

Diagnosis and treatment of allergy to hymenoptera venoms

Robert G. Hamilton^{a,b}

^aDivision of Allergy and Clinical Immunology, Department of Medicine and ^bDepartment of Pathology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

Correspondence to Robert G. Hamilton, PhD, Johns Hopkins Asthma and Allergy Center, Johns Hopkins Dermatology, Allergy and Clinical Immunology (DACI) Reference Laboratory, Room 1A20, 5501 Hopkins Bayview Circle, Baltimore, MD 21224, USA
Tel: +1 410 550 2031; fax: +1 410 550 2030;
e-mail: rhamilt2@jhmi.edu

Current Opinion in Allergy and Clinical Immunology 2010, 10:000–000

Purpose of review

This review overviews select advances reported in the literature from mid-2008 to the present in the diagnosis and management of individuals with immediate-type hypersensitivity to hymenoptera venom(s).

Recent findings

Skin testing remains the principal confirmatory test for sensitization to hymenoptera venoms. Use of dialyzed extracts in North America could enhance the sensitivity of intradermal skin testing. Hymenoptera venom components are overviewed with focus on their use in IgE antibody microarray assays to distinguish true dual sensitization to yellow jacket venom (YJV) and honeybee venom (HBV) proteins from carbohydrate-related cross-reactivity. Mechanisms on how venom induces vascular permeability in the skin following intradermal testing are elucidated and how tolerance is induced following high-dose venom exposure. For management, venom immunotherapy remains the most effective treatment. Use of immunotherapy in large local reactors to reduce morbidity is discussed. Baseline serum tryptase levels have been identified as one potential marker for severe systemic reactions to a subsequent sting.

Summary

Minor enhancements involving dialyzed venoms, component allergens, and microarray systems are poised to improve diagnostic confirmatory assays. Use of venom immunotherapy in large local reactors and tryptase as a predictor of a future severe venom-induced systemic reaction may enhance management programs for stinging insect allergic individuals.

Keywords

allergen, hymenoptera venom, IgE, immunotherapy, in-vivo and in-vitro diagnosis

Curr Opin Allergy Clin Immunol 10:000–000
© 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins
1528-4050

Introduction

Practice parameters for hymenoptera venom allergic patient management are under revision. Thus, the active practice parameters from 2004 comprehensively discuss the consensus diagnostic algorithm and treatment strategies for patients with stinging insect hypersensitivity [1]. This review overviews the recent reports published during the past 2 years that provide new insights into a number of specific issues related to the diagnosis and management of immediate-type hypersensitivity reactions to hymenoptera venoms.

Diagnosis

A number of recent studies have suggested improvements in the design, performance and/or interpretation of hymenoptera venom allergy-related diagnostic tests.

Dialyzed venom in skin testing

The intradermal venom skin test is the recommended diagnostic test for confirming sensitization (IgE antibody

positivity) to hymenoptera venoms [1]. Its diagnostic range is, however, limited with standard venoms because concentrations above 1 µg/ml may cause nonspecific responses. Golden *et al.* [2[•]] reported that yellow jacket venom (YJV), which has been dialyzed to remove irritating small molecular weight amines, allows accurate intradermal skin testing up to 10 µg/ml. Use of dialyzed YJV allowed positive intradermal venom skin test results to be obtained at concentrations of 10 µg/ml or less in 79% of 24 patients with a positive history of allergic reactions, but negative intradermal skin tests using undialyzed YJV. Such dialyzed venoms are available in Europe from one manufacturer, but not yet licensed for use in North America.

Venom-induced neurovascular mechanism of inflammation

Yshii *et al.* [3] studied the mechanism(s) associated with microvascular permeability, edema, and neutrophil influx that follow an intradermal injection of *Polistes* wasp venom into skin. Mass spectrometric analysis identified two peptides with sequence similarity to the C-terminal

region of tachykinin-like peptides. The authors report that increased vascular permeability induced by venom injection into the skin occurs primarily through activation of tachykinin NK(1) receptors by substance P released from sensory C fibers. This in turn induces histamine release from dermal mast cells.

