

Hymenoptera Venom Proteins and Peptides for Diagnosis and Treatment of Venom Allergic Patients

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Abstract: Stings by insects of the order Hymenoptera cause systemic, sometimes life threatening allergic reactions in 1 – 5% of the population in Europe and North America. Responsible for these reactions is an IgE mediated sensitization to proteins of the venoms injected during the stings of social Hymenoptera species, mainly the honey bee (*Apis mellifera*), vespids like *Vespula* sp, *Polistes* sp. and ants, in southern US and central America *Solenopsis invicta* and in Australia *Myrmecia pilosula*.

The venoms of these insects are composed of low molecular weight substances like biogenic amines, cytotoxic and neurotoxic peptides like melittin, apamin, MCD-peptide and mastoparan, and proteins, mostly enzymes like phospholipase A and hyaluronidase, which are major venom allergens.

Immunotherapy with Hymenoptera venoms has been shown to protect 80 to over 95% of patients with a history of systemic allergic sting reaction from further systemic reactions after re-stings. The procedure, safety and efficacy of this treatment and the immune mechanisms involved are discussed.

Since ancient times honey bee venom has been used for the treatment of chronic inflammatory disease, especially arthritis. Anti-inflammatory effects of bee venom have been documented in animal experiments. Most clinical studies suggest an antiinflammatory effect as well, but are uncontrolled. The only few controlled studies could not confirm efficacy of treatment with bee venom so far.

Keywords: Hymenoptera venoms, anaphylaxis, *Apis mellifera*, *Vespula vulgaris*, venom immunotherapy, apitherapy.

INTRODUCTION

Stings by social Hymenoptera insects - like honey bees, vespids or ants – are one of the 3 major causes of anaphylaxis, beside foods and drugs. They were responsible for about 3 fatal reactions per year in Switzerland - 7.5 million inhabitants - over the last 40 years [1]. Extrapolated to Europe with about 500 million inhabitants, an estimated 200 fatalities occur every year; in the whole world with about 7 billion people, several 1000 fatal Hymenoptera sting reactions are to be expected. Most of these fatalities are due to IgE mediated allergic reactions to only single stings, and are a consequence of sensitization to venom proteins following previous stings by the same species. The amount of venom applied by a sting is so small - between 3 and 100 µg [2] - that fatal toxic tissue lesions occur only after several 100 stings.

Hymenoptera venoms have been successfully used for specific immunotherapy of patients with systemic allergic reactions (SAR) following stings, and this treatment protects most patients from further allergic reactions [3]. On the other hand venom of the domestic honey bee has been used for treatment of arthritis and other chronic inflammatory disease since ancient times [4].

I will first present the venom composition of the major stinging Hymenoptera species, then inform about the use of

venom preparations for diagnosis and specific immunotherapy in venom allergic patients, including mechanisms of immune protection induced by this treatment. In a last chapter I will review the use of bee venom proteins and peptides as medications for the treatment of chronic inflammatory disease like rheumatoid arthritis or multiple sclerosis.

COMPOSITION OF HYMENOPTERA VENOMS [5]

The taxonomy of Hymenoptera is shown in Fig. (1). The main species of social Hymenoptera responsible for sting induced allergic reactions are the honey bee (*Apis mellifera*), the yellow jacket (in USA) or wasp (in Europe) (*Vespula* sp. most often responsible are the species *V. vulgaris*, *V. germanica* and *V. maculifrons*) the paper wasp (*Polistes* sp.) and the ants *Solenopsis invicta*, the imported fire ant in America, and *Myrmecia pilosula*, the Australian jack-jumper ant. Occasional allergic reactions have also been described after stings of bumble bees (*Bombus* sp.), various *Dolichovespula* species, and by hornets (*Vespa crabro* and *Vespa orientalis*). The venom of the bumble bee is similar in its composition to that of the honey bee, that of the vespids species *Dolichovespula*, *Vespa* and *Polistes* to that of *Vespula* [5, 6]. I will therefore confine my description to the venoms of *Apis mellifera*, *Vespula vulgaris*, *Solenopsis invicta* and *Myrmecia pilosula*. Venom of the honey bee is obtained by electrostimulation [7], venom of vespids by venom sac extraction [8]. All hymenoptera venoms contain low molecular weight components like biogenic amines, carbohydrates and lipids, peptides and high molecular weight proteins, often enzymes. While biogenic amines and peptides

