

Hypersensitivity to Hymenoptera Venom: Advances in Diagnosis and Implications for Treatment

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Abstract: Diagnosis and treatment of hypersensitivity to Hymenoptera venom took a landmark step forward in the late 1970s with the introduction of venom as an adequate material instead of whole body extracts. Since then, venom immunotherapy (VIT) has provided allergic subjects with a complete protection from fatal anaphylaxis and prevented about 90% of all reactions to stings. The cross-reactivity among some venom components, particularly important in the case of cross-reacting carbohydrate determinants, has often made it difficult to recognize the true causative venom to be used in VIT. Recently, the introduction of purified and recombinant allergens, such as Api m 1 from honeybee venom, Ves v 5 from yellow jacket venom, and Pol d 5 from wasp venom, have allowed a more precise diagnosis with identification of the causative venom component. This paves the way for a patient-tailored VIT in the near future. Another issue which needs to be addressed is the improvement in the safety of VIT with honeybee venom, which is significantly less favourable in comparison to vespid venom. A number of molecular approaches are under investigation in order to achieve this objective. Alternative routes of administration, such as the sublingual and the intralymphatic, have also been proposed, but there are not yet sufficient data available to demonstrate their feasibility. This review also presents patents on new trends in therapies for the management of hypersensitivity to hymenoptera venom.

Keywords: Hymenoptera venom, honeybee, vespids, allergens, cross-reactivity, venom immunotherapy, efficacy, safety.

INTRODUCTION

Insects belonging to the order of the Hymenoptera, such as honey bees (*Apis mellifera*), bumble bees (*Bombus* species), yellow jackets (*Vespula* species), wasps (*Polistes* species), and hornets (*Vespa crabro*, *Dolichovespula* species), are an important cause of hypersensitivity reactions. The prevalence of systemic reactions to Hymenoptera stings varies from 0.5% to 8.9% throughout the world, while large local reactions affect 26.4% of the population [1]. The main factor underlying sensitization seems to be the exposure to stings, as indicated by a prevalence of systemic reactions much higher (up to 32%) in subjects receiving frequent stings such as the beekeepers [2]. Also the fact that males are more frequently subject to Hymenoptera venom allergy (HVA) than females seems related to the higher exposure to stings in males [3]. The risk of fatal events is low, with a mortality comprised between 0.03 and 0.48 cases per million/inhabitants/year in the countries where it has been estimated [1].

The natural history of HVA is characterized by a trend to a spontaneous tolerance; in fact most subjects after an initial systemic reaction do not react to further stings of the same type of insect [4]. The likelihood of having further reactions

is influenced by the kind of reaction, being higher in severe reactions [5] and by the patient's age, with a particularly favourable prognosis for children [6].

For decades, whole body extracts were believed to be an adequate material for diagnosis and treatment. However, the cause of hypersensitivity is the allergenic activity of a number of proteins naturally occurring in venom which are present only in negligible amounts in whole body extracts. These allergens are mainly enzymes such as phospholipase, hyaluronidase, and acid phosphatase. Other important allergens are antigen 5, which is a non-enzymatic basic protein, from vespid venom, and melittin from honeybee venom [7]. Recently, also high molecular weight (≥ 100 kDa) venom components were characterized as dipeptidyl peptidase and were demonstrated as important allergens on the basis of their recognition by the majority of venom-allergic patients. Such allergens were named Api m 5 from *Apis mellifera* and Ves v 3 from *Vespula vulgaris* venom [8]. In recent years the introduction of new *in vitro* techniques and the availability of recombinant venom allergens have led to a significant improvement in the identification of the truly causative allergens in single patients and warrant the research for a patient-tailored immunotherapy.

METHODOLOGY FOR SEARCHING AND SELECTING THE REFERENCES

The medical literature was searched by means of the MEDLINE database for the years 1978 through to 2010

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using the following key words: “Hymenoptera venom”, “insect venom”, “reactions to stings”, “diagnosis”, “treatment”, “venom immunotherapy”, “efficacy”, and “safety”; The search was augmented by scanning the references cited in the articles identified. Only full-length articles in English published in referenced journals were considered. Full copies of the articles were retrieved for analysis and the relevant data extracted. The characteristics of the allergens from hymenoptera venom were searched on the Allergome database.