Component-resolved serological diagnosis

Serological measurements of venom-specific IgE antibodies are an alternative to skin testing for confirming sensitization to hymenoptera venoms. De Graaf *et al.* [4^{••}] discuss proteomic investigations of venoms using two-dimensional polyacrylamide gel electrophoresis to identify protein spots that were subsequently proteolytically digested and identified by tandem mass spectrometry. Peptide mass fingerprinting was used to identify previously unknown micro-heterogeneity, post-translational modifications and protein isoforms of the medically important hymenoptera venom allergens. The authors discuss available native and recombinant forms of venom protein and peptide allergens, with an emphasis on their use in newly developed component-resolved diagnostic microarrays for hymenoptera venom-specific IgE antibody.

Causes of dual honeybee and yellow jacket sensitivity

About 30–50% of hymenoptera venom patients have dual skin test sensitivity to honeybee venom (HBV) and YJV. One area in which serology has a unique strength is in discriminating those patients who have a true sensitivity to venom peptide epitopes in both venoms from those who have dual sensitization that results from IgE anti-carbohydrate cross-reactivity to glycopeptides in their two hyaluronidases: Api-m-2 and Ves-v-2. Focke *et al.* [5[•]] evaluated sera from YJV only positive and HBV–YJV dual positive patients by western blot analysis using inhibition with purified Api-m-2 or isolated bromelain glycopeptides (MUXF-BSA). They concluded that only 10–15% of patients with exclusive YJV sensitivity have IgE antibody-specific for cross-reactive hyaluronidase protein. Moreover, of 83 sera tested by inhibition, 65% reacted only with hyaluronidase through cross-reactive carbohydrate determinants (CCD) and 27% reacted with both CCD and hyaluronidase peptide. Importantly, 97% of YJV-only and YJV–HBV positive sera were detected using YJV Antigen 5 and Phospholipase A1, suggesting that IgE anti-YJV analysis with these two components (in the absence of hyaluronidase) may be sufficient to get an accurate assessment of true YJV and HBV sensitization.

In a separate study, Seppala *et al.* [6[•]] identified at least one alpha 1,3 fucose residue in YJV hyaluronidase (Ves-v-2). They reported that IgE antibodies react to glycosylated native Ves-v-2 but not to nonglycosylated recombinant Ves v 2. Importantly, the glycan structures were not required for induction of T cell and cytokine

responses. Seismann *et al.* [7^{••}] extended these observations using recombinant DNA technology and cell lines that varied in their capacities for alpha 1,3 core fucosylation to produce glycosylated and nonglycosylated forms of Ves-v-2 and Api-m-2 hyaluronidases. These proteins were used in serological studies to discriminate IgE anti-Ves-v-2 and Api-m-2 protein reactivity from those IgE antibodies reacting to cross-reactive glycan structures. Future use of these novel proteins in diagnostic tests for IgE antibody with different levels of glycosylation should provide more accurate identification of the true sensitization of patients with IgE antibodies that react to both honeybee and YJVs.

Muller *et al.* [8] studied the utility of IgE antibody assays [ImmunoCAP-Phadia and ADVIA Centaur: Siemens (available only in Europe)] to discriminate between reactivity to HBV, YJV, cross-reacting carbohydrate determinants (MUXF) and species-specific recombinant major allergens from honeybee (Api-m-1) and YJVs (Ves-v-5). Of 200 sera from skin test positive patients who reported allergic symptoms to both HBV and YJV, carbohydrate-related double positivity was detected using the MUXF in 59 and 32% by ImmunoCAP and ADVIA, respectively. Cross-reacting carbohydrate reactivity was detected in 56% of sera from those patients with double positivity. IgE specific for Api-m-1 protein was detected in 97% of honeybee and 17% of *Vespula* venom allergic patients, while Ves-v-5 specific IgE was detected in 87% of *Vespula* and 17% of bee venom allergic patients. These data encourage the use of Api-m-1 and Ves-v-5 components in serological evaluation of hymenoptera venom-sensitive individuals to identify true double sensitization in support of a clinical indication for immunotherapy with both venoms.

