

EXPERT
REVIEWS

In vitro diagnosis of Hymenoptera venom allergy and further development of component resolved diagnostics

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For most people Hymenoptera stings result in transient and bothersome local inflammatory responses characterized by pain, itching, redness and swelling. In contrast, for those presenting an IgE-mediated allergic reaction, a re-sting may cause life-threatening reactions. In such patients, correct diagnosis is an absolute prerequisite for effective management, i.e. venom-specific immunotherapy. Generally, identification of the offending insect involves a detailed history along with quantification of venom-specific IgE antibodies and venom skin tests. Unfortunately, due to uncertainties associated with both tests, correct diagnosis is not always straightforward. This review summarizes the potentials and limitations of the various *in vitro* tests that are currently being used in the diagnosis of Hymenoptera venom allergy. Particular attention is paid to the potential of novel cellular tests such as basophil activation tests and component-resolved diagnosis with recombinant venom allergens in the diagnostic approach of patients with difficult diagnosis, i.e. cases in whom traditional venom specific IgE and skin tests yield equivocal or negative results. Finally, this review also covers the recent discoveries in the field of proteome research of Hymenoptera venoms and the selection of cell types for recombinant allergens production.

KEYWORDS: allergy • basophil activation • component-resolved diagnostics • Hymenoptera venom • IgE • proteomics • recombinant expression • venomics

For most people, Hymenoptera stings result in transient and bothersome local inflammatory responses characterized by pain, itching, redness and swelling. In contrast, for those presenting an IgE-mediated allergic reaction, a re-sting may cause life-threatening and even fatal reactions [1]. Worldwide Hymenoptera venom allergy is mostly related to stings of vespids, apids and to a lesser degree ants.

According to the European academy of allergy and clinical immunology guidance [2], venom immunotherapy (VIT) should be offered to all subjects who have suffered from a systemic reaction following a Hymenoptera sting and who demonstrate evidence for an IgE-mediated reaction. In contrast, allergy testing should be avoided in patients not suffering from systemic reactions, as VIT is generally

not required in those cases. Anyhow, patients presenting with a systemic reaction after a Hymenoptera sting should undergo basal tryptase measurement (normal value ≤ 11.4 ng/ml) [3–5]. Since an elevated baseline tryptase is an important predictor of severity of reaction, lifelong VIT is recommended in those patients presenting a basal value higher than 11.4 ng/ml. Furthermore, measurement of baseline tryptase offers a noninvasive method to screen patients for a clonal mast cell disease (CMD). Prevalence of CMD in Hymenoptera venom allergy is higher than in the general population going from 1 to 7.9%, with a variable but significant number of patients displaying an elevated basal tryptase but lower 20 ng/ml. This cutoff point is used to perform a bone marrow biopsy [3]. However, there is no

consensus to perform bone marrow biopsy in those patients. Finally, although rarely, an elevated serum tryptase/CMD can be the only finding in patients with systemic anaphylactic reaction after Hymenoptera sting without evidence of IgE-mediated sensitization.

At present, confirmatory diagnosis of Hymenoptera venom allergy is based on thorough history about the clinical outcome and entomological description of the stinging insect along with quantification of venom-specific IgE (sIgE) antibodies or venom skin tests (VSTs). Ideally, diagnosis of a Hymenoptera venom allergy should be documented by unequivocal and concordant venom sIgE and VSTs results. Unfortunately, several studies have revealed that correct entomological identification of the offending insect is not always straightforward. In a recent study, such a definite diagnosis could only be established in 91 of 148 (61%) of the patients with alleged allergy to wasp venom (Yellow Jacket venom [YJV]) [6]. Like in many other series [7–12], difficulties in *in vitro* diagnosis of YJV and honeybee venom (HBV) allergy arose primarily from a lack of specificity of the traditional venom-based sIgE assay with a lot of cases demonstrating double-positive results in the solid-phase assay (see below).

Today, advances have been realized in two *in vitro* diagnostic methods that both have shown promise for improved diagnosis for Hymenoptera venom allergy, that is, flow-assisted basophil activation tests (BATs) and component-resolved diagnostics (CRD) using purified and/or recombinant venom proteins. This review focuses on the drawbacks and promises of the currently applied *in vitro* tests to diagnose Hymenoptera venom allergy.