DIAGNOSIS OF HYMENOPTERA VENOM ALLERGY

The diagnosis of hypersensitivity to Hymenoptera venom is based on the combination of a history of reactions to stings and positive IgE antibody confirmatory test either by venom skin tests or serology. The clinical reactions indicative of hypersensitivity are large local reactions and systemic reactions, which include urticaria/angioedema, dyspnea, vomiting and diarrhoea, and fall in blood pressure with collapse, incontinence and loss of consciousness [9]. Such reactions are generally induced by the IgE-mediated mechanism in response to the injection of venom allergens by the sting. Toxic reactions caused by multiple stings (more than 50) may also occur with similar manifestations, and require the same medical treatment [9]. The IgE-mediated mechanism may be revealed by intradermal testing with venom or by measurement of specific IgE in serum [10]. The diagnosis is easy when a patient has recognized the culprit insect, for example because of the loss of the stinger (typical of honey bees) and there is a positive result to intradermal test with the corresponding venom. However, in most patients the recognition is uncertain and skin tests show multiple positive results, making the choice of venom to be used for specific immunotherapy difficult [11]. For decades, in such cases venom immunotherapy (VIT) was performed using all venoms eliciting a positive skin test. In 1993, Hamilton and colleagues demonstrated by the RAST-inhibition technique that 36.4% of patients showing a positive IgE antibody result to *Vespula* species and *Polistes* species were really allergic only to *Vespula* species, because the positivity to *Polistes* species was due simply to cross-reactive allergens. These individuals were thus candidate for VIT with *Vespula* species venom alone [12]. Theoretically, identification of the culprit insect should have been obtained by a challenge test, that is, a sting from a living insect under medical control, but this test is not recommended because of the potential risk of severe reactions and the insufficient reproducibility with vespid stings [13].

Major advances in identifying the causative venom allergens were provided by the availability of purified isolated native allergens and the introduction of recombinant allergens [14]. The venom from honey bee has been extensively investigated [15]. The most relevant major allergen is phospholipase A2 (PLA2) [16], thus named Api m 1 according to the current allergen nomenclature [17]. PLA 2 is a weakly basic glycoprotein with a molecular weight (MW) of 19 kDa that accounts for 12-15% of the dry weight of bee venom. It specifically recognizes the sn-2 acyl bond of phospholipids and catalytically hydrolyzes the bond releasing arachidonic acid and lysophospholipids. When

splitting the cell membrane phospholipids PLA2 has cytotoxic effect. It was recently demonstrated that PLA2 from the apid *Bombus ignitus* functionally binds cell membranes and can induce apoptotic cell death [18]. In addition, bee venom had genotoxic effect on human lymphocytes in both a time and venom dose dependent manner [19]. This ability could explain the long claimed anti-inflammatory and anti-cancer effect of bee venom [20, 21]. The other bee venom components identified as allergens are hyaluronidase, acid phosphatase, melittin, dipeptidylpeptidase, cystein-rich trypsin inhibitor, serin protease, carboxylesterase, serin carboxypeptidase, and carbohydrate-rich protein-icarapin [10]. Table 1 reports all the allergens from bee venom thus far reported.

