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Review

Bee, wasp and ant venomics pave the way for a component-resolved diagnosis of sting allergy

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ARTICLE DATA

ABSTRACT

With the complete sequencing of its genome, the honey bee is now a preferred model organism for Hymenoptera species, also with respect to venomous studies. Major pitfalls in proteomic profiling are: i) highly abundant proteins masking low-copy number proteins; ii) high heterogeneity in proteomes due to isoforms, protease activity and PTMs; iii) the inability for protein function assignment. If genomic information is not available, proteins still might be identified through cross-species protein identifications or MS/MS data-based de novo sequencing techniques. Venomous approaches discovered several new proteins and peptides from honey bees, bumble bees, ants and different wasp species, and some of these constituents were proven to be of immunological significance. Further digging in the proteome/peptidome will yield more so-called "venom trace elements" with only a local function in the venom duct or reservoir or released by leakage of the gland tissue. An impressive list of recombinant venom proteins has become available from a diverse range of Hymenopterans. Protein microarray allows the determination and monitoring of allergic patients' IgE reactivity profiles to disease-causing allergens using single measurements and minute amounts of serum. The information the physician will get from such a single run will largely exceed the output from current IgE capturing tools using whole venom preparations.

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1. Introduction

The venoms from stinging bees and wasps are important weapons in the defence of the colony or themselves. A single sting will kill other insects instantly, but will only cause a transient and bothersome local inflammatory reaction characterized by pain, redness and swelling in humans (Fig. 1, 2). Massive envenomation, for instance by the more aggressive Africanized bees, can produce both immediate and delayed toxic reaction in man [1], sometimes leading to the death [2]. Nevertheless, fatality due to bee venom is more frequently related to allergic responses of the victim than to envenomation.

Worldwide Hymenoptera venom allergy is generally caused by the stings of vespids of the genera *Vespula* (e.g., *Vespula vulgaris*, *V. germanica* and *V. maculifrons*), *Vespa* (*Vespa crabro*), *Dolichovespula* (*Dolichovespula maculate*, *D. media*) and *Polistes* (*Polistes gallicus*, *P. dominulus*, *P. annularis*, *P. exclamans*) and of apids of the genera *Apis* (*Apis mellifera*, *A. cerana*, *A. dorsata*) and *Bombus* (*Bombus terrestris*, *B. pennsylvanicus*). Two ant genera are also of importance: *Solenopsis* (*Solenopsis invicta*, *S. geminate*) and *Myrmecia* (*Myrmecia pilosula*) (Fig. 3). Hymenoptera venom allergy is an IgE-mediated allergic hypersensitivity of non-atopic origin [3] and the most frequent clinical patterns are: (i) large local reactions exceeding 10 cm in diameter and 24 h in duration and (ii) rapid-onset (usually within 10 min after sting) generalized immediate-type hypersensitivity reactions such as pruritus, urticaria, angioedema, nausea, vomiting, diarrhea, rhinoconjunctivitis, bronchospasm, hypotension, cardiovascular collapse and unconsciousness [4]. Systemic reactions have been reported to occur in 0.8–5% of the general population [5,6]; they may be severe and even life-threatening with 0.09–0.45 deaths per million within the general population [7].

Generally, physicians rely upon quantification of specific IgE antibodies and skin tests to diagnose venom allergy. Unfortunately, these tests lack absolute sensitivity and specificity, making the diagnosis of Hymenoptera venom allergy not always straightforward [8]. Indeed, up to 50% of the diagnostic test output is double positive to both bee and vespid venoms. This can be explained by truly double sensitization if the patient was stung by both bees and wasps, or by cross-reactivity between allergens of the two venoms, particularly between the carbohydrate epitopes they share [9]. As the patient cannot always provide the entomologic identification of the culprit, it sometimes remains obscure which life-saving venom-specific immunotherapy should be started. Promising in vitro test methods based on the venom-specific stimulation of basophils are increasingly introduced, and pushed the sensitivity and specificity of the diagnostic tools further upwards [10,11]. However, serologic as well as effector-cell based diagnosis of venom allergy is currently performed with whole venom preparations, containing in addition to allergens, other non-allergenic components.

At the best, current diagnosis of bee or wasp venom allergy only permits the identification of a given allergen source, but not of the molecular entities involved in the adverse immunological reactions.