Quantification of venom-specific IgE

Generally, confirmation of a clinical suspicion of a Hymenoptera venom allergy starts with quantification of venom sIgE antibodies along with appropriate VST. However, as already exemplified in the introductory paragraph, both these tests fail to demonstrate an absolute accuracy and to correctly predict the outcome. First, it should be kept in mind that both tests can yield false-negative results. In a recent series of 308 patients with YJV allergy, a combination of titrated skin prick and intradermal tests correctly identified 282 of 301 (93.7%) of the cases, whereas the conventional YJV sIgE assay, that is, not spiked with recombinant Ves v 5 (see below) yielded a sensitivity of 83.4% [13]. Similarly, in our series, sensitivity of the conventional YJV sIgE assay was 88.5%, and no culprit was delineable in 7% of the patients with a compelling history of YJV allergy [6]. Second, tests can show a limited sensitivity that is related to the nature of applied venom allergen extract. As a matter of fact, conventional and rVes v 5-spiked venom sIgE assays and VST have been using whole-venom extract preparations that harbor highly cross-reactive structures that can significantly impede diagnosis by causing clinically irrelevant sIgE results. Indeed, although the vast majority of patients are allergic to only a single venom, up to two-thirds of the patients display double-positive sIgE results to both YJ and HB venom but generally not in skin testing [6–12]. Today, after more than a decade of intense research, it has emerged that the most

important reason for the extensive immunological *in vitro* cross-reactivity between both venoms is due to the presence of structurally related N-linked carbohydrate determinants (CCDs) rather than from homologous peptide epitopes such as found in Ves v 2 from YJV and Api m 2 from HBV (recently reviewed in [14]). Actually, important allergen components from both venoms such as Api m 1, Api m 2, Ves v 1 and Ves v 3 are glycoproteins that undergo a post-translational glycosylation and harbor asparagine (N)-linked glycans carrying a core -1,3-fucose that is highly immunogenic for humans. Moreover, this core α -1,3-fucose moiety is not restricted to YJ, HB and bumblebee venom, but is ubiquitously present in plant glycoproteins to form a typical complex form N-glycans together with a β -1,2-xylose. Specific IgE antibodies directed against these glycan pan-epitopes provide the basis of an extensive *in vitro* cross-reactivity that extends beyond insect venoms but also involve pollen, plant-derived food and natural rubber latex from *Hevea*. Hence, their designation as cross-reactive CCD [15]. Among a Flemish population, sensitization to CCD was detected in about one-quarter of the patients with pollen allergy and one-fifth of patients with a genuine Hymenoptera venom allergy, particularly HBV allergy [16]. For a recent review about the origin and diagnostic consequences of CCD sensitization in Hymenoptera venom allergy, the reader is referred elsewhere [14]. From this review, it is clear that because of the highly deceptive nature of anti-CCD IgE antibodies, prudence should be called upon interpretation of double-positive YJV and HBV sIgE results. Actually, all patients showing doubtful results should be offered additional testing to discriminate between true double sensitization and mere *in vitro* cross-reactivity. Strategies that can be adopted to detect and circumvent the CCD issue, such as the use of glycan biomarkers [17] and BAT as well as molecular diagnostics applying nonglycosylated species-specific allergen components, are discussed below. Unlike YJV and HBV, venoms of different *Polistes* species have recently been demonstrated to be devoid of any α 1,3-core fucosylation and hence to allow CCD interference free diagnostics [18].

Cellular tests

For decades, functional *in vitro* assays have focused on T-lymphocyte proliferation and transformation [19] based upon the incorporation of [[methyl-3H]³]thymidine and basophil histamine as well as sulphidoleukotriene release. However, the time-consuming and cost-consuming two-step approach (cell incubation and quantification of mediators in the supernatant) as well as poor sensitivity have limited their entrance in mainstream use.

In 1981, Nakagawa *et al.* [20] were the first to report on flow cytometric analysis of human basophil degranulation in response to stimulation with HBV. However, it was only in the early 1990s that the discovery of the basophil activation marker CD63 [21] has induced the development of the currently applied flow cytometric technique to quantify allergen-specific *in vitro* activation of basophils [22]. As displayed in TABLE 1, during the last