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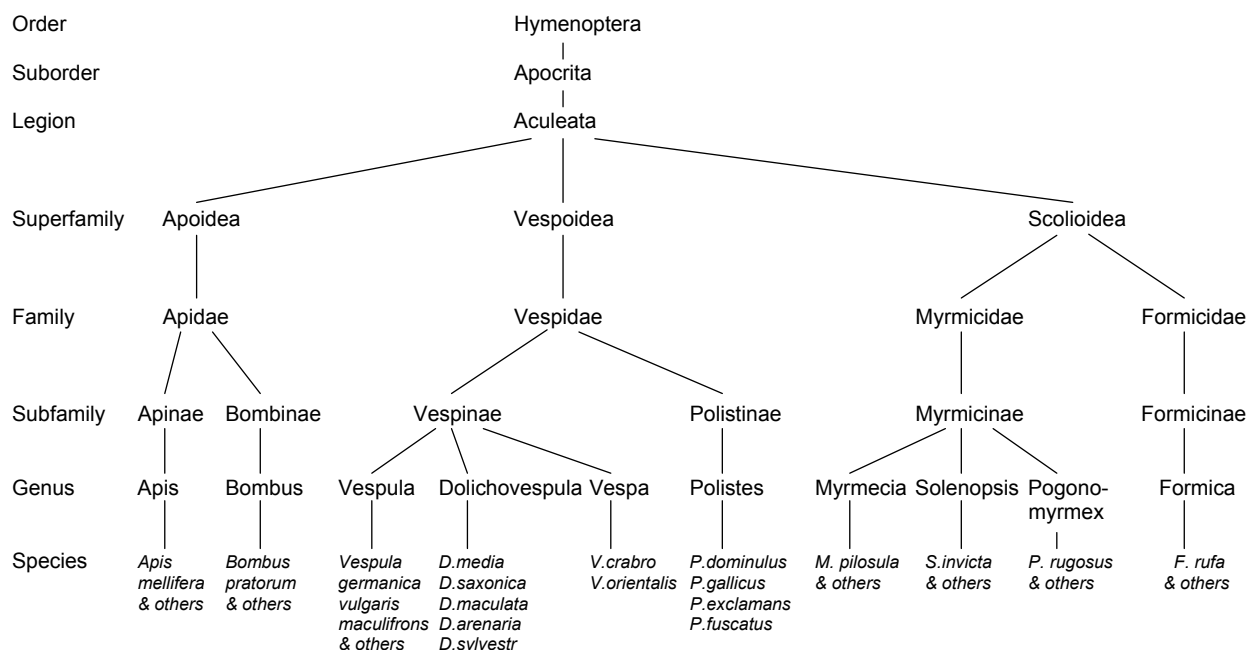


Fig. (1). Taxonomy of hymenoptera.

are primarily responsible for local pain and inflammation at the sting site and systemic toxic effects following multiple stings, allergic sensitization is most often due to proteins.

Venom of the Honey Bee *Apis mellifera* [9,10]

Low Molecular Weight Components and Peptides

Bee venom (BV) contains the low molecular components histamine, dopamin, noradrenalin, free amino acids, oligopeptides, carbohydrates, lipids and pheromones, that on the whole make up for about 25% of the dry weight of whole BV.