Protein microarrays have recently been introduced as promising tools for the simultaneous assessment of specific IgE antibodies against multiple recombinant or purified natural allergens involved in food or pollen allergy [12–15]. The concept of using separate allergens to determine the patients sensitization profile was termed “component-resolved diagnosis” (CRD). Originally aimed at providing the basis for patient-tailored forms of immunotherapy [16], this approach was found to have several other advantages related to the diagnostic test requirements (little amounts of serum), performances (sensitivity, specificity), standardization (concentration, structural integrity, batch-to-batch variation) and

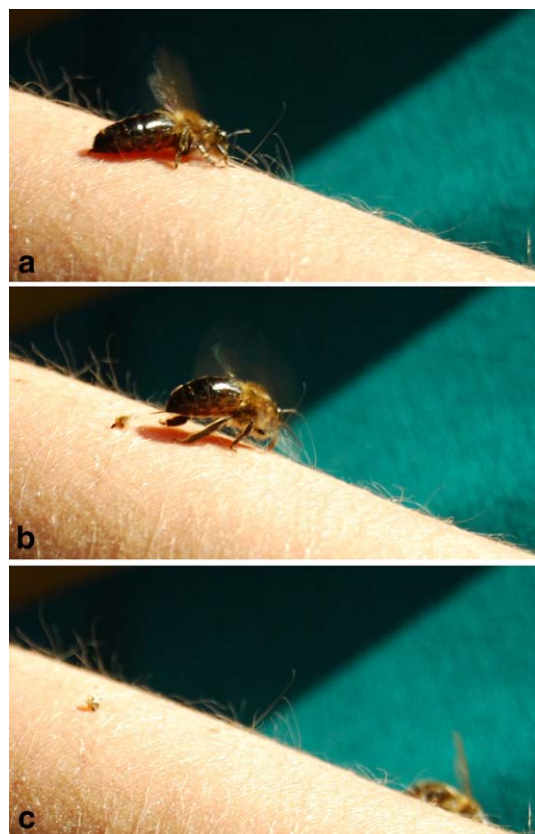


Fig. 1 – Stinging honey bee (a–c). The honey bee carries a barbed stinger that remains stuck in human elastic skin (a). When the bee attempts to escape after the sting, the whole sting apparatus and venom reservoir remain in the victim (b) and may continue to inject venom (c). Wasps, yellow jackets, hornets, and bumblebees can sting their victims multiple times.



Fig. 2 – The normal reaction to a bee sting is characterized by pain, redness and swelling. Some parts of the body are more sensitive than others and swelling is most noticeable on the face.

interpretation (risk assessment on the likelihood and severity of allergic reactions) [17].

In the present paper the recent developments in the field of Hymenoptera venomics are reviewed. First we stand still by the more technical aspects of venomics. Thereafter we will comment on how the information gained from recent venom studies can be applied in the diagnosis of bee, wasp and ant allergy.

2. Venom approach

2.1. Venom collection

Venomics start with sampling of the venom, a crucial step in the experimental set-up as it may obscure the output of the ultimate analytical stage of the experiment by untrue hits or identification failures due to contaminants or sample degradation, respectively. Honey bee venom sampling is well documented [18], and two methods are traditionally retained for analytical purposes: the dissection of the venom glands (including the reservoir) and the so-called “electric milking”. When large quantities of venom are wanted, electric milking is the most suitable. Here a large number of bees are stimulated by mild, high-frequency electric shocks on their way out of the hive, causing them to sting through a nylon net and to deposit a droplet of venom onto a glass plate. Air-dried venom powder is then scraped off. However, from similar techniques with spiders it is known that contamination by saliva or digestive tract fluids may occur [19]. Honey bee salivary α -glucosidase