decade, the technique has repeatedly proven to be a reliable instrument in the diagnosis of Hymenoptera venom allergy, provided that optimal stimulation conditions are applied, and it is performed in an experienced laboratory. Besides, the BAT has been demonstrated to constitute a valuable asset in cases demonstrating double-positive [11,12,23] or negative venom sIgE and/or skin test results [23,24]. FIGURE 1 displays a representative plot of a patient with double-positive sIgE results for YJ and HBV. It has been stated that the BAT fails to discriminate between systemic reactors and large local reactions. However, this is not surprising given the pathogenic mechanism of large local reactions. Up to 70% of the patients with large local reactors have been demonstrated to display positive venom sIgE and/or VST results [25]. As a matter of fact, the criticism that BAT not able to discriminate between systemic and local reactors seems moot, since in the current European Academy of Allergy and Clinical Immunology guidance, there is no rationale for testing the latter [26]. There is no firm evidence that BAT might discriminate between mild and more severe systemic reactors. Whether the BAT might add to the diagnosis in patients with mastocytosis with negative results of traditional tests seems controversial with pro [27] and cons [28]. Alternatively, it has been shown that BAT unlike sIgE to be helpful in monitoring VIT [29–31]. Most recently, novel basophil activation techniques analyzing alternative readouts such as intracellular signaling molecules [32,33] and intracellular histamine content [34] have also been applied to diagnose venom allergy and monitor VIT. Today, the main drawbacks of BAT that have hampered its entrance in mainstream use is the need for fresh whole blood and the test being not readily accessible and requiring particular expertise.

Component-resolved diagnostics

In 2009, almost a decade after the introduction of the BAT into the field of venom allergy [35], Müller *et al.* [10] demonstrated that the recombinant species-specific major allergens Ves v 5 from YJV and Api m 1 from HBV, both produced in *Escherichia coli* and therefore not being glycosylated, could help to discriminate between genuine allergy and cross-reactivity. Since, the added value of rVes v 5, rVes v 1 and rApi m 1 (r stands for recombinant) in the diagnosis of Hymenoptera allergy has been confirmed by several authors [6,11,13,36–40]. In patients with allergy to YJV, the diagnostic sensitivity of a combination of the currently available YJV allergen components rVes v 5 and rVes v 1 has been reported to be as high as 92–96%, whereas the frequency of sensitization to Api m 1 in HBV allergy appears to vary between 58 and 80% [6,37,41,42]. Besides, in two of these studies, it has been demonstrated that the newly developed YJV solid-phase assay complemented with rVes v 5 has a substantially higher sensitivity than the traditional YJV test and that the spiking native venom with rVes v 5 does not hamper correct diagnosis in patients monosensitized to Ves v 1 [6,13]. In a pilot study, sIgE reactivity to the commercially available rApi m 1 was detected in 72%, to rApi m 2 in 48%, to rApi m 3 in 47%, to nApi m 4 and rApi m 5 in 23% and to rApi m 10 in 62%. Positive results to at least one

Table 1. Basophil activation tests in wasp and bee venom allergy.

Antigen	Reference test	Sens	Spec	N	Ref.
Wasp, bee, hornet	H	100	100	55	[35]
Wasp, bee	H	91	100	35	[76]
Wasp	H	92	80	70	[29]
Wasp	H	85	83	87	[77]
Bee	H	91	100	87	[77]
Wasp, bee, polistes	H	91	100	34	[78]
Bee	H	100	100	12	[79]
Wasp	H	97	100	39	[79]
Wasp	H	99 [†]	100	94	[30]
Wasp	H	87	97	84	[80]
Bee	H	90	95	58	[80]
Wasp [*]	H	100	100	32	[32]

[†]Taking into account the nonresponders to positive control stimulation and allergen, the sensitivity of the basophil activation test is 84%.

^{*}This study, for the first time, uses the intracellular signaling molecule p38 MAPK as a read out for basophil activation in Hymenoptera venom allergy.

H: History (clinical outcome and entomologic identification); N: Number of enrolled patients and (stung) control individuals; Sens: Sensitivity; Spec: Specificity.

allergen were detected in 94% of all HBV allergic patients. A positive result to at least one of the HBV-specific allergens (Api m 1, 3, 4 or 10) was detected in 90% of the patients. In wasp venom allergic patients, no IgE reactivity was demonstrable to rApi m 1, rApi m 2, rApi m 3, nApi m 4 and rApi m 10 and only in 1 of 40 to rApi m 5 [43]. Finally, from a Spanish series, it has emerged that CRD can be helpful to discriminate between *Vespula vulgaris* and *Polistes dominula* allergy, provided that the antigen 5 (Ves v 5, Pol d 5) and phospholipases (Ves v 1, Pol d 1) of both vespid species are used [44]. For a review on wasp and bee venom proteins and peptides for diagnosis of venom allergic patients, see [45].

