

New Developments in the Diagnosis and Treatment of Hymenoptera Venom Allergy

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Key Words

Antihistamines · Honeybee · Hymenoptera venom allergy · Mastocytosis · Recombinant venom allergens · Venom immunotherapy · Vespula

Abstract

According to most textbooks, diagnostic tests with Hymenoptera venoms are reliable, and immunotherapy with these venoms in Hymenoptera-venom-allergic patients leads in near to 100% to full protection. Careful analysis of the literature shows however that the specificity of diagnostic tests is far from perfect and that both efficacy and tolerance, especially in patients receiving honeybee venom immunotherapy, are still suboptimal. The major allergens of honeybee and vespine venoms are now available in recombinant form. Preliminary trials analyzing diagnostic tests with recombinant allergens in honeybee venom allergy are promising: the specificity is clearly increased in both skin testing and in determining venom-specific IgE antibodies when compared to natural venom allergens. An important recent finding is the frequent association of severe Hymenoptera venom allergy and elevated basal serum levels of the mast-cell-specific enzyme tryptase. Elevated levels are found in up to 30% of the patients with a history of severe shock reactions following Hymenoptera stings. The current find-

ings indicate that basal tryptase levels indicating an increased mast cell load are much more frequent than previously thought and are a risk factor for severe or even fatal sting reactions. Premedication with antihistamines in the initial phase of venom immunotherapy reduced both local and systemic allergic side effects in several controlled studies. In a retrospective analysis of one of these trials it was found that reexposure during immunotherapy resulted in significantly more systemic allergic reactions in patients on placebo than on antihistamine premedication, suggesting that initial antihistamine premedication might increase the efficacy of venom immunotherapy. Different ways of allergen modification for venom immunotherapy have been proposed. While the results with chemical modifications were not convincing, recent studies with T-cell epitope peptides from the major bee venom allergen phospholipase A₂ look promising. Patient-tailored cocktails of recombinant venom allergens or isoforms thereof may be another possibility in the future. A number of prospective studies analyzing the duration of venom immunotherapy required for long-term protection have been published in the last decade. While most patients are still fully protected 1 year after discontinuation of therapy, relapses may occur in up to 20% of patients reexposed many years after treatment. Various risk factors for such relapses have been identified.

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Introduction

Allergic reactions to Hymenoptera stings are one of the major reasons for IgE-mediated anaphylaxis. According to epidemiologic studies in Europe, the United States and Australia, between 1 and 4% of an unselected adult population report systemic allergic reactions to stings by honeybees or vespids [1, 2]. Fatal sting reactions are rare; still more than 100 deaths mostly from single Hymenoptera stings are estimated to occur in Europe every year [3].

Since the introduction of Hymenoptera venoms instead of the previously used whole body extracts in the diagnosis and treatment of this allergy in the late 70s [4–6], Hymenoptera allergy is often considered a model for IgE-mediated allergy. Diagnostic tests are thought to be reliable and specific immunotherapy with venoms highly effective. However, the specificity of the main diagnostic tests, skin tests and venom-specific IgE antibodies is far from perfect. Up to 20% of individuals with no history of systemic sting reactions have positive tests. On the other hand, only 30–50% of those with positive tests will react to a subsequent sting by the respective insect [7]. According to a sting provocation test during venom immunotherapy the complete protection rate is around 95% for patients allergic to vespid stings but only 80–90% for those allergic to honeybee venom [7, 8]. Systemic allergic side effects to immunotherapy injections may occur in 20–40% of patients during immunotherapy with honeybee venom and in 5–10% during immunotherapy with vespid venoms [8]. There is thus a considerable potential for improvement of both diagnosis and immunotherapy of Hymenoptera venom allergy.