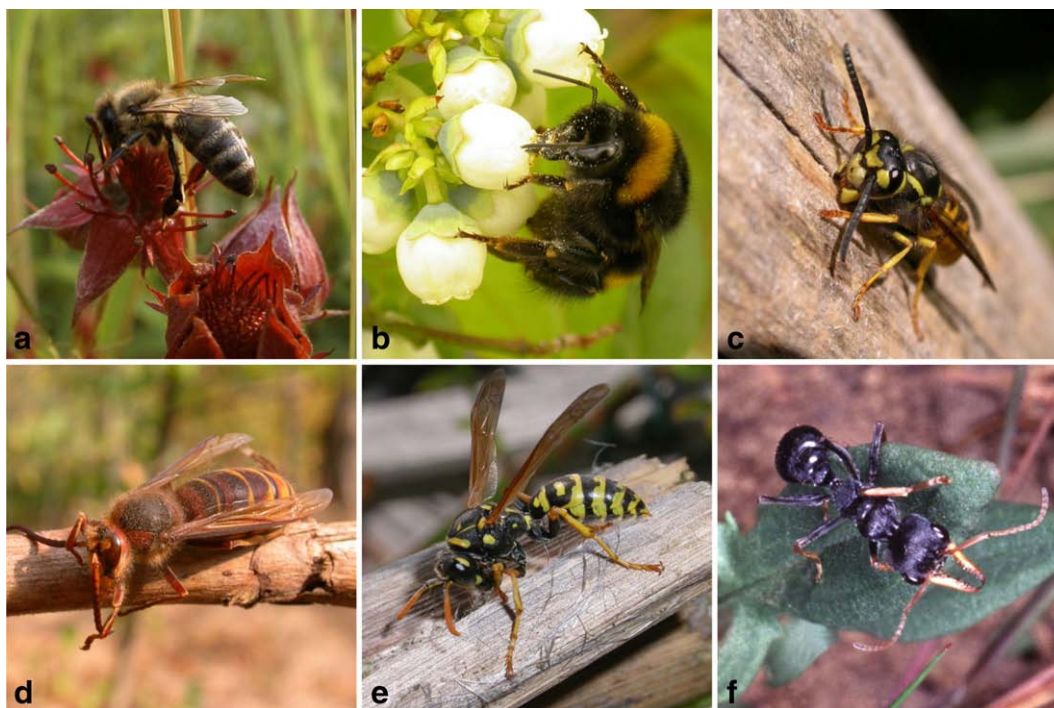


Fig. 3 – Some stinging Hymenopteran species: *Apis mellifera* (a), *Bombus terrestris* (b), *Vespa germanica* (c), *Vespa cabrio* (d), *Polistes dominulus* (e) and *Myrmecia pilosula* (f) [pictures provided courtesy and remain copyright of respectively Informatiecentrum voor Bijenteelt, Ghent University (a); Biobest N.V. (b); Devalez Jelle (c–e); and Prof Simon GA Brown, University of Western Australia (f)].

has long been misrecognized as venom constituent due to such a sample contamination [20]. Fortunately, current mass spectrometry techniques require only minute amounts of sample permitting safer sampling techniques. The venom collected from a single specimen can give access to a wealth of information [21], but on its turn lacks individual variation related to the bee/wasp genetic constitution [22] or age [23], or caste differences [24], or moment and place of sampling [25]. When venom gland and reservoir are dissected, the venom can be released by a simple centrifugation step. Preferentially, this step is preceded by a washing step in physiological solution, in order to prevent contamination by hemolymph that stuck on the outside. Peiren et al. [26] collected venom by dissecting the whole sting apparatus and emptying the reservoir by gently compressing (manual milking). The droplet of venom that appeared at the top of the stinger was immediately stabilized in denaturing and reducing buffer. A recent proteomic study of honey bee venom gland tissue revealed a number of protein spots that might serve as markers for leakage of cytoplasmic or structural components in future experiments: glutathione-S-transferase S1 isoform A (BI513885) and two endocuticular proteins (GB16211-PA, GB18929-PA) [27].

2.2. Proteomics

Hymenopteran venoms have been intensively studied in the past and still, at present, they are subjected to modern venom profiling studies. Originally, crude venom extracts were separated via chromatography and interesting fractions, i.e. purified peptides and proteins, were further biochemically characterized using bio-assays, Edman degradation chemistry, spectroscopy and eventually X-ray crystallography [28]. Although these techniques showed to be useful in the initial characterization of venom compounds, they are single compound oriented, time-consuming and little sensitive. This contrasts sharply with novel profiling techniques: in a mass spectrometric based approach Favreau et al. were able to detect venom peptides from a single specimen of *Bombus lapidarius* (bumble bee) [21].