Among the peptides **melittin** is by far the most important, responsible for around 50% of the dry weight of whole BV. The 26 amino acid peptide has a molecular weight of 2.84 kd and has a strongly basic, hydrophilic C-terminal and a hydrophobic N-terminal end, thus showing the structure of an invert soap. This not only explains its surfactant activity as a true detergent, but also its extreme membrane-toxicity on a cellular and subcellular level, which results in a strong cytotoxic, hemolytic and also antimicrobial effect. Its interaction with cellular organelle membranes induces liberation of enzymes from lysosomes or mediators from granules of effector cells like mastocytes and basophil leukocytes, which potentiate its toxic effects. Usually peptides of less than 3 kd are not allergenic. However, in aqueous solution part of the melittin presents as tetramers [11] and this may explain its activity as a minor allergen inducing specific IgE antibodies.

Apamine is also a basic peptide, consisting of 18 amino acids and having a molecular weight of 2.03 kd. It comprises about 2% of the dry weight of whole BV. It is strongly basic

and has a high sulphur content. In animal experiments it was identified as a neurotoxin and shown to target potassium channels by blocking receptor-mediated calcium-activated potassium permeability in many cells such as neurons, smooth, skeletal and heart muscles [10,12]. In high doses in animal experiments it induces hypermotility, hyperexcitability and tonic clonic cramps due to central nervous damage.

Mast cell degranulating peptide (MCD-peptide, peptide 401) is structurally closely related to apamine, but even more basic and comprises about 1% of the dry weight of whole bee venom. It consists of 22 amino acids and has a molecular weight of 2.59 kd. Its histamine releasing potency from mast cells is even 10 to 100 times stronger than that of melittin. Like apamin it blocks potassium channels and is also a neurotoxin [10]. Besides it was shown to have antiinflammatory effects in animal models of inflammation induced in rats by carrageenan injections and in adjuvant arthritis [13].

Further peptides which comprise less than 1% are **tertiapin**, a 2.46 kd, basic molecule, which also blocks potassium channels [14] and has mast cell degranulating and neurotoxic effects; and **secapin**, 2.87 kd with unknown activity.

Allergenic Proteins (Table 1a) [5, 6, 15]

Phospholipase A2 (Api m1) is a 16 kd enzyme with one carbohydrate epitope which comprises 10 – 15% of dry weight of BV and hydrolyses fatty acids of membrane phospholipids. It is definitely the most important major allergen of BV. Specific serum IgE antibodies can be detected in over 95% of patients with SAR to honey bees if looked for within a year after a SAR [16,17].

Table 1a. Allergens in Honey Bee (*Apis mellifera*) Venom

Allergen	Molecular Weight	Glycosylation	Major/Minor Allergen*	Recombinant Form Published	References
Phospholipase A2 Api m1	16 kd	yes	major	yes	Dudler [15]
Hyaluronidase Api m2	43 kd	yes	major	yes	Soldatova, Markovic-Housley [17,18]
Acid phosphatase Api m3	45 kd	yes	minor	yes	Grunwald [19]
Melittin Api m4	2.8 kd	no	minor	yes	King [27]
Dipeptidylpeptidase Api m5	102 kd	yes	?	ND	de Graaf [20], Blank [21]
Api m6	8 kd	no	minor	yes	Kettner [22]
CUB serine protease Api m7	39 kd	yes	?	yes	Winningham [23]
Carboxylesterase Api m8	70 kd	?	minor	ND	de Graaf [20]
Icarapin	29 kd	?	?	yes	Peiren [24]

*Major allergen: $\geq 50\%$ of allergic patients have specific IgE against.

Minor allergen: $< 50\%$ of allergic patients have specific IgE against.

Hyaluronidase (Api m2) is a 43 kd, glycosylated enzyme which comprises about 2-3% of the dry weight of BV. It splits mucopolysaccharides like hyaluronic acid, primarily in the connective tissue of the skin, thereby increasing the penetration of venom peptides and proteins into deeper layers. It is also a major allergen inducing specific IgE antibodies in more than 50% of BV allergic patients [18,19].

Acid phosphatase (Api m3) is a 45 kd, glycosylated enzyme which comprises about 1% of the dry weight, and is probably a minor allergen, since only 37% of BV allergic patients had specific IgE to the recombinant protein in a recent study [20].