Despite the identification and expression of various venom allergen compounds of fire ant (*Solenopsis invicta*) such as Sol i 1, Sol i 2, Sol i 3 and Sol i 4 [46,47], jack jumper ant (*Myrmecia pilosula*) such as Myr p 1.010 (pilosulin 1) [48] and its predominant form Myr p 1.0102 ([Ile⁵]pilosulin 1) [49] and [50], the major allergen Myr p 2 (pilosulin 3) [49,50] and Myr p 3 (pilosulin 4.1) [50,51] and Chinese needle ant (*Pachycondyla chinensis*) such as Pac c 3 [52] to our or knowledge, no readily available component-specific solid-phase assays have been developed, and data on the clinical relevance of these allergen components remain scarce (for review: [53]). Allergens that have been characterized and cloned are summarized in TABLE 2.

Allergen discovery

The characterization of novel allergens is an important step toward the improvement of CRD. Compounds are incorporated

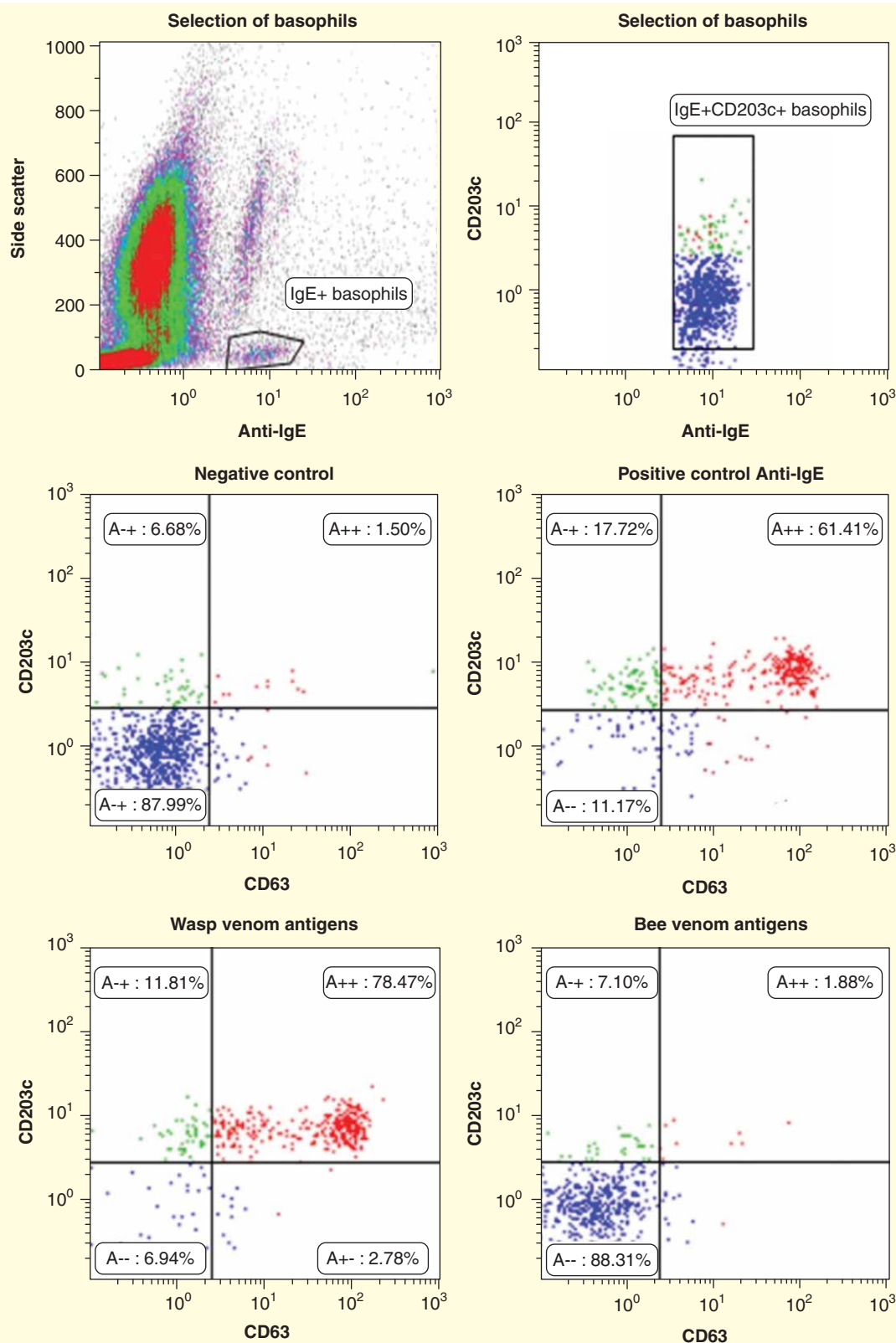


Figure 1. Representative basophil activation plots of a patient with allergy to wasp venom who demonstrates double-positive specific IgE results for yellow jacket venom and honeybee venom as a result of sensitization to cross-reactive carbohydrates. Selection of peripheral blood basophils according to side scatter, surface-bound IgE and spontaneous expression of the lineage-specific marker CD203c [Top panels]. Activation of basophils is quantified according to upregulation of CD203c and appearance of CD63. Stimulation with negative and positive control stimulation [mid panels], wasp venom [bottom left], bee venom [bottom right].

Table 2. Allergen components Hymenoptera venom.

Species	IUIS nomenclature	Allergen	MW (kDa)	Glycosylated
Wasp (<i>Vespula vulgaris</i>)	Ves v 1[†]	Phospholipase A1	35	-
	Ves v 2a and b	Hyaluronidase	42	+
	Ves v 3	Dipeptidylpeptidase	100	+
	Ves v 4	CUB protease		
	Ves v 5[†]	Antigen 5	25	-
	Ves v 6	Vitellogenin	200	+
Polistes	Pol d 1	Phospholipase A1	34	
	Pol d 4	Serine protease	33	+
	Pol d 5[†]	Antigen 5 family	23	