With the recent high throughput genome sequencing efforts, more and more complete genomes are becoming publicly available and therefore stimulating large-scale protein mapping experiments. In a classical bottom-up proteomic approach [29,30], protein extractions are separated by SDS-PAGE or 2D-PAGE (Fig. 4), after which individual protein bands and spots are proteolytically digested and subsequently identified using peptide mass fingerprinting (PMF) or/and tandem mass spectrometry (MS/MS) with database searching algorithms as MASCOT [31] or SEQUEST [32]. With the introduction of shotgun proteomics [33], using multidimensional LC combined with modern mass spectrometers, proteomes are divulging their secrets at an increasing rate as well as revealing new bottle-necks and challenges for future studies. Micro-heterogeneity, post-translational modifications, protein isoforms and the complexity of protein networks, however, make deeper research in proteomics necessary, evolving in new proteomic domains as peptidomics, glycomics and phosphoproteomics.

With the complete sequencing of its genome, *Apis mellifera* (honeybee) [34] is now a preferred model organism for Hymenoptera species. A pilot study on the bee venom proteome, using 2D-PAGE, confirmed most of the previous characterized venom proteins [26,27]. Another study, on *A. mellifera* royal jelly, showed that combined 2D-PAGE and shotgun approaches result in similar, yet partially complementary protein identifications [35]. Additionally, both studies reveal some of the major pitfalls in proteomic profiling: i) highly abundant proteins masking low-copy number proteins; ii) high heterogeneity in proteomes due to isoforms, protease activity and PTMs; iii) the inability for protein function assignment.

Although MS-based protein profiling is highly dependent on genomic information, several studies report protein identifications from organisms without sequenced genomes. Song et al. published a proteomic analysis on *Plutella xylostella* larvae parasitized by *Cotesia plutellae*, a solitary endoparasitoid wasp [36]. In total, they detected 152 proteins differentially expressed over several time points after parasitization, from which 26 could be identified even though the genome of *P. xylostella* has not been sequenced. The MS/MS spectra were searched against the NCBI database using MASCOT or MSBLAST [37] resulting in protein hits emanating from both homolog proteins in related species as from *P. xylostella* ESTs, deposited in NCBI from previous cDNA studies. Also important, they demonstrated that quantitative real-time PCR data of the sapin 2 mRNA does not follow the same expression profile as the sapin 2 protein, thereby supporting the assumption that mRNA expression patterns not always correlate with protein abundance. In a similar study, Charneau et al. identified 58 proteins from the saliva proteome of *Triatoma infestans* [38]. MS/MS data were submitted for database searching using MASCOT. Unmatched spectra were further interpreted with the PepNovo software tool [39] and

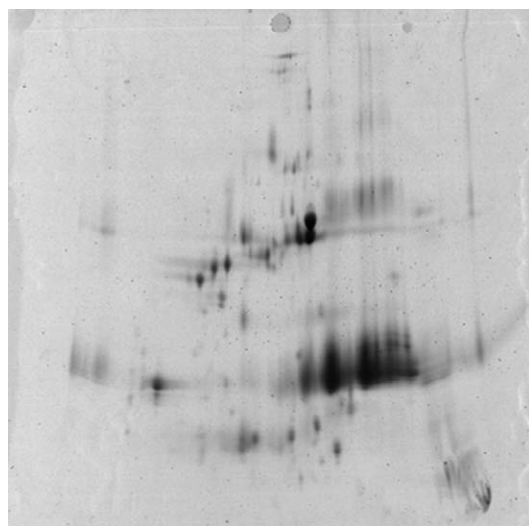


Fig. 4 – In a classical bottom-up proteomic approach, protein extractions are separated by 2D-PAGE after which individual protein spots are proteolytically digested and subsequently identified by mass spectrometry. The shown 2D-gel is from bumble bee (*Bombus terrestris*) venom.

these de novo amino acid sequences were subsequently submitted for identification on the MSBLAST tool.

Conclusively, if genomic information is not available, proteins still might be identified through cross-species protein identifications. Usually, homolog proteins from strongly related species only differ by a few amino acids, still allowing identification by PMF or MS/MS [40]. As homology decreases, automated database searching fails and MS/MS data need de novo sequencing techniques combined with database blasting for proper identification and annotation. It should be considered that for protein identifications from organisms without sequenced genomes, the power of shotgun proteomics is significantly reduced compared to 2D-PAGE or related approaches. Obviously, the connectivity between peptides from the same proteins is lost in peptide centered approaches, resulting in lower identification scores compared to 2D-PAGE where a complete set of peptides contribute to homology based identification. This was recently discussed in a review on proteomic approaches for non-model plants [41].