Dipeptidylpeptidase (Api m5) is a recently identified 102 kd, glycosylated enzyme [21, 22], to which specific IgE has also been detected in part of the BV allergic patients. Further, probably minor BV allergens are melittin (Api m4), Api m6 [23], CUB serine protease (Api m7) [24], carboxylesterase (Api m8) [21] and icarapin [25], each making up for less than 1% of the dry weight of BV.

Venom of the Yellow Jacket (USA) or Wasp (Europe) *Vespa* sp.

Low Molecular Weight Components and Peptides

Vespa venom (VV) contains a number of low molecular weight components like histamine, serotonin, catecholamines, carbohydrates, amino acids, polyamines and volatile pheromones. Several VV kinins, oligopeptides with biological activities similar to bradykinin, have been described [26]. Besides a hemolysin and **mastoparan**, a mast cell degranulating tetradecapeptide were found. Mastoparan also releases serotonin from platelets, insulin from pancreatic cells and catecholamines from chromaffin cells and elevates intracellular calcium ions. Most interesting it also modulates activities of proteins like regulatory G-proteins, calmodulin, phospholipases and protein kinases [27].

Allergens of Vespa Venom (Table 1b)

Major allergens of VV, to which the majority of VV allergic patients have specific IgE antibodies are **phospholipase A1** (Ves v1) and **antigen 5** (Ves v5). Phospholipase A1 is a non glycosylated, 34 kd enzyme which is structurally not related to BV phospholipase A2 [6]. Therefore there is no crossreactivity between these two major allergens. Antigen 5 is a non glycosylated, 23 kd allergen with unknown biological activity, which is also specific for VV as compared to BV [17]. **Hyaluronidase** (Ves v2) is a 38 kd, glycosylated enzyme [28] which crossreacts with BV hyaluronidase both because of an about 50% identity of the peptide structure and, probably more important, the presence of specific IgE to crossreacting carbohydrate determinants (CCDs), which are not only found in insect but also in plant allergens and are generally considered to be of little clinical relevance. Although IgE to hyaluronidase are found in more than 50%, these are only in a minority of patients due to peptide epitopes but more often to CCDs. Therefore VV hyaluronidase is today considered as a minor VV allergen [29]. More recently a glycosylated 100 kd VV allergen has been identified as a **dipeptidylpeptidase** (Ves v3) [21, 22], which is probably a minor allergen and may crossreact with the dipeptidylpeptidase (Api m5) of BV.

A number of BV and VV allergens have been cloned and expressed as recombinant allergens (Tables 1a and 1b). Already today some can be used for component resolved diagnosis, and hopefully in future also for immunotherapy of Hymenoptera venom allergic patients [5, 16-22].

Venoms of the Ants *Solenopsis invicta* and *Myrmecia pilosula* (Table 1c)

Solenopsis Invicta the Imported Fire Ant

Stings by the imported fire ant are a major cause of SAR in southern USA and central America. The venom contains

Table 1b. Allergens of Wasp/Yellow Jacket (*Vespa* sp.) Venom

Allergen	Molecular Weight	Glycosylation	Major/Minor Allergen	Recombinant form Published	References
Phospholipase A1 Ves v1	34 kd	no	major	Yes	King [27]
Hyaluronidase Ves v2	38 kd	yes	minor	yes	King [27]
Dipeptidylpeptidase Ves v3	100 kd	yes	?	yes	de Graaf [20] Blank [21]
Antigen 5 Ves v5	23 kd	no	major	yes	Skov [28]

*Major allergen: $\geq 50\%$ of allergic patients have specific IgE against.