Venom allergens from vespids include phospholipase A1, hyaluronidase, dipeptidylpeptidase, and antigen 5 (Table 2). Only antigen 5 appears to be species-specific, while all other allergens show a variable degree of cross-reactivity, which involves different vespid species for phospholipase A1, and extends to also honeybee venom for hyaluronidase and dipeptidylpeptidase. Recent studies modified the concepts about these allergens. In fact, the newly discovered dipeptidylpeptidase was demonstrated as an important allergen in both honeybee and yellow jacket venom [8], while the role of hyaluronidase was reduced. Recently, assessing the *in vitro* positivity to this allergen in a population of patients allergic to solely yellow jacket venom (n = 31) and allergic patients showing an *in vitro* double positivity to honey bee and *Vespula* venoms (n = 105), it was demonstrated that IgE binding to hyaluronidase was mainly due to carbohydrate component. In fact, while in the first group (*Vespula* monosensitized patients) only one bound hyaluronidase, 87% of double-positive patients bound this allergen [22]. Delving deeper into the positivities to hyaluronidase by means of IgE immunoblotting inhibition technique, the authors pointed out that 65% of double-positive patients reacted to cross-reacting carbohydrate determinants (CCD), 27% either to CCD or peptide epitopes and a bare 8% only to peptide epitopes. Instead, antigen 5 and phospholipase A1 were each recognized by around 90% of sera from both groups, while together they identified 87% patients. This lead the authors to suggest that hyaluronidase is a minor allergen in yellow jacket venom and that component-resolved diagnosis with antigen 5 and phospholipase A1 would detect virtually all patients with yellow jacket venom allergy [22]. This suggestion is in line with Muller *et al.* who investigated two highly selected populations formed by 100 patients allergic solely to honeybee or yellow jacket. Several double positivities were found, most of them (56%) caused by CCD. In this study rVes v 5 was recognized by 97% of yellow jacket-allergic patients, in contrast with the 17% of honeybee-allergic patients; while rApi m 1 was recognized by 97% of honeybee-allergic patients, in contrast with the 17% of yellow jacket-allergic patients. This shows that these allergens represent a highly selective marker for specific venom allergy, moreover *in vitro* positivity to both rApi m1 and rVes v 5 indicates true double sensitization [23].

The role of CCDs in apparent pluri-sensitization to venom has emerging importance. CCDs are naturally occurring in glycoproteins from plants (including pollens and vegetable foods) and in Hymenoptera venoms. Their identification as molecules inducing IgE cross-reactivity

Table 1. Allergens from Honeybee Venom.

Allergen	Function	Molecular Weight (Kilodaltons)	Name According to the Allergen Nomenclature	Access Number in Allergome
Phospholipase A2	Enzyme	16	Api m1	45
Hyaluronidase	Enzyme	39	Api m 2	46
Acid phosphatase	Enzyme	43	Api m 3	47
Melittin	Peptide (surfactant activity)	3	Api m 4	48
Dipeptidylpeptidase IV	Enzyme	100	Api m 5	5754
Cystein-rich trypsin inhibitor	Trypsin inhibitor	8	Api m 6	49
Serine protease	Enzyme	39	Api m 7	732
Carboxylesterase	Enzyme	70	Api m 8	5756
Serin carboxipeptidase	Enzyme	60	Api m 9	6189
Carbohydrate-rich-protein-icarapin	Unknown	50-55	Api m 10	8204

Table 2. Allergens from Vespid Venom.

Vespid	Allergen	Function	Molecular Weight (Kilodaltons)	Name According to the Allergen Nomenclature	Access Number in Allergome
<i>Vespula vulgaris</i>	Phospholipase A1	Enzyme	34	Ves v 1	668
<i>Vespula vulgaris</i>	Hyaluronidase	Enzyme	42	Ves v 2	669
<i>Vespula vulgaris</i>	Dipeptylpeptidase IV	Enzyme	100	Ves v 3	5767
<i>Vespula vulgaris</i>	Antigen 5	Unknown	23	Ves v 5	670
<i>Dolichovespula arenaria</i>	Phospholipase A1	Enzyme	34	Dol a 1	2448
<i>Dolichovespula arenaria</i>	Hyaluronidase	Enzyme	42	Dol a 2	2449
<i>Dolichovespula arenaria</i>	Antigen 5	Unknown	23	Dol a 5	327
<i>Vespa crabro</i>	Phospholipase A1	Enzyme	34	Ves c 1	672
<i>Vespa crabro</i>	Hyaluronidase	Enzyme	42	Ves c 2	1678
<i>Vespa crabro</i>	Antigen 5	Unknown	23	Ves c 5	673
<i>Polistes gallicus</i>	Phospholipase A1	Enzyme	34	Pol g 1	1208
<i>Polistes gallicus</i>	Hyaluronidase	Enzyme	38	Pol g 2	1209
<i>Polistes gallicus</i>	Serine protease	Enzyme	33	Pol g 4	1210
<i>Polistes gallicus</i>	Antigen 5	Unknown	23	Pol g 5	708