2.3. Glycomics

Because of the allergic response and the intention for future vaccine development, it is important to discriminate between carbohydrate- and protein-based epitopes. Several studies indicated that both are able to inflict immune responses [42,43]. In glycomics, several strategies have been developed for high-throughput screening of glycomes. Using the precursor ion scan function on triple quadrupole mass spectrometers, proteomes can be screened for glycosylated peptides [44,45]. Due to the complexity and heterogeneity of glycan structures, additional techniques were developed to focus more on structure determination. In general, glycans are chemically or enzymatically cleaved from the proteins, labeled with APTS or PA (for negative charge) and analyzed using CE-MS or LC-MS [46]. A glycoproteomic analysis on yellow jacket venoms by Kolarich et al. characterized the N-linked glycans on hyaluronidase and its newly identified homologue [47]. Comparison of these glycans between other wasp and bee venoms showed high homology and partially explains the cross-reactivity in in-vitro immunological tests.

2.4. Peptidomics

Besides (glyco)proteins, peptides carry out important biological processes in venom. Their activities are very diverse and range from neurotoxic and inflammatory to anti-bacterial. Due to their physicochemical properties, peptides can directly be analyzed with mass spectrometric means. This so-called peptidomics, its experimental design and instrumentation are reviewed in Boonen et al. [48]. In wasp venoms, several peptides are described and include mastoparans, protonecins, chemotactic peptides and waspkinin, a bradykinin-homologue. Whereas chemotactic peptides recruit macrophages and polymorphonuclear leukocytes near the site of stinging, mastoparans and protonecins were proven to act as mast cell degranulating peptides responsible for histamine release. Mastoparans are believed to sustain an amphipathic, α -helical conformation permitting the formation of porin structures in membranes and so releasing histamine. Besides,

these peptides are assumed to trigger histamine release via G-protein coupled receptor cascades as well and, considering the venom proteome, mastoparans are thought to regulate some venom enzymes as phospholipase A2. Several mastoparan and protonecins homologues have been sequenced from different wasp species using Edman degradation and tandem mass spectrometry [49–51]. Although amino acid sequence within these peptides is mainly conserved, amino acid substitution seem to alter the bio-activity of the considered peptide showing an interesting basis for further use in pharmacological applications [51,52]. Turillazo et al. identified two new mastoparan-like peptides from *P. dominulus* with strong anti-bacterial properties, dominulin A and dominulin B. Although MALDI-MS analysis directed on wasp body parts prove these peptides mainly to be located on the wasps cuticle, they were also shown to be present in the venom [53]. Although peptidomes are mainly characterized via HPLC, Wiese et al. published a gel-based protocol for studying the peptidome of the jack jumper ant (*M. pilosula*) [54]. Using one-dimensional acid urea PAGE and two-dimensional AU-SDS PAGE, they were able to identify 3 new disulfide bridge linked dimers by comparing non-reduced and reduced/alkylated venom.

The peptidome of bee venom has also been largely elucidated, consisting mainly of apamin (a neurotoxin), a mast cell degranulating peptide and melittin. The latter is considered a bee venom allergen (api m 4). In a very recent publication, Franscese et al. report a MALDI imaging experiment in rat tissue stung by *A. mellifera* [55]. In a time-point experiment they were able to record the distribution of several venom peptides and proteins in stung tissue. In the same experiment, they pointed out the appearance of rat peptides and proteins who were previously identified as markers for the inflammatory event evoked by bee venom. More studies like this in the near future, will play key-roles in the elucidation of the physiological processes in stinging accidents and its attending immune/allergic response.