New Developments in Diagnosis

Recombinant Venom Allergens

The cDNA of a number of major venom allergens has been cloned and recombinant allergens were expressed in various systems. These data are summarized in table 1 [9–18]. Clinical studies with recombinant venom allergens are still scarce. For the major allergen of honeybee venom, phospholipase A₂, comparable allergenic activity of recombinant and natural purified allergen has been demonstrated in subcutaneous skin testing [19], binding to venom-specific IgE antibodies [20] and induction of histamine release from peripheral blood leukocytes [21]. The allergenic activity of recombinant hyaluronidase expressed in a prokaryotic system, *Escherichia coli*, was clearly inferior to that of the natural purified allergen. Therefore, another expression system, Baculovirus-infected insect cells, which through transmembranous secretion provides posttranslational changes such as refolding and glycosylation, was chosen [17]. It resulted in an allergenic activity similar to that of the natural purified allergen. This indicates that such posttranslational changes are essential for the correct conformation of the molecule and its IgE-binding epitopes. On the other hand, inhibition studies have invariably shown that even highly purified natural allergens contain trace amounts of other venom allergens, as illustrated in figure 1: All commercially available preparations of the purified honeybee venom phospholipase A₂ inhibit IgE binding to recombinant hyaluronidase in up to 80–100%, although at 1–3 log higher concentrations than the homologous allergen. Recombinant allergens will therefore be superior to highly

Table 1. Recombinant venom allergens

Venom	Allergen	cDNA cloning	Expression
<i>D. maculata</i>	antigen 5	Fang et al. [9] (1988)	<i>E. coli</i>
	phospholipase A ₁	Soldatova et al. [10] (1993)	<i>E. coli</i>
	hyaluronidase	King et al. [11] (1996)	<i>E. coli</i>
<i>Vespula</i> spp.	antigen 5	Monsalve et al. [12] (1999)	<i>E. coli</i> , yeast
		Suck et al. [13] (2000)	<i>E. coli</i>
	phospholipase A ₁	King et al. [11] (1996)	<i>E. coli</i>
	hyaluronidase	King et al. [11] (1996)	<i>E. coli</i>
<i>Apis mellifera</i>	phospholipase A ₂	Kuchler et al. [14] (1989)	<i>E. coli</i>
		Dudler et al. [15] (1992)	
	hyaluronidase	Gmachl and Kreil [16] (1993)	Baculovirus system
	acid phosphatase	Soldatova et al. [17] (1998)	
		Soldatova et al. [18] (2000)	Baculovirus system

purified natural preparations regarding the determination of the true relevance of an individual allergen. Finally, recombinant allergens provide increased specificity for diagnostic testing (table 2). While 15% of individuals with no history of allergic reactions to honeybee stings react to whole-bee venom, none of these 20 patients reacted to recombinant phospholipase A₂, recombinant hyaluronidase or a panel of recombinant phospholipase A₂, recombinant hyaluronidase and synthetic melittin. If all relevant allergens, in the case of honeybee venom also acid phosphatase, are added to such a panel, its sensitivity should equal that of whole-bee venom [22].

Systemic Mastocytosis and Hymenoptera Venom Allergy

Systemic Hymenoptera sting reactions have been reported by several authors in patients with urticaria pigmentosa or other forms of systemic mastocytosis [23–25]. Venom-specific IgE antibodies were detected in most but not all of these patients. This suggests that non-IgE-mediated mediator release from increased mast-cell numbers and consequent anaphylactoid Hymenoptera sting reactions may occur in some of these patients. In others with detectable venom-specific IgE antibodies, the increased mast-cell load may result in more severe IgE-mediated anaphylactic reactions. In one paper reporting on 10 patients with a combination of urticaria pigmentosa and systemic Hymenoptera sting reactions, all 10 had a history of severe shock reactions, most of them with loss of consciousness [24]. All 10 patients were submitted to venom immunotherapy, whether IgE antibodies were detectable or not, and tolerated the treatment well. Six of the 7 patients reexposed to stings of the respective insect were completely protected while 1 developed minor systemic allergic symptoms. On the contrary, Oude Elberink et al. [25] have described 2 patients with systemic mastocytosis and Hymenoptera venom allergy who died from *Vespula*