Similar allergens were identified in other *Vespula* sp. (*V. germanica*, *V. flavopilosa*, *V. maculifrons*, *V. pennsylvanica*, *V. squamosa*), *Dolichovespula* sp. (*D. maculata*), *Vespa* sp. (*V. orientalis*, *V. magnifica*, *V. mandarinia*), and *Polistes* sp. (*P. annularis*, *P. dominulus*, *P. exclamans*, *P. fuscatus*, *P. metricus*) [10]

dates back to 1981 [24]. In a recent study, 66 patients with double positivity of tests with honeybee and vespid venom were evaluated for the presence of specific IgE for oilseed rape and MUXF3 (N-glycan chain composed of 2 mannose, 1 xylose, 1 fucose and 2 N-acetylglucosamine molecules). In 29 patients the double positivity was due to cross-reactivity, as demonstrated by inhibition tests, and high levels of IgE to such CCDs were detected; 5 patients were sensitized to honeybee venom and 24 to vespid venom [25]. In general the N-glycans found on most hymenoptera venom proteins

possess a number of non-mammalian features rendering them potentially immunogenic. However, the supposed hallmark of CCDs on insect venom allergens comprises carbohydrates carrying α -1,3-linked core fucose residues. IgE with specificity for such glycotopes represents the underlying basis with all proteins possessing CCDs cross-reactivity [24].

Venom from formicids, namely from fire ants, is also a cause of allergic reactions, especially in USA [26]. The two major allergens are phospholipase A1, named Sol i 1, and

antigen 5, named Sol i 5, after the scientific name *Solenopsis invicta*, but there are also other allergens present. For example, the crystalline structure of the allergen Sol i 3 was shown to be similar to that of Ves v 5 but no cross-reactivity between the two allergens was detected, probably because of different epitopes in some molecule regions [27].

Further advances in recognizing the role of venom allergens are likely to be provided by venomomics, that is, the application of proteomics to venoms [28]. By this approach, a number of new proteins and peptides from Hymenoptera venom have been identified and evaluated for immunological significance. *In vitro* techniques such as two-dimensional polyacrylamide gel electrophoresis, tandem mass spectrometry and peptide mass fingerprinting have allowed previously unknown micro-heterogeneity to be detected, including post-translational modifications and protein isoforms of Hymenoptera venom allergens [10]. The increasing availability of recombinant venom proteins and their use by the protein microarray technique will be able to provide the IgE reactivity profile in single patients [29].

Such component-resolved diagnosis obtained by a unique test on a small amount of serum has the optimal characteristics to become the reference standard for *in vitro* diagnosis of HVA, but currently it is used by a very low number of physicians. However, appropriate use of single recombinant allergens may achieve similar data to reach the correct diagnosis. When conventional diagnosis by skin tests and/or *in vitro* IgE tests using whole venoms show apparent pluri-sensitization, the physician has two choices. The first is to perform VIT with all the venoms eliciting a positive response; this choice has the drawback of increasing the cost of the treatment because of the number of venoms that are used which eventually have been proved unnecessary. The additional risk with this strategy is sensitizing the patient to allergenic venom proteins to which the patient is not initially sensitive. The second choice is to limit the number of venoms for VIT, for example on the basis of clinical history data or venom inhibition serology. This choice has the drawback of exposing truly pluri-sensitized patients to potentially severe reactions if they receive a sting from a venom not used by their VIT.