Minor allergen: $< 50\%$ of allergic patients have specific IgE against.

an oily phase with dialkylpiperidines and other alkaloids which comprise more than 95% of the dry weight and are no allergens, but responsible for the local sting reactions with blister formation [5]. Four proteins have been identified as allergens: **Phospholipase A1** (Sol i1) is a glycosylated 37 kd protein and a major allergen which shows some crossreactivity with phospholipase A1 of vespid venoms, based on a partial peptide sequence identity, while crossreactivity with BV is only based on CCDs [30]. **Sol i3** is a 26 kd major allergen and a member of the antigen 5 family, showing 44-50% identity to antigen 5 from vespids [31], and is responsible for crossreactivities based on peptide epitopes. **Sol i2** and **Sol i4** [30-32] are both minor allergens, related to each other but not to proteins from other hymenoptera venoms.

***Myrmecia pilosula* the Jack Jumper Ant**

Stings by the jack jumper ant are an important cause of SAR in Australia [33]. Its venom contains, besides low molecular components like histamine, a number of peptides and also proteins. Enzymatic activities include phospholipase A, hyaluronidase, acid and alkaline phosphatase [34]. Several mostly minor allergens binding to IgE of allergic patients have been identified [35]. **Pilosulin 3** (Myr p2) a 5.6 kd peptide is the only major allergen identified so far, binding serum specific IgE in 42 of 54 (78%) patients with a history of SAR to ant stings. Two

other peptides, **pilosulin 1** (Myr p1) a 6.1 kd molecule and **pilosulin 4.1** (Myr p3) are minor allergens, being recognized by specific IgE in 33% and 17% respectively, of the 54 ant allergic patients. Moreover 2 proteins with unknown biologic functions of 25.6 kd and 89.8 kd were recognized by specific IgE of 46% and 37% respectively, of the patients and thus qualify as minor allergens.

HYMENOPTERA VENOMS FOR DIAGNOSIS AND TREATMENT OF SYSTEMIC ALLERGIC STING REACTIONS

Epidemiology and Clinical Symptoms of Hymenoptera Venom Allergy [15, 36]

According to epidemiologic studies in different areas of the world between 57 to over 90% of human beings are stung by Hymenoptera insects at least once in their life and 1 to 5% suffer from systemic allergic reactions (SAR) during lifetime, which are most often IgE mediated.

Symptoms during such acute reactions are starting within minutes to about half an hour after the sting and can manifest in the skin with itching, flush, urticaria or angioedema; in the gastrointestinal tract with abdominal cramps, nausea, vomiting and diarrhea; in the respiratory tract with dyspnoe, hoarseness, asthma or pulmonary edema; and in the cardiovascular system with dizziness, drop in blood pressure, arrhythmia, collapse and unconsciousness [36]. They usually

Table 1c. Allergens of Ant Venoms (*Solenopsis invicta*, *Myrmecia pilosula*)

Allergen	Molecular Weight	Glycosylation	Major/Minor Allergen	Recombinant Form Published	References
Phospholipase A1 Sol i1	37 kd	yes	major	yes	Hoffman [30]
Sol i2	14 kd		minor	yes	Schmidt [31]
Antigen 5 Sol i3	26 kd		major	yes	Schmidt [32]
Sol i4	20 kd		minor		Hoffman [30]
Pilosulin 1 Myr p1	6.1 kd		minor		Wiese [35]
Pilosulin 3 Myr p2	5.6 kd		major		Wiese [35]
Pilosulin 4.1 Myr p3	8.2 kd		minor		Wiese [35]

disappear within several hours. Lasting morbidity like myocardial or cerebrovascular infarction following severe shock reactions [37] as well as fatalities, may however occur occasionally [1].

Diagnosis

Within a year after a systemic allergic sting reaction specific IgE antibodies to venom proteins can be detected routinely in the serum (e.g. by UNICAP, Phadia, Uppsala, Sweden) and by intracutaneous skin tests in near to 100% of patients [17]. Their specificity is however limited: Controls without a history of systemic sting reactions present positive tests in 10 to 20% [15], maybe because of sensitization following the last well tolerated sting, probably more often due to sensitization to crossreacting carbohydrate determinants (CCDs), which are also present in many plant allergens [29]. Cellular tests like histamine or leukotriene release from basophils (CAST, cellular allergen stimulation test), and by activation of surface markers of basophils like CD63 or CD203 following exposure to the venom (BAT, basophil activation test) are also very sensitive and may be more specific than skin tests and specific IgE, but are much more expensive. Moreover, their predictive value in relation to a sting challenge has not been analyzed in a large number of patients so far.