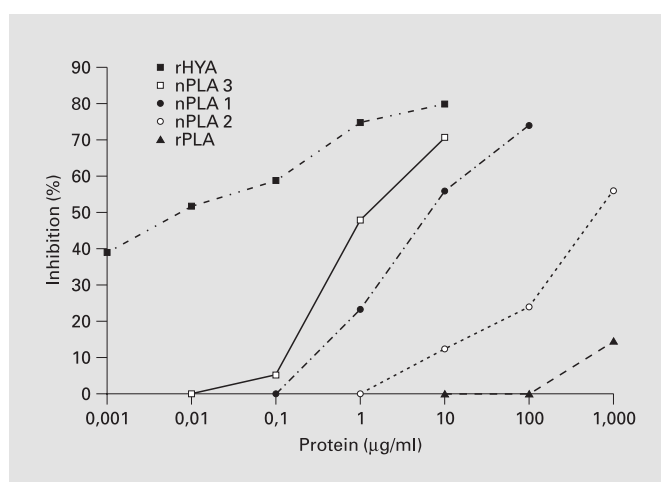


Fig. 1. Inhibition of IgE binding to recombinant hyaluronidase by recombinant (r) and three commercial preparations of natural purified (n) phospholipase A₂ (PLA) from bee venom in comparison with the homologous allergen, recombinant hyaluronidase (rHYA).

stings 3 and 8 years after stopping venom immunotherapy with *Vespula* venom. In one of these patients, immunotherapy had been given at the usual maintenance dose for 5 years, in the other however it had to be stopped prematurely at a lower maintenance dose because of repeated systemic allergic side effects. These observations support the notion of an increased severity of Hymenoptera sting reactions in patients with systemic mastocytosis.

Very recently Ludolph Hauser et al. [26] have estimated tryptase serum levels in a large number of Hymenoptera-allergic individuals and reported an elevated basal tryptase level in almost 30% of those with a history of shock reactions. Systemic mastocytosis, mostly not of the urticaria pigmentosa type, could be detected in the majority of these patients. In a similar study (table 3) we were

Table 2. Specific IgE to whole-bee venom (BV), natural purified (n) and recombinant (r)/synthetic (s) BV allergens phospholipase A₂ (PLA), hyaluronidase (Hya) and melittin (Mel), as well as to a combination of the three recombinant/synthetic allergens (panel)

Population	Percent with specific IgE to							
	BV	nPLA	rPLA	nHya	rHya	nMel	sMel	panel rPla + rHya + sMel
Patients (n = 85)	96	86	78	88	71	66	28	87
Controls (n = 20)	3	1	0	0	0	0	0	0

Table 3. Serum tryptase levels in patients with systemic allergic reactions to Hymenoptera stings

Symptoms	Patients	Tryptase serum levels			
		>13.5	%	arithmetic mean value*	SD
Cutaneous only	24	0	0	5.81	2.4
Respiratory	33	3	9	7.36	4.1
Mild cardiovascular	26	2	8	6.6	4.9
Severe shock	22	4	18	8.6	5.2

* p = 0.02, group 1 vs. group 4.

Table 4. Premedication with anti-histamines during early immunotherapy: double-blind, placebo-controlled trials

Author/medication	Patients	Allergen protocol	Reduction of side effects	
			local	systemic
Berchtold et al. [29] (1992) Terfenadine	52	bee venom rush	yes	yes (U, AE)
Herman and Melac [30] (1996) Cetirizine	140	bee venom rush	yes	no
Brockow et al. [31] (1997) Terfenadine	121	hym venom rush	yes	yes
Reimers et al. [32] (2000) Fexofenadine	54	bee venom ultra rush	yes	yes (U, AE)

U = Urticaria; AE = angioedema.

able to confirm these results in 108 patients with systemic Hymenoptera sting reactions [27]. These observations indicate that an increased basal tryptase level, reflecting an increased mast-cell load and thus probably systemic mastocytosis, is far more frequent than previously thought of. It also seems to be a significant risk factor for severe or even fatal anaphylactic reactions to Hymenoptera stings and maybe other allergens. Further confirmation of these observations will probably imply that patients with a combination of systemic mastocytosis and Hymenoptera sting anaphylaxis will have to stay on venom immunotherapy for life.

