following an initial wheal-and-flare, a large local reaction may develop several hours later. This includes erythema and edema that extends more than 10 cm from the initial sting site. This reaction is thought to occur in up to 30% to 50% of IFA stings.

Systemic allergic reactions can manifest all the symptoms of anaphylaxis including generalized erythema, urticaria, angioedema, nausea, vomiting, diarrhea, laryngeal edema, asthma, as well as shock and death. Anaphylaxis to the IFA sting is thought to occur in up to 1% of stings (61). In 1989, a survey of 29,300 physicians reported a total of 32 deaths thought to be secondary to anaphylaxis induced by ant stings (62). Although the species of ant was not identified in most cases, *Solenopsis* and *Pogonomyrmex* species were implicated in these deaths. Postmortem case reports of deaths following IFA stings describe findings of acute pulmonary changes and cerebral vascular congestion compatible with shock due to anaphylaxis. Neurological sequelae due to the IFA are rare but include mononeuropathy, focal motor, and grand mal epileptic seizures (63,64).

Allergens of the IFA

The venom of the IFA is unique when compared with other Hymenoptera. The venom of the IFA, unlike other Hymenoptera venoms, has an extremely low protein content in the aqueous fraction, less than 0.1%, with a prominence of toxic alkaloids. The aqueous component contains the allergenic proteins. Alkaloids comprise 95% and the aqueous 5% of the venom. The alkaloids are responsible for the hemolytic, bactericidal and cytotoxic properties that result in formation of a sterile pustule. This alkaloid portion, however, is nonallergenic (65). The venom of the harvester ant, *Pogonomyrmex*, more closely resembles that of the flying Hymenoptera and consists of 73% protein. *Solenopsis* and *Pogonomyrmex* proteins do not cross-react (65). IFA whole body extract (WBE), unlike the WBE of other members of Hymenoptera, contains the clinically important allergens responsible for hypersensitivity (65). Both IFA WBE and venom produce positive skin tests in sensitized individuals. Skin testing with IFA venom is more sensitive and specific than IFA WBE. The venom is also thought to be 10 times more potent and better tolerated for skin testing, and RAST testing with IFA venom more sensitive than WBE. IFA venom, however, is not commercially produced currently leaving IFA WBE as the only available option for testing and immunotherapy. Similarly, WBE and not venoms are available for *Pogonomyrmex* species.

Diagnosis

There is a high rate of false-positive results when skin testing is performed on patients in endemic regions. Therefore, only patients who have experienced a systemic allergic reaction following an ant sting should undergo skin testing with IFA WBE. Skin testing should be done at least thirty days after the systemic reaction. A prick-puncture test with IFA WBE is performed first, and if there is no response, it is followed by serial intradermal testing beginning with a 1:1,000,000 weight/volume (*w/v*) dilution. A great majority of patients who are sensitive react before reaching a 1:500 *w/v* dilution (66). In vitro tests for IFA IgE should be obtained in history positive skin test negative patients.

Immunotherapy

With other Hymenoptera (honeybee, wasp, hornet and yellow jacket), children, who have had only a cutaneous systemic reaction (generalized erythema, urticaria, and/or pruritus), are not candidates for immunotherapy (8,9). However, with IFA, there are no data to indicate that children, who have had only a cutaneous reaction to an IFA sting, will not respond to subsequent stings with a more serious systemic reaction. Therefore, patients of all age groups, with positive skin or in vitro tests, should receive IFA immunotherapy, regardless of the severity of their systemic reaction to IFA.

IFA immunotherapy is begun with 0.05 mL of the highest dilution of WBE that produces a positive skin test (usually 1:10,000 or 1:100,000 *w/v*). The dose is increased with each injection, either weekly or biweekly. Once a maximum tolerated dose or 0.5 mL of a 1:10 *w/v* is achieved, the interval between injections is extended to every four to six weeks.