Venom allergens

A list of the hymenoptera venom allergens that have been reported is presented in Table 1. The number of component venom allergens either in native or recombinant form continues to grow. As discussed in the previous section, a number of these component venom allergens have been used together with carbohydrates (MUXF and oilseed rape) in research studies to investigate the frequency of individuals with true dual yellow jacket and honeybee sensitivity [4^{••},5[•],6[•],7^{••},8,9[•]]. Structural features of a number of hymenoptera venom allergens have also been identified. For instance, the crystalline structure of recombinant Sol i 3 from fire ant venom (Table 1) has been shown to be similar to that of Ves v 5, with an antiparallel beta sheet that is surrounded on both sides by alpha helices [10]. Principal differences between the two molecules reside in the solvent exposed loop regions that contain amino acid insertions that contribute to the observed lack of cross-reactivity between Sol i 3 and Ves v 5. These data are important for understanding

Table 1 Reported hymenoptera venom allergens

Honeybee	<i>Apis mellifera</i> (Api-m)	
	Api-m-1	Phospholipase A2
	Api-m-2	Hyaluronidase
	Api-m-3	Acid phosphatase
	Api-m-4	Mellitin
	Api-m-5	Dipeptidylpeptidase IV
	Api-m-6	Cysteine-rich trypsin inhibitor
	Api-m-7	CUB serine protease
	Api-m-8	Carboxylesterase
	Api-m-9	Serine carboxypeptidase
	Api-m-10	carbohydrate-rich protein-Icarapin
Africanized honeybee	<i>Apis mellifera scutellata</i> (Api-me)	
Indian honeybee	<i>Apis cerana</i> (Api-c)	
	Api-c-1	Phospholipase A2
	Api-c-2	Hyaluronidase
Giant honeybee	Api-c-4	Melittin
	<i>Apis dorsata</i> (Api-d)	
	Api-d-1	Phospholipase A2
Little-red dwarf honeybee	Api-d-4	Melittin
	<i>Apis florae</i> (Api-fl)	
Indian/oriental honeybee	Api-fl-4	Melittin
	<i>Apis cerana</i> (Api-c)	
	Api-c-1	Phospholipase A2
Bumblebee	Api-c-2	Hyaluronidase
	Api-c-4	Mellitin
	<i>Bombus pennsylvanicus</i>	
	Bom-p-1	Phospholipase A2
Bumblebee	Bom-p-2	Hyaluronidase
	Bom-p-3	Acid phosphatase
	Bom-p-4	Serine Protease
	<i>Bombus terrestris</i>	
White-faced hornet	Bom-t-1	Phospholipase A2
	Bom-t-4	Serine Protease
	<i>Dolichovespula maculata</i> (Dol-m)	
Yellow hornet	Dol-m-1	Phospholipase A1
	Dol-m-2	Hyaluronidase
	Dol-m-5	Antigen 5
	<i>Dolichovespula arenaria</i>	
European hornet	Dol-a-1	Phospholipase A1
	Dol-a-2	Hyaluronidase
	Dol-m-5	Antigen 5
	<i>Vespa crabro</i> (Vesp c)	
Oriental hornet	Vesp-c-1	Phospholipase A1
	Vesp-c-2	Hyaluronidase
	Vesp-c-5	Antigen 5
	<i>Vespa orientalis</i> (Vesp-o)	
Giant Asian hornet	<i>Vespa mandarinia</i> (Vesp-m)	
	Vesp-m-1	Phospholipase A1
	Vesp-m-5	Antigen 5
Giant hornet	<i>Vespa magnifica</i> (Vesp-ma)	
Asian hornet	<i>Vespa velutina</i> (Vesp-v)	
Yellow jacket	<i>Vespula vulgaris</i> (Ves-v)	
	Ves-v-1	Phospholipase A1
	Ves-v-2	Hyaluronidase
	Ves-v-3	Dipeptidylpeptidase IV
Yellow jacket	Ves-v-5	Antigen 5
	<i>Vespula flavopilosa</i> (Ves-f)	
	Ves-f-1	Phospholipase A1
	Ves-f-2	Hyaluronidase
Yellow jacket	Ves-f-5	Antigen 5
	<i>Vespula germanica</i> (Ves g)	
	Ves-g-1	Phospholipase A1
	Ves-g-2	Hyaluronidase
Eastern yellow jacket	Ves-g-5	Antigen 5
	<i>Vespula maculifrons</i> (Ves-m)	
	Ves-m-1	Phospholipase A1
	Ves-m-2	Hyaluronidase
Yellow jacket	Ves-m-5	Antigen 5
	<i>Vespula pensylvanica</i> (Ves-p)	
	Ves-p-1	Phospholipase A1
	Ves-p-2	Hyaluronidase
	Ves-p-5	Antigen 5

Table 1 (continued)