New Developments in Treatment

Several approaches have been studied in order to improve safety and efficacy of venom immunotherapy. Some of these will be described in the following:

Premedication with Antihistamines

Allergic side effects to venom immunotherapy (both local and systemic) occur most often during the initial phase of this treatment. Therefore, antihistamine premedication has been recommended during the up dosing phase [28]. A number of prospective double-blind placebo-controlled studies (table 4) [29–32] have invariably shown a significant reduction of local reactions to venom injections, and in three of four studies the systemic allergic side effects of the urticaria angioedema type were also significantly reduced. The question however arose, whether such premedication could not affect the efficacy of venom immunotherapy. Alterations in T-cell reactivity have been observed during immunotherapy and are thought to be important for the protection induced by this treatment: a decrease of T-cell proliferation following allergen stimulation and a shift of the cytokine secretion pattern from TH2 to TH1 or TH0 have been described [33, 34]. It is well known that T cells bear histamine receptors [31], which, depending on their binding, could influence immunotherapy-induced alterations. In a retro-

Table 5. Terfenadine premedication during rush immunotherapy with bee venom: long-term results

Patients	Premedication	
	terfenadine	placebo
Originally treated	26	26
Follow-up at 3 years	24	23
Reexposed	20	21
Field sting/challenge	9/11	8/13
Systemic reaction	0	6
$\chi^2 = 7.41$; $p < 0.01$.		

spective study we therefore analyzed the long-term results of our first double-blind placebo-controlled study on antihistamine premedication during rush immunotherapy with bee venom [29]. Table 5 shows that of the 52 patients originally involved, 26 had been premedicated with terfenadine or placebo, respectively, during the initial 3 weeks of venom immunotherapy. Forty-seven of them were reevaluated after 3 years of venom immunotherapy. Seventeen had been reexposed to a field sting and 24 agreed to a sting provocation test during reassessment. Of the 41 thus reexposed patients (20 on original premedication with terfenadine and 21 on placebo), 6 developed a systemic allergic reaction. All 6 were in the placebo group [36]. These results indicate that antihistamine premedication during early venom immunotherapy does not affect the efficacy of this treatment but may rather increase it. The mechanism involved could be interference with histamine binding to H1 receptors on T cells. Further prospective studies are needed to confirm these results.

Allergen Modification

This concept postulates that allergens can be modified in a way to reduce or even destroy conformational IgE binding B-cell epitopes which are responsible for allergic side effects, while linear T-cell epitopes, which mediate protective immunity, are preserved.

Chemical modifications, e.g. by coupling of venom proteins with polyethylene glycol, were in fact able to reduce allergenicity and thus systemic allergic side effects of venom immunotherapy. However in a double-blind placebo-controlled study, efficacy indicated by the tolerance of a sting provocation test seemed reduced [37].

The availability of recombinant venom allergens offers several possibilities to analyze this concept further [38]: Conformational epitopes have been shown to be strongly

Table 6. Results of provocation tests after 2 months of immunotherapy with T-cell epitope peptides of honeybee venom phospholipase A₂ in bee-venom-allergic patients

Patient No.	Reaction after provocation	
	10 µg of PLA ₂ s.c.	live bee sting
1	none	local only
2	none	local only
3	local immediate and delayed swelling	local only
4	none	local, after 15 min pruritus, single urticarial wheals, mild angioedema
5	local immediate	local, after 25 min erythema of face, single urticarial wheals on chest, mild angioedema of eyelids

reduced in unrefolded recombinant allergens [19] and may also be destroyed by point mutations in the B-cell epitope region. Such preparations were however not yet studied in clinical trials for immunotherapy with Hymenoptera venoms.