Honey bee (<i>Apis mellifera</i>)	Api m 1[†]	Phospholipase A2	16–20	+
	Api m 2	Hyaluronidase	43	+
	Api m 3	Acid phosphatase	49	+
	Api m 4	Mellitin	3	-
	Api m 5	Dipeptidylpeptidase	102	+
	Api m 6	Protease inhibitor	7–8	-
	Api m 7	CUB serine protease	39	+
	Api m 8	Carboxyl esterase	70	+
	Api m 9	Carboxypeptidase	60	+
	Api m 10	Icarapin	29	+
	Api m 11	MRJP 8	65	+
		MRJP 9	60	+
	Api m 12	Vitellogenin	200	+
Bumblee bee (<i>Bombus pennsylvanicus</i>)	Bom p 1	Phospholipase A2	16	+
	Bom p 4	Protease	28	
Bumblee bee (<i>Bombus terrestris</i>)	Bom t 1	Phospholipase A2	16	+
	Bom t 4	Protease	28	
Ants (<i>Solenopsis invicta</i>)	Sol i 1	Phospholipase A1B	34	+
	Sol i 2			-
	Sol i 3	Antigen 5 family	24	-
	Sol i 4			-
Ants (<i>Pachycondyla chinensis</i>)	Pac c 3	Antigen 5 family	24	
Ants (<i>Myrmecia pilosula</i>)	Myr p 1.010	Pilosulin 1	6	-
	Myr p 1.020	[Ile ⁵] pilosulin 1	6	-
	Myr p 2	Pilosulin 3	5.6	-
	Myr p 3	Pilosulin 4.1	8.2	-

Major allergen components are indicated in bold.

[†]Anno 2013 available for component-resolved diagnostics as nonglycosylated recombinant proteins Phadia Thermo Fisher.

IUIS: International Union of Immunological Sciences; MRJP: Major royal jelly protein.

Data taken from [81].

into the International Union of Immunological Societies official list of allergens [54] in case IgE binding is demonstrated by five sera of patients allergic to the respective allergen source or in case of IgE binding by at least 5% of all tested sera of patients allergic to the respective allergen source. The identification of novel allergens is often performed by 2D-PAGE separation of the source extract, followed by immunoblotting using sera of allergic patients and protein identification of IgE-recognized spots by mass spectrometry. Alternatively, individual proteins are purified or produced as recombinants, and their IgE recognition is analyzed by ELISA, spot blot or western blot, followed by confirmatory *in vitro* cellular tests such as the BAT.