A two-day rush protocol for IFA immunotherapy has been studied in a small population of patients and shown to be safe and efficacious (67). Of 58 patients, only three patients (5.2%) experienced a mild systemic reaction during the two-day rush protocol, a final dose of 0.3 mL of 1:100 *w/v*. When 56 patients (total of 112 stings) had sting challenges on day 22, all (98.2%) tolerated the sting except one. He complained of lightheadness, which resolved without intervention. Prophylactic pretreatment with H1 and H2 antihistamines and glucocorticosteroids did not reduce the systemic reaction rate associated with rush immunotherapy, which was underpowered with only three reactions (67).

IFA immunotherapy can be discontinued when the individual becomes negative on repeat skin testing. Otherwise, the decision to discontinue such therapy after five years is determined by the physician in consultation with the patient, since no data exist when IFA immunotherapy can be discontinued when skin tests remain positive.

Only whole ant body extracts are used for ant immunotherapy. However, a double-blind placebo-controlled crossover study on VIT in patients allergic to the jack jumper ant, *Myrmecia pilosula* was reported from Tasmania, Australia (68). Of 29 patients on placebo, 21 (72%) developed a systemic allergic skin reaction, while only one of the 35 (3%) on ant venom developed a mild urticarial reaction when purposely stung. When the placebo group switched to VIT, all 26 patients except one (mild urticaria) tolerated a sting challenge. Ant venom

immunotherapy to the jack jumper ant, *Myrmecia pilosula*, could offer some benefit to prevent life-threatening sting anaphylaxis in southeastern Australia. Such impressive evidence of the efficacy of VIT to the jack jumper ant, *Myrmecia pilosula*, supports more research investment in this area. Currently, ant venom products are not commercially available for immunotherapy.

NEW APPROACHES TO IMMUNOTHERAPY

Insect venom allergy is often considered as a model to study IgE-mediated reactions. Diagnostic tests (skin tests and RAST) are reliable and specific VIT is safe and effective. However, the specificity of skin tests with insect venom extracts and tests for venom specific serum IgE antibodies are far from perfect. Up to 20% of individuals with no history of systemic sting reactions have positive tests and only 30% to 75% (2,3,24,68) of those with positive tests and a history of a systemic sting reaction, react to a subsequent sting by the incriminated insect. In addition, following a sting challenge about 95% of patients allergic to vespid stings are completely protected as compared to only 80% to 90% (11,24) of those allergic to honeybee venom to a sting provocation test during VIT. Systemic allergic side effects to honeybee-venom immunotherapy injections may occur in 20 to 40% of patients and 5% to 10% to vespid venom immunotherapy. Thus, there is room for considerable improvement to diagnose and treat Hymenoptera allergy.

RECOMBINANT VENOM ALLERGENS

A number of honeybee, vespid, and ant allergens in recombinant form are available today (Table 6) (69,70). The IgE binding capacity of these recombinant allergens correlates closely with that of their respective natural purified preparations. Some disparities, however, revealed by RAST-inhibition and western blot studies indicate that all natural preparations are contaminated with trace amounts of other venom allergens. Recombinant allergens, therefore, should be superior to highly purified natural preparations when the true clinical relevance of the individual allergen is determined.

The use of recombinant cocktails is also promising for diagnosis. Venom-specific IgE antibodies were measured in 85 bee venom allergic patients with positive and 20 nonallergic controls with negative skin tests. None of the negative controls reacted to a recombinant cocktail containing phospholipase A2, hyaluronidase, and melittin as compared to 15% who reacted to the whole bee venom, indicating superior specificity of the recombinant cocktail. On the other hand, 87% of the patients were positive with the recombinant cocktail versus 95% with the whole bee venom (69). The somewhat lower sensitivity of the cocktail could probably be improved by the addition of further relevant bee venom allergens such as acid phosphatase and protease in the recombinant forms. The sensitization pattern of an individual patient can be determined once all relevant allergens of a venom are available in recombinant form. A

Table 6 Recombinant Hymenoptera Venom Allergens [76–77]