Southern yellow jacket	<i>Vespula squamosa</i> (Ves-s)	
	Ves-s-1	Phospholipase A1
	Ves-s-2	Hyaluronidase
	Ves-s-5	Antigen 5
Paper wasp	<i>Polistes annularis</i> (Pol-a)	
	Pol-a-1	Phospholipase A1
	Pol-a-2	Hyaluronidase
	Pol-a-5	Antigen 5
European paper wasp	<i>Polistes dominulus</i> (Pol-d)	
	Pol-d-1	Phospholipase A1
	Pol-d-4	Serine Protease
	Pol-d-5	Antigen 5
Paper wasp	<i>Polistes exclamans</i> (Pol-e)	
	Pol-e-1	Phospholipase A1
	Pol-e-2	Hyaluronidase
	Pol-e-4	Serine Protease
	Pol-e-5	Antigen 5
Wasp	<i>Polistes fuscatus</i> (Pol-f)	
	Pol-f-5	Antigen 5
Wasp	<i>Polistes gallicus</i> (Pol-g)	
	Pol-g-1	Phospholipase A1
	Pol-g-2	Hyaluronidase
	Pol-g-4	Serine Protease
	Pol-g-5	Antigen 5
Paper wasp	<i>Polistes hebraeus</i> (Pol-he)	
Wasp	<i>Polistes metricus</i> (Pol-m)	
	Pol-m-5	Antigen 5
Red fire ant	<i>Solenopsis invicta</i> (Sol-i)	
	Sol-g-1	Phospholipase A1
	Sol-g-2	
	Sol-g-3	Antigen 5
	Sol-g-4	
Tropical fire ant	<i>Solenopsis germinata</i> (Sol-g)	
	Sol-g-2	
	Sol-g-3	Antigen 5
	Sol-g-4	
Black imported fire ant	<i>Solenopsis richteri</i> (Sol-r)	
	Sol-r-1	Phospholipase A1
	Sol-r-2	
	Sol-r-3	Antigen 5
Brazilian fire ant	<i>Solenopsis saevissima</i> (Sol-s)	
	Sol-s-2	
	Sol-s-3	Antigen 5
Southern fire ant	<i>Solenopsis xyloni</i> (Sol-x)	
Reddish-brown European ant	<i>Formica rufa</i> (For-c)	
Pharaoh ant	<i>Monomorium pharaonis</i> (Mon-p)	
Red bulldog ant	<i>Myrmecia gulosa</i> (Myr-g)	

Extracted from the Allergome.org.

the structural basis of the fire ant venom allergen specificity and cross-reactivity.

While HBV allergens have been extensively investigated, relatively little is known about bumblebee venom allergens. Xin *et al.* [11] identified *Bombus ignitus* phospholipase A2 cDNA. They showed that mature Bom-i PLA2 contains 10 conserved cysteine residues and a calcium-binding site analogous to other bee PLA2s. The authors used immunofluorescence staining to show that PLA2 from Bom-i functionally binds cell membranes and can induce apoptotic cell death. This study provides a rationale for the traditional use of HBV to treat a variety of diseases because of its known anti-inflammatory and anticancer effects. Gajski and Garaj-Vrhovac [12] extends the observation that bee venom inhibits proliferation and induces apoptosis of cancer cells. They studied its effects

on human lymphocytes. Using a single-cell gel electrophoresis assay, the authors report the genotoxicity of HBV on peripheral blood human lymphocytes, with the magnitude of the DNA damage being time and venom dose dependent.

Detection of venom allergens in tissues and foods

A matrix-assisted laser desorption/ionization mass spectrometry imaging system has been experimentally used to map the diffusion and distribution of Api m 1, 4 and 6 allergens and two venom toxins (apamine and mast cell degranulating peptide) following envenomation of various tissues by live honeybees [13[•]]. This procedure may provide a novel manner for studying the rate of absorption, thus facilitating the optimization of venom administration strategies during immunotherapy.

Armentia [14] reported that food challenges of patients experiencing allergic symptoms following wine consumption were negative with sulfites, wine additives and aged-wines but positive with young wines. Challenge positive subjects were shown to be serologically positive to hymenoptera venoms, despite no history of a previous sting. The author speculates that sensitization may have been induced by chronic oral consumption of venoms in wine.