The cellular immune response to venom proteins in this situation is characterized by activation of T-helper 2 (TH2) cells with secretion of the cytokines IL-4, IL-5 and IL-13 [38, 39].

Management of Hymenoptera Venom Allergy

Emergency Medication

Emergency treatment consists of general measures like shock positioning, intramuscular injection of adrenaline, infusions for volume substitution, i.v. injections of antihistamines and corticosteroids, oxygen supply and if necessary resuscitation.

Besides instructions on how to avoid further stings, all patients receive emergency medication for self application in case of a re-sting: An Epipen[®] autoinjector containing 0.3 mg of adrenaline for i.m. application and antihistamine and corticosteroid tablets [3].

Immunotherapy with Hymenoptera Venoms (VIT)

Immunotherapy with electrostimulated venom of the honey bee and venom sac extracts of vespids and the Australian ant *Myrmecia pilosula* was shown to be effective in 3 controlled studies comparing it with either placebo or wholebody extracts of the respective insects (Table 2a) [40-42]. In one of these studies wholebody extracts were not more efficient than placebo. The high efficacy of VIT was confirmed by numerous prospective studies with sting challenges during VIT (Table 2b) [43-46]: More than 95% of vespid venom allergic patients and 80 - 90% of bee venom allergic patients do not develop any systemic allergic reactions if resting during VIT. If systemic symptoms occur these are usually much less severe than before treatment [3, 36]. VIT is started with very low venom doses of 0.1 µg or even less of the respective venom. The dose is then continuously increased up to a maintenance dose of 100 µg, corresponding to the amount of venom injected by 1 to 2 honey bee stings and more than 10 vespid stings. Systemic allergic side effects may occur, especially during the dose increase phase, more often during BV than VV treatment [44], and therefore VIT requires close supervision for at least 30 minutes after each injection. In order to get longterm protection monthly injections of the maintenance dose are recommended in the first year and injections every 6 to 8 weeks from the second year with a total duration of 5 years [3].

Mechanisms of Protection Induced by VIT [38]

An imbalance between T-helper 1 (TH1) and T-helper 2 (TH2) allergen specific lymphocytes in favour of TH2 cells is today accepted as the basis for hymenoptera venom allergy. TH2-cells stimulated by the venom allergens secrete the cytokines IL-4, IL-5 and IL13 which induce specific IgE production by B-cells, and are essential for the survival, differentiation and activity of effector cells of the allergic reactions, like mast cells, basophils and eosinophils.

Several mechanisms of protection induced by VIT have been observed:

- 1) Blocking IgG antibodies: Heavily exposed beekeepers, who experience more than 100 stings a year, have very high levels of specific IgG, especially IgG4 antibodies to BV proteins and tolerate bee stings very well [47]. Moreover, in several studies highly allergic patients who developed repeated SAR

Table 2a. Controlled Studies of Venom Immunotherapy

Author	Immunotherapy with	No pts	Systemic Reaction at Reexposure	p
Hunt 1978 [40]	Bee or Vespula venom	19	1 (5.3%)	< 0.01
	Bee or Vespula WBE	11	7 (63.6%)	< 0.01
	placebo	12	7 (58.3%)	
Müller 1979 [41]	Bee venom	12	3 (25%)	< 0.03
	Bee WBE	12	9 (75%)	
Brown 2003 [42]	Ant venom	35	1 (2.9%)	< 0.0001
	placebo	29	21 (72.4%)	

WBE = wholebody extract.