The immunological characterization of low abundant venom proteins is mostly based on tests with recombinant homologs,

as here sufficient quantities of pure proteins can hardly be obtained using purification strategies [55]. In addition, the recombinant technology allows to select an expression host that produces recombinants containing the preferred post-translational modifications. Seen the clinical relevance of CCDs, the choice whether recombinants should be produced with or without glycosylation needs to be considered with great care. Several cell lines are available, which allow the recombinant production of CCD-lacking proteins. However, prokaryotically produced, nonglycosylated proteins may suffer limitations regarding folding, solubility, activity and IgE epitope conservation, while yeasts produce aberrant glycosylations. Insect cell lines provide expression hosts that are phylogenetically as close as possible to the parental organism, making

them suitable for recombinant insect venom protein production. In addition, expression in insect cells mostly results in secretion of biologically active and soluble proteins, usually in glycosylated form. This added glycosylation is more authentic than that from other hosts, although variation between different insect cell types has been noticed. Seismann and co-workers [56] showed that HighFive (*Trichoplusia ni*) and Sf9 (*Spodoptera frugiperda*) cells both produce N-linked glycosylations, but HighFive glycosylations include α -1,3-core fucosylations, which are lacking in Sf9 cells. These fucose residues are known as the only CCDs of Hymenoptera venoms. Consequently, the evaluation of the allergenicity of novel hymenopteran venom proteins is preferably executed using the baculovirus-mediated infection of Sf9 insect cells, as this cell line produces the natural insect-specific post-translational modifications, but without CCDs, which interfere with the identification of proteinous epitopes.

Seen the large numbers of venom proteins recently discovered that still are awaiting for full immunological characterization, this research domain would benefit from high-throughput recombinant expression systems. One can argue that cell-free protein synthesis could be an option [57]. And in case of the study of less common sting allergies, where the few available patient sera should be treated with caution, one would benefit from miniaturization of the immunological assay, requiring only minutes of the precious serum. Here, the protein microarray technology could be valuable [58,59].

Unraveling the venom proteome

A prerequisite to deepen the potential of CRD is a thorough knowledge of the venom protein composition and the species-specific/cross-reactive venom components of a broad range of stinging hymenopteran species [60]. Although for some species several major allergen components have been characterized, an in-depth knowledge of their venom composition is often lacking. The proteomic technologies used to explore the venom compositions have gone through a remarkable evolution. Most research conducted in the 1970s and 1980s relied on single compound-oriented, time-consuming and low sensitive techniques, identifying only a few, primarily high abundant compounds. Venom constituents were often isolated by chromatographic means followed by bioassays to determine their function. Only since 2000, mass spectrometry (MS) was used for profiling of Hymenoptera venoms [61]. The shotgun proteomics strategy, based on digesting proteins into peptides and sequencing them using tandem MS, has been widely adopted. Many early 'venome' studies combined 2DE with mass spectrometric identification, for instance, MALDI-TOF/TOF [62,63]. However, this gel-based approach lacks dynamic range and sensitivity to allow the detection of low abundant compounds. MS studies of in-liquid digested liquid chromatography (LC) venom fractions may overcome these issues and gain deeper insights in venom proteomes/peptidomes. Moreover, sample pretreatment using a combinatorial peptide ligand library (CPLL) can significantly improve the coverage of the proteomic analyses as was demonstrated in the latest HBV MS study, performed in the context of the re-

sequenced honeybee genome [64,65]. The solid-phase CPLL consists of a bead-bound set of possibly 64 million different hexapeptides, which are bound by constituents of the protein mixture upon incubation. High abundant proteins saturate their high-affinity binding sites and excess protein is washed away, while low abundant proteins are enriched by concentration on their specific affinity ligands. This technology is commercially available since 2007 under the trade name ProteoMiner and has been used in studies of the 'deep' venom proteome of two snake species, namely the Western diamondback rattlesnake (*Crotalus atrox*) [66] and the African puff adder (*Bitis arietans*) [67,68], which led to the discovery of a large number of proteins previously undetected in these proteomes.