In patients with apparent double sensitization to honeybee and yellow jacket venom, the use of recombinant Api m 1, Ves v 1 and Ves v 5 may yield the correct identification of the causative venom [30]. In patients with apparent double sensitization to yellow jacket and wasp (*Polistes*) venom, the use of Ves v 5 and Pol d 5, that is, the species-specific allergens, will help identify which of the two vespids is responsible. Such allergens must be used in patients with apparent sensitization to honeybee and vespid (including both yellow jacket and wasp) venom. In one study, the use of non-glycosylated recombinant Api m 1, Api m 2, and Ves v 5 ruled out the IgE binding to carbohydrate epitopes and allowed to identify the sensitizing venom [31].

In any case, the role of CCDs in causing cross-reactivity can be assessed using MUXF3 or (if this is not available) bromelain. In fact, the use of MUXF3 is preferable because it has 7 glycosylated side chains, while bromelain has only 1 glycosylated side chain. The MUXF3 however is less readily

available. It is apparent that methods to remove CCDs from venom will further improve the diagnostic process [32].

The opposite diagnostic problem is the occurrence of negative skin tests and *in vitro* tests in patients with a history of systemic reactions to stings [33], because in such cases VIT is not clinically indicated. Once it has been excluded that inadequate techniques were used, possible explanations are: 1) the loss of sensitivity over time; 2) the variability of venom skin tests [34]; 3) the presence of very low amounts of specific IgE not detected by tests; 4) the failure to test the truly causative venom specificity [35]; and 5) the possibility that the reaction was due to a non IgE-mediated mechanism. The latter is true for patients with mastocytosis [36], which is increasingly recognized as an important factor favouring anaphylaxis to insect stings and may be revealed by measurement of tryptase, the marker of mast cell degranulation [37]. In the other instances, in the past the only advisable procedure was to repeat the tests to detect a possible shift to positivity, but currently there are other approaches. One is using dialyzed venom in skin testing, from which irritating low molecular weight substances have been removed. This allows venom concentrations exceeding the recommended limit of 1 mcg/ml to be employed without inducing false positive intradermal skin test responses [10]. Golden *et al.* reported that around 80% of patients with a history of systemic reactions to stings and negative skin tests to standard venom showed a positive result to intradermal skin tests at concentration of 10 mcg/ml or less [38]. Another valid diagnostic alternative is the basophil activation test (BAT), by which a substance (e.g. Venom) is tested with whole blood specimens by flow cytometry, for its capacity to activate the basophil as assessed by the expression of the surface marker CD63 [39]. Performing BAT showed a positive response to bee or wasp venom in 60% of patients with negative intradermal skin tests [40]. It was reported that CCDs may affect the results of this test as well; in fact BAT with CCD-depleted venoms was positive only with the venom eliciting a positive intradermal test [41].

TREATMENT OF HYMENOPTERA VENOM ALLERGY

Usually, subjects experiencing allergic reactions to Hymenoptera stings are given precautions to reduce the risk of being stung [9] but this can not rule out the possibility of this ever happening in the future. Other preventive measures could include the use of insect repellents or venom absorbers applied at the site of the sting. There are patents on the insect repellent Dihydronepetalactone [42] and on a venom absorber located on a vacuum suction tube [43], which require validation in future controlled studies.

When a systemic reaction to a sting occurs, an emergency treatment is needed. The principal medical treatment for anaphylactic reactions is epinephrine by intramuscular administration, preferably in the thigh muscle. This is the only measure which can rapidly reverse the effects of the massive release of mediators by mast cells [44, 45]. Various studies have demonstrated that a delay in administering epinephrine is associated with an increase in complications, such as biphasic anaphylaxis (which is often more severe), and mortality [46-48]. The failure to use epinephrine is often

caused by the fear of adverse effects, but the risk of severe side effects is extremely low compared to the risk of fatal anaphylaxis if epinephrine is not used [49]. Antihistamines and glucocorticosteroids are frequently used to treat anaphylaxis as first line drugs instead of epinephrine, but recent Cochrane systematic reviews have demonstrated that there is no evidence to support their administration in anaphylactic reactions [50, 51].