Table 2b. Prospective Studies with Sting Challenges During Venom Immunotherapy

Author	Systemic Allergic Reactions to Challenge	
	Bee Venom Allergic pts	Vespula Venom Allergic pts
Gillman 1980 [43]	4/18 (22%)	not done
Müller 1992 [44]	34/148 (23%)	5/57 (9%)
Golden 1992 [45]	not done	4/147 (3%)
Mosbech 1987 [46]	not done	0/19 (0%)

following VIT injections, could be protected by prophylactic intravenous passive immunotherapy with beekeeper gammaglobulins [48-50]. During VIT specific IgG, especially IgG4 antibodies increase strongly within 2 weeks and stay elevated during the whole treatment, whereas skin sensitivity and specific IgE antibodies - after an initial increase - decrease steadily during prolonged VIT, resulting in a strong decrease of the ratio of specific IgE to specific IgG4, and consequently to inhibition of mast cell activation by interaction of venom allergens with effector cell bound specific IgE (Fig. 2). There is however no close correlation between the IgG titer and the clinical efficacy of VIT [3, 51].

- 2) In heavily exposed beekeepers and in patients on VIT the *in vitro* immune response in lymphocyte cultures following stimulation with venom proteins reveals the presence of CD4+ T-regulatory cells which secrete IL-10 and thus suppress the activity of specific TH2 cells, leading to inhibition of secretion of cytokines IL-4, IL-5 and IL-13 from TH2-cells and consequently of specific IgE production, whereas venom specific IgG4 are increased [38]. Moreover the survival, proliferation and activity of effector cells is decreased.
- 3) Immediately after a 1 day ultra rush protocol of VIT histamine and leukotriene release from venom stimulated basophils is already significantly decreased, suggesting a reduction of effector cell releasability [39]. This explains very early protection observed in occasional patients already after reaching maintenance dose during rush or ultrarush VIT [52], at a time when the induction of T-regulatory cells may not yet be completed. This change in releasability of effector cells during very early VIT may thus not be due to suppression of TH2-cells by T-regulatory cells, but rather by mediator depletion through the high allergen doses applied over a short period during the ultra rush and rush protocols over 1 to 5 days.

BEE VENOM FOR PHARMACEUTICAL TREATMENT OF CHRONIC INFLAMMATORY DISEASE

Bee venom has been used since ancient times in Egypt, Babylon, China, Greece, and Rome as a medical treatment for arthritis [4]. The relatively easy availability of domestic honey bees to apply stings to the patient may have favoured this kind of treatment which is practiced up to our time, especially in eastern Europe and China, and is also called

apitherapy. Since the early 20th century experimental animal and *in vitro* studies were initiated to explain the postulated pharmacologic effects of BV and its components [4]. A number of mostly uncontrolled clinical trials have been published on apitherapy for rheumatic arthritis and multiple sclerosis.

Antiinflammatory Effects of Bee Venom

In several trials on rats it could be shown, that rat paw edema and inflammation following carrageenan injection, and adjuvant arthritis could be inhibited by whole BV, but not by melittin, apamin or phospholipase A2 alone [53-55]. Several possible explanations for this observed antiinflammatory effect have been suggested:

- In one study MCD-peptide was made responsible for this antiinflammatory effect and an interference of this peptide with the metabolism of arachidonic acid and thus creation of inflammatory eicosanoids suggested [13].
- An alternate explanation was the observation of an increased corticosteroid release from adrenal glands [56] after BV injection. In dogs this effect is however blocked by hypophysectomy indicating a primary effect of injected BV on the release of adrenocorticotrophic hormone from the hypothalamus/hypophysis axis [57].
- In another *in vitro* study a potent dose dependant inhibition of oxygen radical production from stimulated neutrophils, which are thought to play a role in chronic inflammation, was observed by whole BV and melittin [58].
- More recently it was reported that during *in vitro* studies with rat macrophages and human synovial cells whole BV and melittin block LPS-mediated activation of the transcription factor NFκB, which is of utmost importance in the upregulation of proinflammatory genes [55, 59]. A study in human fibroblast-like synoviocytes could however not confirm this finding [60].