Species	Allergen	MW kDa
<i>Apis mellifera</i>	Api m 1 phospholipase A2	16–20
	Api m 2 hyaluronidase	43
	Api m 3 acid phosphatase	49
<i>Vespula vulgaris</i>	Ves v 1 phospholipase A1	35
	Ves v 2 hyaluronidase	45
	Ves v 5 antigen 5	25
<i>Dolichovespula maculata</i>	Dol m 1 phospholipase A1	35
	Dol m 2 hyaluronidase	45
	Dol m 5 antigen 5	25
<i>Polistes annularis</i>	Pol a 5 antigen 5	25
<i>Solenopsis invicta</i>	Sol i 2	30
	Sol i 3 antigen 5	25

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patient-tailored cocktail containing all the allergens to which the patient has IgE antibodies could then be prepared for immunotherapy. The mostly conformational B-cell epitopes have been shown to be reduced in unrefolded or point-mutated recombinant allergens. Cocktails of such preparations will have a strongly reduced reactivity to IgE antibodies fixed on effector cells, therefore, will induce much less mediator release and will be theoretically safer. Their capacity to interact with T cells, thus inducing protective immunologic effects, will be preserved. So far no clinical trials on immunotherapy with recombinant venom allergens have been performed.

In another study, a recombinant chimeric protein consisting of overlapping fragments of the bee venom allergens, phospholipase A2, hyaluronidase, and melittin, was engineered and shown to have strongly reduced allergenicity but preserved T-cell epitopes (71).

T-Cell Epitope Peptides

Peptide immunotherapy has been studied experimentally both in cat and bee venom allergy (72). Its goal is to provide effective immunotherapy without sideeffects, by destroying IgE reactive conformational B-cell epitopes, but preserving the linear T-cell epitopes. Peptides can be prepared synthetically or expressed as recombinant fragments. In one study, three short linear peptides of 11–18 amino acids of phospholipase A2 (the major bee venom allergen) were identified that were unable to bind to the respective specific IgE-antibodies in sera from bee venom allergic patients. However, they induced strong proliferation of their T lymphocytes in vitro. Immunotherapy with an equimolar mixture of these major synthesized T-cell epitope peptides was performed in five bee venom allergic patients in a preliminary trial. No side effects were observed. A honeybee sting challenge after 10 weeks of peptide immunotherapy resulted in complete protection of three and partial protection of two patients (73). In vitro studies on lymphocyte cultures of the patients suggested the induction of phospholipase A2 specific tolerance by this form of peptide immunotherapy.

In another study longer synthetic peptides consisting of 45 to 60 residues and covering the whole molecule of phospholipase A2 were used in a phase I clinical trial and shown to induce an increase in IL-10, IFN γ and allergen specific IgG4, but caused some mild adverse reactions (74). In patients treated with a mixture of 4 to 18 residues peptides with high affinity to commonly expressed MHC (major histocompatibility complex) class II molecules, no side effects and significantly reduced late phase reactions were observed (72).

DNA-Vaccination

This technique consists of the injection of DNA-plasmids that encode the allergen and induce a TH1 response. Successful DNA-vaccination of phospholipase A2 sensitized mice with the corresponding DNA-sequence has been reported (75). Protection against anaphylaxis was complete when vaccination was done before sensitization but only 65% of the mice survived when it was performed after intraperitoneal sensitization.

SALIENT POINTS

- VIT is highly effective for Hymenoptera venom allergy.
- Indication for VIT is based on a history of systemic allergic reactions to Hymenoptera stings and positive diagnostic tests.
- Rush and ultrarush protocols for immunotherapy provide more rapid protection than conventional protocols but may be associated with more side effects.
- Most patients remain protected for many years after stopping VIT of three to five years.
- Immunotherapy with whole body vaccines of the fire ant (*Solenopsis invicta*) and probably other ants appears to be effective, in contrast to other Hymenoptera, where only venoms induce protection.
- The most promising new approaches to VIT include treatment with modified recombinant allergens, T-cell epitope peptides and DNA vaccination.

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