The optimal treatment of allergic reactions to Hymenoptera stings is VIT. This valuable therapy was introduced in the late 70s by two controlled studies using vespid venom [52] and honeybee venom [53], respectively. More recently, another double-blind placebo-controlled study demonstrated the efficacy of VIT with ant venom [54]. For ethical reasons, following single demonstrations by double-blind trials, subsequent studies were conducted in an open fashion. A meta-analysis performed in 2000 on available studies confirmed the clinical efficacy of VIT [55]. VIT is indicated in adults with systemic reactions of any severity, while children are generally admitted only when respiratory or cardiovascular reactions are present, as skin reactions have a very good prognosis [6, 56]. VIT has also been recommended for some occupationally-exposed individuals (e.g. beekeepers) who experience severe large local reactions.

The build-up phase of VIT may be performed with a number of schedules, including conventional, clustered, rush schemes in 3-4 days and ultrarush schemes in a few hours [56]. Then, the common maintenance dose of 100 mcg is administered at monthly intervals, but in the ensuing years the intervals may be progressively increased to 3 months maintaining efficacy and safety [57]. Of note, to date no fatal reaction to stings has been reported while the patient is receiving VIT. Concerning overall efficacy, VIT prevents any kind of reaction in more than 90% of treated patients, though a lower rate has been reported for honeybee venom [58]. Most residual reactions are slight moderate, however also severe reactions have been reported. In the case of incomplete protection, doubling the maintenance dose to 200 mcg is recommended, even though in rare cases higher doses are needed [59].

VIT has a good safety profile and notably no fatal reaction to treatment has been reported. This represents a difference when compared to immunotherapy with inhalant allergens. However, as has emerged in a number of studies, VIT with honeybee venom has a lower safety record compared with vespid venom. In fact, the rate of side-effects is higher than 20% with honeybee venom while is 2-3% with vespid venom [60]. Thus, it is necessary to find a solution to the lower safety record issue for bee venom for VIT. A number of approaches have been tried: 1) using venom allergoids, that is, monomethoxy-polyetilen glycol-modified venom, resulted in better safety but lower efficacy [61]; 2) using retard, alum-adsorbed preparations gave a significantly lower rate of large local reactions but not of systemic reactions [62-64]; 3) similar results showing significant reductions only in large local reactions were obtained using purified vs. nonpurified venoms [65]; 4) preventive medication with antihistamines reduced both local and systemic reactions [66, 67]; the treatment with the anti-IgE antibody omalizumab prevented severe systemic reactions allowing

the maintenance dose to be reached [68, 69]. As far as alum-adsorbed preparations are concerned, it should be taken into account that a possible association between alum (and other adjuvants) and autoimmune/inflammatory conditions was recently suggested [70].

A recent study has reported that baseline serum tryptase levels may be useful as predictors of side effects during the build-up phase of VIT; however a significant association was detected for wasp venom but not for bee venom VIT [71]. Of note, another study showed that tryptase levels decline during long-term VIT (median time 4.2 years, range 2-12 years), as measured by a 2.5% decrease per year. The authors suggested that such decrease could be induced by a dampened mast cell function or a decline in mast cell burden during VIT [72]. These observations are related to the issue of mastocytosis in HVA. This particular condition is not as rare as once believed and recent studies have demonstrated that VIT is effective and relatively safe (the incidence of side-effects is increased, especially with vespid venom) in venom-allergic patients with mastocytosis [73]. In particular, in studies on patients with mastocytosis a much higher incidence of severe anaphylaxis following hymenoptera stings than in the normal population was observed. In patients with HVA, elevated baseline tryptase is strongly associated with severe anaphylaxis. During VIT, most patients with mastocytosis are protected from further sting reactions. Based on these observations, in all patients with anaphylaxis following hymenoptera stings baseline serum tryptase should be measured.