Another approach is that of using synthetic linear T-cell epitopes of major venom allergens for immunotherapy. For the most important allergen of bee venom, phospholipase A₂, major T-cell epitopes have been identified by several groups [39–41]. These linear short peptides did not bind IgE antibodies to phospholipase A₂ but were able to stimulate T cells from bee venom-allergic individuals in vitro. In a pilot study [42], 5 bee venom-allergic patients were treated with increasing doses of an equimolar mixture of three major T-cell epitopes of phospholipase A₂ over 2 months to a total dose of 397 µg protein. All tolerated these injections without any local or systemic side effects. The efficacy of this treatment is shown in table 6. All 5 patients tolerated a first provocation test with 10 µg of natural purified phospholipase A₂, corresponding to the approximate amount of allergen contained in one bee sting, without any systemic allergic symptoms. One week later, 3 of the patients tolerated a provocation test with a live bee sting without any systemic symptoms while 2 developed minor cutaneous symptoms, which were much less severe than before treatment. Serological studies indicated that the 2 with incomplete protection were sensitized to other bee venom allergens besides phospholipase A₂ as well. Immunotherapy with T-cell peptides from all allergens to which the patient is sensitized may thus be required in order to achieve complete protection by peptide immunotherapy.

Table 7. Parameters affecting the risk of developing allergic reactions after stopping venom immunotherapy

Parameters indicating an increased risk	Parameters not associated with increased risk
Severe pretreatment reactions	Sex
Allergic reactions to immunotherapy shots	Atopic constitution
Allergic reactions to stings during immunotherapy	Specific IgE any time
3 years vs. >5 years of immunotherapy	Specific IgG any time
Repeated re-stings after stopping	
Adults vs. children	
Bee venom vs. <i>Vespula</i> venom allergy	

Duration of Venom Immunotherapy

The length of treatment required for long-term protection has been addressed in a number of studies which observed patients for their reactions to a sting provocation test 1–3 years after stopping venom immunotherapy [43–48]. These studies indicate that after treatment for at least 3 years, 83–100% of the patients were still completely protected 1–3 years after stopping the treatment. More recently, three studies [49–51] analyzed long-term protection after stopping immunotherapy. In a first study with repeated sting provocation test in 74 patients treated during 5 years, Golden et al. [49] reported 7 (10%) who developed minor systemic allergic symptoms following the

sting. In a second paper analyzing field re-stings up to 7 years after stopping treatment, 5 of 26 reexposed patients (19 %) developed systemic reactions again, some of them were severe [50]. Lerch and Mueller [51] reported on 200 patients reexposed mostly by field stings during up to 7 years after stopping venom immunotherapy. Twenty-five or 12.5% developed a systemic reaction again, 16% of those being allergic to bee venom and 7.5% of those to *Vespula* venom. Table 7 summarizes the influence of various parameters on the re-sting reaction risk after stopping immunotherapy.

The risk of a relapse of systemic-allergic sting reactions seems highest in patients with a history of very severe pretreatment reactions, particularly in those who developed also systemic allergic reactions to venom immunotherapy injections. Multiple reexposures after stopping immunotherapy are also associated with an increased relapse rate. On the other hand, venom-specific IgE or IgG antibody levels before, during or after immunotherapy are not associated with the relapse rate. In some studies, in contrast to others, an increased risk was described in patients with a high skin sensitivity either before treatment or at discontinuation. In conclusion, very severe pretreatment reactions, systemic-allergic side effects to treatment injections and high exposure are parameters associated with an increased relapse rate which may indicate the need for prolonged venom immunotherapy exceeding the 3–5 years usually proposed.

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