Diagnostic cellular assays

A number of reports [15,16] have re-examined the utility of basophil activation test as an alternative confirmatory assay for monitoring sensitization to hymenoptera venoms. Cited limitations of the flow cytometric basophil activation test remain the current lack of a unified standardized protocol and the difficulty of getting the whole blood specimen to a clinical laboratory in a timely manner to insure viability of the basophils. When performed with a validated procedure and interpreted by an experienced clinician, the basophil activation test can aid in clarifying history positive cases that have negative skin and serological tests for venom-specific IgE.

Tolerance by high-dose venom exposure

Meiler *et al.* [17] used high-dose bee venom exposure in bee keepers as a model to study the mechanisms of T cell tolerance to allergens in healthy individuals. They report that repetitive stings in nonallergic beekeepers induces diminished T cell related cutaneous late phase swelling to bee stings in parallel with suppressed allergen-specific T cell proliferation and T helper type 1 and Th2 cytokines secretion. Rapid switching and expansion of IL-10 producing T regulatory 1 cells and the generation of cytotoxic T lymphocyte-associated antigen 4 suppressive factors are involved. These factors induce and maintain tolerance following chronic high dose allergen exposure in nonallergic individuals. T cell regulation returns to baseline levels by 2–3 months after a bee sting.

Varga *et al.* [18] examined the development of protective serum inhibitory activity as a result of bee venom immunotherapy in children. They used an IgE-facilitated allergen binding assay to show that sera obtained during venom immunotherapy significantly inhibited allergen–IgE binding to B cells compared with sera obtained from individuals after immunotherapy was withdrawal and with no venom exposure (normal controls). Their examination of the time course of IgE and IgG4 antibody and serum inhibitory changes in relation to clinical improvement led them to the conclusion that a significant reduction in venom-specific IgE antibody levels may be more relevant for long-term clinical efficacy of venom-immunotherapy than development of serum inhibitory activity.

Relevance of total IgE and specific activity in hymenoptera venom allergy

Practice parameters support the testing of serum for venom-specific IgE antibody only when there is a need to confirm sensitization for symptomatic patients. The clinical relevance of a positive venom-specific IgE antibody measurement in an asymptomatic patient remains unclear. Sturm *et al.* [19] studied the frequency of asymptomatic sensitization to hymenoptera venoms in individuals who tolerated stings in the past and had neither a history of local or systemic sting reactions. They observed that the rate of positive venom-specific IgE antibody levels in individuals with no history of hymenoptera venom allergy increased in proportion with their total IgE. They proposed that increased cross-reactive carbohydrate antibodies in individuals with high total IgEs and sensitized to multiple allergens may have contributed to this trend.

The percentage of the total IgE that is specific for a given allergen (the IgE-specific activity) may, however, be a more relevant parameter to explain this relationship with total IgE since it increases as the total serum IgE decreases [20[•]]. As the IgE-specific activity increases, there is a higher probability that IgE antibodies with the same specificity will reside close to each other on effector cells and thus increase the probability of mediator release following subsequent allergen exposure. Importantly, more than 50% of venom sensitized individuals had a venom-specific IgE to total IgE ratio >4%, which has been shown by Johansson *et al.* [21] to have clinical implications in Xolair-treated subjects. This is a higher frequency than any other of the 182 allergen specificities evaluated in the study [20[•]].

Management

Of the four management strategies for hymenoptera venom allergic patients (avoidance, pharmacotherapy, immunotherapy, and anti-IgE therapy), immunotherapy is possibly the most efficacious. Finegold has published an excellent review of issues up to 2008 related to stinging insect allergy immunotherapy [22]. His review examines issues related to patient selection, evaluation testing, venom immunotherapy dosage, schedules-intervals of therapy, the impact of concomitant medications, remote therapy, and discontinuing treatment. The current review examines observations published subsequent to the Finegold review that deal with general topic of hymenoptera venom allergic patient management.

Clinical indication for immunotherapy in large local reactors

To date, venom immunotherapy has been recommended for patients who exhibit systemic reactions following an inadvertent hymenoptera sting. Golden *et al.* [23^{••}]

suggested expanding the clinical indications for venom immunotherapy to include large local reactors where insect stings cause significant morbidity and impair the quality of life. Individuals with previous venom-induced large local reactions and positive venom skin tests underwent a sting challenge and then received venom immunotherapy up to 4 years. After 7–11 weeks of treatment, a repeat sting challenge showed the size and duration of the large local reaction had decreased 42–53% in treated patients and 18% in untreated controls. This increased up to a 70% decrease by 4 years of venom immunotherapy. For occupationally and recreationally exposed individuals (farmers, campers, horseback riders), immunotherapy improves a large local reactor's quality of life as reported by Oude Elberink *et al.* [24]. Severino *et al.* [25] studied the epidemiology and risk factors associated with venom-induced large local reactions. They advise an alternative to venom immunotherapy which is issuing a prescription for auto-injectable adrenaline to patients who experience large local reactions, since 5–10% of large local reactors will progress to experiencing a systemic reaction following a re-sting.