The modular arrangement of MALDI and ESI with different types of mass analyzers has resulted in a wide variety of mass spectrometric instrumentation. Fourier transform (FT)-based mass spectrometers provide the highest performance in mass resolution and mass accuracy [65,69,70] as they measure m/z values as frequencies, which can be obtained more accurately than any other experimental parameter. It has so far only been used in the studies of the Hymenopteran venoms of the honeybee [64,65] and of the parasitoid wasp *Nasonia vitripennis* [71,72]. In case of the HBV, the combination of an improved genome assembly (Amel_4.5), a new gene annotation set (OGSv3.2), CPLL sample pretreatment and shotgun LC-FT-ion cyclotron resonance (ICR), MS analysis resulted in an unexpectedly rich venom composition of in total 102 proteins and peptides [64,65]. Another mass analyzer that matches the resolved power and mass accuracy of the FT-ICR technology is the Orbitrap technology [73]. The venom compositions of the Africanized honeybees and two European subspecies, *A. m. ligustica* and *A. m. carnica*, have been studied applying a shotgun LC-MS/MS analysis incorporating such an Orbitrap mass analyzer [74]. In-depth proteomic insights have also been obtained for the venoms of the neotropical social wasps, *Agelaia pallipes pallipes* and *Polybia paulista*, and the red imported fire ant *S. invicta*.

However, an important drawback of the FT-ICR and Orbitrap technologies is that because of their high sensitivity, they may also identify low or even extremely low abundant compounds with no toxic functions, collectively named 'venom trace molecules' [60]. These include a group of common secretory proteins exerting essential functions in the extracellular space, for example, immunity-related proteins and apolipoproteins. However, most trace molecules are typical secretory pathway proteins being unintentionally released due to an inefficient retrieval and retrograde transport within the secretory pathway of the highly active secreting venom gland tissue [64,65]. In case of the HBV, they represent more than half of the IDs (58/102), which might obscure the following selection of putative new allergens. Moreover, at present, it is not known whether these trace molecules evoke seroconversion.

Finally, it should be noted that the analytic power of these FT-ICR and Orbitrap mass spectrometers soon will be supported by an exponentially growing number of available insect genomes. The 5000 arthropod genomes initiative aims at

sequencing and analyzing the genomes of species having beneficial and negative effects on humankind [75].

Expert commentary

This review aims at summarizing the evidence for BAT and CRD with recombinant venom allergens in patients with difficulty of diagnosis. As a matter of fact, during the last decade, it has emerged that correct entomological identification of the responsible insect is not always easy and that incorrect diagnosis might jeopardize patient's life. The most important reason for misdiagnosis, undoubtedly, is the presence of double-positive venom sIgE results because of sensitization to complex asparagine (N)-linked glycans carrying a core α 1,3-fucose moiety. Both BAT and recombinant proteins free from such glycans produced in prokaryotic cells, yeasts or Sf9 (*Spodoptera frugiperda*) insect cells have increasingly been shown to constitute reliable techniques to improve correct diagnosis in patients with double-positive venom sIgE results. Despite the efficiency of VIT, the mechanisms and biomarkers associated with clinical efficacy remain elusive. Studies monitoring conversion of venom sIgE and VST have consistently demonstrated these tests to be of poor predictive value and generally fail to reflect induction of clinical tolerance. In contrast, evidence is emerging BAT not only to constitute a tool to take the sting out of difficult cases but also to enable monitoring successful treatment.

Five-year view

In the next 5 years, we predict the expansion and mainstream application of recombinant venom components in the *in vitro*

diagnosis of IgE-mediated Hymenoptera venom allergy. Minimizing the number of patients with negative or equivocal test results should finally lead to an improved therapeutic management. We hope that novel basophil activation assays that allow simultaneous quantification of immunophenotypic alterations, changes in signal transduction and release of mediators on a single cell level will further unveil the effects of VIT on effector cells. Basic research on the proteome of Hymenoptera venoms is a prerequisite to deepen the potential of CRD. This research domain will benefit from the new generation of extremely powerful mass spectrometers and the 5000 arthropod genomes initiative aims at sequencing and analyzing the genomes of species having beneficial and negative effects on humankind [79].

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Key issues

- Hymenoptera venom allergy constitutes a potential life-threatening condition with considerable risk of diagnostic error.
- Although correct diagnosis is a prerequisite for Hymenoptera venom immunotherapy, correct entomological identification of the culprit insect is not always straightforward.
- It is increasingly recognized that novel tests such as component-resolved diagnostics and basophil activation tests can improve diagnosis, particularly in patients who display negative or equivocal results for traditional venom sIgE tests and venom skin tests. However, additional collaborative studies are required before these can enter mainstream application.
- Despite the high efficiency of venom immunotherapy, the mechanisms and biomarkers associated with clinical efficacy remain elusive. Studies monitoring basophil responsiveness have demonstrated this test to be of better predictive value than venom skin tests and sIgE that generally fail to reflect induction of clinical tolerance.

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