3. Application in allergy diagnosis

3.1. Allergen discovery

In Table 1 the Hymenoptera venom proteins and peptides are listed that were newly discovered by one of the venomous approaches. Remarkably, peptidomics yielded the most new venom constituents with 19 peptides identified in bumble bees (*B. lapidarius*), ants (*M. pilosula*) or different wasp species (*P. dominulus*, *P. major major*, *P. rothneyi*, *P. paulista* and *P. exigua*). Particularly, the finding of new peptides, so-called pilosulins, in the venom of jack jumper ant (*M. pilosula*) was proven to be of immunological significance. Wiese et al. [54,56] found a new variant (pilosulin 3.1) of the already known allergen pilosulin 3 (Myr p 2), a heterodimer of pilosulin 3a and pilosulin 3b, differing in an additional glycine on the 3b chain. Pilosulin 4.1, a homodimer of 8198 Da, was recognized by 9 out of 54 (17%) sera from patients with a history of jack jumper ant sting allergy and was designated as the minor allergen Myr p 3

Table 1 – Hymenoptera venom proteins/peptides recently found by venom studies and their contribution to allergy

Species ^a	Common name	Protein/peptide	Allergen ^b	References
<u>Agelaia pallipes</u>	Wasp	Antigen 5	sIgE	[62]
		Serine protease	sIgE	[62]
<u>Apis mellifera</u>	Honey bee	Acid phosphatase	Api m 3	[28]
		Dipeptidylpeptidase IV	Api m 5	[28]
		Carboxylesterase	Api m 8	[28]
		Icarapin	sIgE	[26,28]
		PVF-1		[26]
		MRJP9		[26]
		Hexamerin-2		[28]
		MRJP8		[27]
<u>Bombus lapidaries</u>	Bumble bee	Bombolitin 6		[21]
		Bombolitin 7		[21]
		Bombolitin 8		[21]
<u>Myrmecia pilosula</u>	Australian jumper ant	Pilosulin 3.1	var. Myr p 2	[54]
		Pilosulin 4.1	Myr p 3	[54]
		Pilosulin 5		[54]
<u>Polistes dominulus</u>	Mediterranean paper wasp	Dominulin A		[53]
		Dominulin B		[53]
<u>Polistes gallicus</u>	Wasp	Phospholipase	Pol g 1	[57]
		Antigen 5	Pol g 5	[57]
		Hyaluronidase	sIgE	[57]
		Protease		[57]
<u>Polistes major major</u>	Wasp	PMM1		[80]
		PMM2		[80]
		PMM3		[80]
<u>Polistes rothneyi</u>	Wasp	Polistes-mastoporan-R1		[51]
		Polistes-mastoporan-R2		[51]
		Polistes-mastoporan-R3		[51]
		Polistes-protonectin		[51]
<u>Polybia paulista</u>	Wasp	Polybia-MPI		[49]
		Polybia-CP		[49]
<u>Protopolybia exigua</u>	Wasp	Protopolybia-MP I		[50]
		Protopolybia-MP II		[50]
		Protopolybia-MP III		[50]

^a Underlined species names are not yet included in the IUIS allergen list.

^b IUIS nomenclature of allergens was provided; sIgE, specific IgE response in patients with allergy detected; var., variant of a known allergen.

according to the IUIS (International Union of Immunological Societies) nomenclature.

The proteomic approach revealed homologues of some common wasp allergens in *P. gallicus* and *Agelaia pallipes*. In *P. gallicus* venom phospholipase, antigen 5, hyaluronidase and protease were characterized by mass spectrometry and specific binding to IgE [57]. Based on an EAST (Enzymatic Allergo Sorbent Test, Allercoat™ 6, Biorad), phospholipase and antigen 5 were proven to be strong allergens, similar to those in other vespids [58–60]. A smaller amount of IgE against hyaluronidase was found, confirming the lower allergenic activity of this protein [61]. The former two are now designated as Pol g 1 and Pol g 5, respectively. Antigen 5 and serine protease from the venom of the South American wasp *A. pallipes* were identified as the major antigenic proteins using sera from three *A. pallipes*-sensitized-patients [62].