Clinical Studies on Apitherapy

A number of studies on treatment of arthritis with BV, applied either by stings, injections of collected venom or acupuncture, report about positive results [61-64]. However, these studies were either uncontrolled [61, 62], insufficiently controlled [63] or the patient population was ill defined [64]. In 2 studies [65, 66] no significant effect was found. One of

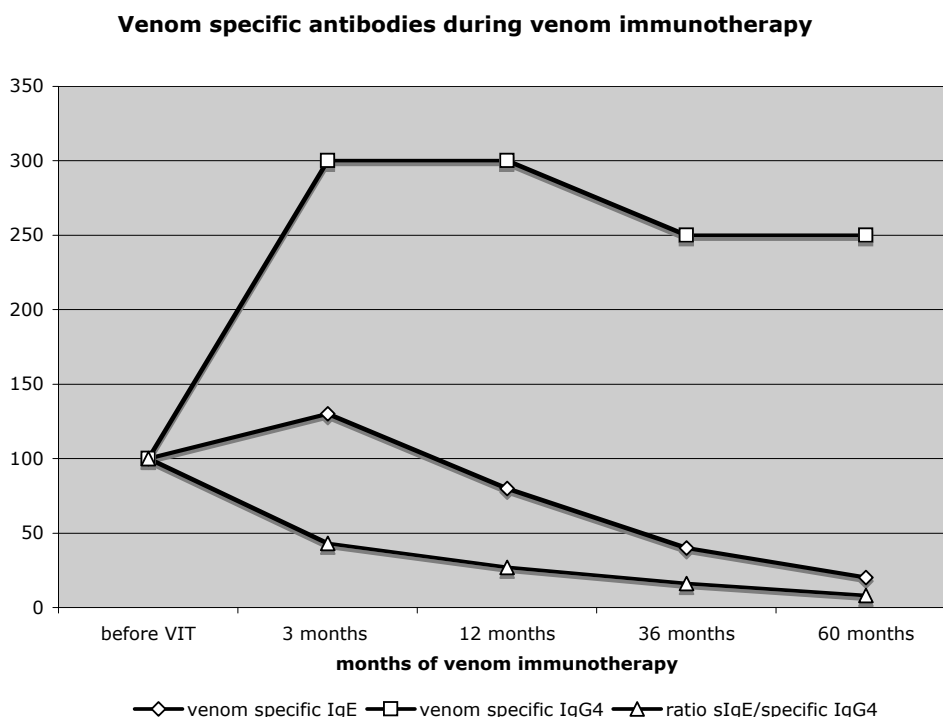


Fig. (2). Schematic course of venom specific IgE, IgG4 and ratio of specific IgE/specific IgG4 before starting and during venom immunotherapy.

them was a controlled study on 33 patients with rheumatoid arthritis: improvement was observed in 29% of the BV and 33% of the placebo treated patients [66].

Honey bee venom has also been used for treatment of multiple sclerosis. One uncontrolled study on 51 patients with primary or secondary progressive multiple sclerosis reported significant improvement of clinical symptoms under treatment with several weekly injections of BV over 12 months [67]. However, in a more recent randomized crossover study over 6 months in 26 patients with relapsing multiple sclerosis, a positive effect could not be confirmed. During the application of bee stings 3 times a week over 6 months, the number of new gadolinium enhancing brain lesions in the MRI was not different from that of a subsequent or earlier 6 months phase with no bee-stings in both groups. There was also no significant difference in change of symptom scores during the 2 periods [68].

In summary there are undoubtedly antiinflammatory and possibly immunoregulatory effects of BV and some of its components in animal experiments and *in vitro* studies, and several possible mechanisms for these effects have been suggested. Based on several case reports and uncontrolled studies in arthritis and other inflammatory disease, a beneficial effect of treatment with bee stings or injected BV has also been suggested in humans. This was however not confirmed in the few controlled studies so far.

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