Concerning optimal duration of VIT, 3-5 years is the currently recommended duration, based on observations from several studies showing long-lasting protection from stings after 3-5 years of treatment [74-77]. Results taken from the latest study observed that out of 181 patients who had received VIT for more than 3 years, 100 (55.2%) were stung after discontinuing the treatment. At the time of the first sting after stopping VIT, 92 patients had a local reaction and 8 had a systemic reaction. Of 40 patients who were stung more than once after stopping VIT, 7 (17.5%) had more severe reactions after subsequent stings. All the patients reported that their reactions after ending VIT were milder than before treatment [77]. However, in patients with systemic reactions to VIT or not completely protected from stings longer durations are recommended [56]. In particular, patients with mastocytosis must be managed with special care, because there are reports of fatal reactions to stings following discontinuation of VIT [78]; this strongly suggests that treatment should be life-long [73].

All the properties reported above support the validity of VIT as a valuable treatment. Further research is still required to improve its efficacy and safety using new materials based on tolerogenic peptides, recombinant allergens, chimeric proteins, and DNA vaccination. Table 3 reports the materials thus far investigated [79-86]. Recombinant allergens are the most likely material to be introduced in a near future, fulfilling the concept of patient-tailored immunotherapy [87].

Recently, additional routes of administration have been proposed. In a "proof-of-concept" study sublingual immunotherapy with bee venom was able to reduce large local reactions [88]. However, the sublingual route is currently not

Table 3. New Materials Proposed for Venom Immunotherapy.

Author (Reference)	Material	Purpose
Müller <i>et al.</i> [79] Fellrath <i>et al.</i> [80]	Long synthetic peptides derived from phospholipase A2	Increasing efficacy and safety
Spertini <i>et al.</i> [81]	Contiguous overlapping peptide fragments from bee venom forming an amino acid sequence	Increasing efficacy
Forster <i>et al.</i> [82]	Natural and recombinant enzymatically active or inactive phospholipase A2	Increasing efficacy
King <i>et al.</i> [83]	Recombinant allergens with reduced allergenicity but retaining immunogenicity	Increasing safety
Kusseby <i>et al.</i> [84]	Fusion protein composed of the 2 major bee venom allergens phospholipase A2 and hyaluronidase	Increasing safety
Karamloo <i>et al.</i> [85]	Chimeric protein including phospholipase A2, hyaluronidase and melittin fragments with overlapping amino acids	Increasing efficacy and safety
Jilek <i>et al.</i> [86]	DNA vaccination by phospholipase A2 sequence-carrying DNA plasmids.	Increasing efficacy and safety

recommended for VIT [89]. Intralymphatic injection of phospholipase A2 elicited an immune response even stronger than the subcutaneous injection in an experimental model [90], but as yet there are no clinical studies on this interesting approach.

CURRENT & FUTURE DEVELOPMENTS

For decades, whole body extracts were believed to be an adequate material for use in the diagnosis and treatment of patients allergic to Hymenoptera stings. However, hypersensitivity is induced by allergenic proteins that are naturally present in negligible amounts in whole body extracts. An important milestone was reached in the late 1970s for diagnosis and treatment with the introduction of purified venoms as diagnostic and treatment material. Since then, VIT has provided allergic subjects with complete protection from fatal anaphylaxis and prevented about 90% of reactions of any kind to stings. The cross-reactivity among some venom components, with particular importance for cross-reacting carbohydrate determinants, has made it difficult to identify the causative insect for selection of the proper venom to be used in VIT. Recently, the introduction of purified and recombinant allergens, such as Api m 1 from honeybee venom, Ves v 5 from yellow jacket venom, and Pol d 5 from wasp venom, have permitted a more precise diagnosis with identification of the causative venom component(s). This has paved the way for patient-tailored VIT in the near future. Another challenge is improvement in the safety of VIT with honeybee venom, which is significantly less favourable compared with vespine venom. A number of molecular approaches are under investigation to fulfill this goal.

CONFLICT OF INTEREST

Cristoforo Incorvaia is a scientific consultant for Stallergenes, Milan, Italy. The other authors declare they have no conflict of interest.

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