Far the most progress has been made in the characterization of the constituents of honey bee venom. In 2005 Peiren et al. [26] found three proteins never described in Hymenoptera venoms before: a member of the platelet-derived and vascular endothelial growth factor protein family (PVF-1), the second protein showed no homology with any known protein, and was named icarapin later on [63], and thirdly, a protein resembling major royal jelly

protein (MRJP) 8, but designated MRJP9 by Albert et al. [64]. Subsequently, it was demonstrated that recombinant icarapin was recognized by IgE from 4 out of 5 bee venom allergic patients, all beekeepers [63]. In 2006 Hoffman [28] was able to unravel approximately half of the amino acid sequence of bee venom acid phosphatase by mass spectrometry, permitting further completion by a PCR based approach. The acid phosphatase activity of bee venom was already described three decades earlier [65], and its allergenic characteristics were then already known (designated as allergen B). According to the IUIS nomenclature the enzyme is now named Api m 3. Recombinant Api m 3 was recognized by IgE antibodies of pooled serum in Western blots and by 37% of the individual sera of honeybee venom-sensitized patients in an ELISA (Enzyme-linked Immunosorbent Assay) [66]. In the laboratory of D.R. Hoffman four more venom proteins were identified [28]: carboxylesterase, an enzyme also found by Kettner et al. [67], carbohydrate-rich protein, (synonym for icarapin) also discovered by Peiren et al. in the previous year [26], hexamerin-2, an arylphorin insect storage protein and lastly, dipeptidylpeptidase IV a membrane-bound enzyme involved in the processing of melittin in the venom duct. In the meantime, two of these proteins received an IUIS allergen name: dipeptidylpeptidase IV (Api m 5) and carboxylesterase (Api m 8). Finally,

in a proteomic study of venom gland tissue another member of the MRJP protein family, MRJP8, was unambiguously identified. Because of the presence of a signal peptide it was suggested that MRJP8, like MRJP9 (see above), is secreted in the venom reservoir [27].

The question now raises whether the actual honey bee venom profile can be considered complete. We do not think so. At first, several 2D-PAGE spots of bee venom remained unidentified [26] and the low molecular weight range should be further explored. Secondly, the molecular work revealed another striking feature of the venom components: their protein heterogeneity. Api m 6, a minor bee venom allergen, exists as four isoforms of 7190, 7400, 7598 and 7808 Da, respectively, differing in their primary structure at the amino and carboxy-terminus by a maximum of six amino acids [68]. Recently, it was shown that Api m 6 derives from a single locus, and that substantial protein-level variation is due to allelic variation [22]. Cloning of icarapin also revealed different transcript variants, but here the cause was alternatively spliced exons [63]. Further study is needed to understand the complexity of this venom protein heterogeneity and its immunological significance.

It is also remarkable that the biological function of some of the newly discovered low abundant venom constituents do not correspond anymore with the principal function of venom as defence weapon. For instance, dipeptidylpeptidase IV, a membrane-anchored enzyme that sequentially cleaves dipeptides ending in proline or alanine in order to activate melittin into the venom duct [28], has probably no function once it is injected in the victim. It seems reasonable to believe that further digging in the proteome/peptidome of Hymenoptera venoms will eventually yield more of these “venom trace elements” occurring in extreme low quantities, and which have only a local function in the venom duct or reservoir (maturation and stabilization of the secreted, protection and recovery of the tissue) or which are normal cell components, released by leakage of the gland tissue.

Nevertheless, the case of dipeptidylpeptidase IV demonstrates that these trace elements can be immunologically relevant.

3.2. Recombinant allergens

At present, an impressive list of recombinant venom constituents has become available, covering a diverse range of Hymenopteran species of origin (Table 2). In fact, the recombinant technology has become a grateful tool to evade problems related to the traditional chromatographic purification of venom constituents. Moreover, excessive amount of “venom trace elements” can easily be produced in a vector of choice, permitting to select whether the end-product should undergo secondary modifications (prokaryotic/eukaryotic), and where and how it should be tagged in order to permit easy one-step affinity purification [63,69]. Biologic activity and IgE recognition of venom allergens expressed in eukaryotic expression systems were shown to be comparable with those of natural enzymes [70,71]. While diagnostic tests with bee venom are more sensitive, the specificity with recombinant allergen (PLA2, prokaryotic) is clearly increased as compared with natural bee venom [72]. This lower sensitivity can be improved by making diagnostic cocktails of recombinant (PLA2 and hyaluronidase) and synthetic (melittin) venom allergens, without losing specificity [73]. Genetic engineered fusion proteins composed of two (Api m 1/2) [74] or three (Api m 1/2/3) [75] venom allergens have been constructed for usage in allergen-specific immunotherapy or as a vaccine, respectively. For more information on recombinant proteins and peptides as therapeutic reagents for arthropod allergies we refer to the recent review by Ramos et al. [76].

3.3. Component-resolved diagnosis

There are different IgE capturing and effector-cell based tools for diagnosing allergic diseases that permit the use of separate

Table 2 – Recombinant Hymenoptera venom allergens

Species	Protein/peptide	Accession number	MW (kDa)	Allergen	Vector ^a	References ^b
<i>Apis mellifera</i>	Phospholipase A2	NP_001011614	16	Api m 1	P	[81]
	Hyaluronidase	NP_001011619	43	Api m 2	P, E	[70,82]
	Acid phosphatase	AAV57281	47	Api m 3	E	[83]
	Dipeptidylpeptidase IV	NP_001119715	100	Api m 5	ND	IUIS
	CUB serine protease	NP_001011584	39	Api m 7	P	[84]
	Carboxylesterase	NP_001119716	70	Api m 8	ND	IUIS
	Icarapin	ABF21077	29		P	[63]
			58	Api m 1/2	P	[74]
			66	Api m 1/2/3	P	[75]
				Api c 4	P	[85]
<i>Apis cerana cerana</i>	Melittin	CAH05131	3		P	
<i>Dolichovespula maculata</i>	Hyaluronidase	P49371	45	Dol m 2	P	[86]
	Antigen 5	P10736	23	Dol m 5	P	[87,88]
<i>Myrmecia pilosula</i>		Q07932	7.5 or 5.5	Myr p 1	P	[89]
	Pilosulin-3	AAB47023	8.5 or 2-4	Myr p 2	P	[90]
<i>Polistes annularis</i>	Antigen 5	AAA29793	23	Pol a 5	E	[91]
<i>Solenopsis invicta</i>		P35775	14	Sol i 2	E	[92]
		P35778	26	Sol i 3	E	[93]
				Sol i 4	E	[94]
<i>Vespula vulgaris</i>	Phospholipase A1	P49369	34	Ves v 1	P	[94]
	Hyaluronidase	P49370	38	Ves v 2	P	[94,95]
	Dipeptidylpeptidase IV	ACA00159	100	Ves v 3	ND	IUIS
	Antigen 5	Q05110	23	Ves v 5	P, E	[91,96]

^a P, prokaryotic expression vector; E, eukaryotic expression vector; ND, no data.

^b IUIS, information from the IUIS list of allergens at <http://www.allergen.org/Allergen.aspx>.

allergens, but only the microarray allows the determination and monitoring of allergic patients' IgE reactivity profiles to disease-causing allergens using single measurements and minute amounts of serum. First attempts to microarray Hymenoptera venom allergens included Api m 1, Api m 2, Ves v 5, Ves g 5 and Pol a 5 in a set-up with 96 recombinant or natural allergen molecules representative of most important allergen sources [77]. Nowadays protein chips can be spotted with thousands of proteins or peptides, permitting to analyses the IgE responses against a tremendous variety of allergens. The venom allergens from different bee, wasp and ant species can be offered on a single chip, allowing to differentiate the species that has stung based on species-specific markers. Moreover, the recombinant technology will permit to microarray in parallel the different allelic variants of each allergen, together with their unglycosylated and truncated forms. The former could be valuable to elucidate the geographic location where insects with such a genetic constitution occur; the latter will allow to discriminate between carbohydrate- and protein-based cross reactivity, if this would exist. The information the physician will get from such a single run will largely exceed the output from current IgE capturing tools using whole venom preparations.

4. Future prospects

New separation techniques are constantly being developed with increasing sensitivity, resolution and dynamic range based on nano-, chip- and UPLC-technology. Combined with novel high resolution mass spectrometry, these tools will become very useful in future venomomics. In a study by Escoubas et al. over a thousand peptides were identified in a single LC-MALDI experiment on spider venoms [78]. Similar results were published by Fox et al. using several top-down and bottom-up approaches on snake venom combined with FT-ICR MS [79]. These new technologies are all very promising tools for the further and hopefully final characterization of Hymenoptera venoms. Despite these recent developments in proteomic research, venomomics remains laborious as genomic data on most Hymenoptera species and by extension venomous animals in general is scarce and protein identifications are most often based on homology database searching, de novo approaches, Edman degradation or cDNA sequencing.

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