Predictive value of baseline mast cell tryptase

One age-old quest has been searching for a serological marker which could predict the development of a severe allergic reaction following a hymenoptera sting in a sensitized individual. A number of studies have identified baseline serum tryptase levels as one possible marker. Ruëff *et al.* [26**] performed a multicenter observational study to examine the association between baseline serum tryptase concentrations and other variables routinely recorded during patient evaluation with the frequency of past severe anaphylaxis following a field sting. They studied the patient's age and sex, the type of culprit insect, medications, prior system reactions and tryptase level in serum. Of 962 patients enrolled, 21.4% had a severe anaphylactic reaction defined by anaphylactic shock, loss of consciousness or cardiopulmonary arrest. The frequency of the severe reaction increased significantly with higher baseline tryptase concentrations, older age, male sex, or treatment with angiotensin-converting enzyme inhibitors.

Dugas-Breit *et al.* [27] examined changes in mast cell tryptase concentration during venom immunotherapy. The baseline serum tryptase level was measured before and at 4-week intervals at least twice during venom immunotherapy. They showed a small but continuous decline in the serum tryptase level with venom immunotherapy, suggesting a dampening in mast cell function or a decline in mast cell burden.

Guenova *et al.* [28] examined the utility of baseline tryptase levels in the elderly who based on epidemiology studies are more prone to have severe anaphylactic reac-

tions. They observed that sting reaction severity increased significantly with increased age and tryptase levels. Moreover, basal serum trypase levels increased continuously with age, indicating that mast cell load is at least partially responsible for the observed increased tendency for more severe allergic reactions in the elderly.

Conclusion

Diagnosis of hymenoptera venom sensitization can be enhanced through the use of dialyzed venoms in intradermal skin testing and component venom allergens in microarray assays for venom-specific IgE serology. More specifically, newly developed vespid and honeybee hyaluronidase (Api m 2, Ves v 2) possibly with and without glycosylation and Api m 1 and Ves v 5 may be particularly useful together in future diagnostic assays to differentiate true dually sensitized patients (yellow jacket, honeybee) from those who exhibit carbohydrate-related cross-reactivity. In the management of hymenoptera venom patients, immunotherapy of large local reactors to reduce morbidity and the use of baseline tryptase levels as a possible predictor for severe allergic reactions need to be considered in future management strategies.

Acknowledgements

This work has been supported by internal funds from the Johns Hopkins University DACI Reference Laboratory. Appreciation is extended to Dr. David Golden for his review and critique of this paper.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

- 1 Moffitt JE, Golden DBK, Reisman RE, Lee R, *et al.* Stinging insect hypersensitivity: a practice parameter update. *J Allergy Clin Immunol* 2004; 114:869–886.
- 2 Golden DB, Kelly D, Hamilton RG, *et al.* Dialyzed venom skin tests for identifying yellow jacket allergic patients not detected using standard venom. *Ann Allergy Asthma Immunol* 2009; 102:47–50.
- This paper discusses the utility of dialyzed venom to enhance the diagnostic sensitivity of the intradermal skin test without adversely altering diagnostic specificity.
- 3 Yshii LM, Souza GH, Camargo EA, *et al.* Characterization of the mechanisms underlying the inflammatory response to *Polistes* (paper wasp) venom in mouse dorsal skin. *Toxicon* 2009; 53:42–52.
- 4 De Graaf DC, Aerts M, Danneels E, *et al.* Bee, wasp and ant venomomics pave the way for component resolved diagnosis of sting allergy. *J Proteomics* 2009; 72:145–154.
- Review of established and novel venom protein/peptide allergens.
- 5 Focke JC, Leonard R, Jarich R, *et al.* Reassessing the role of hyaluronidase in yellow jacket venom allergy. *J Allergy Clin Immunol* 2010; 125:184–190.
- Study describing a strategy for identifying true yellow jacket venom sensitivity in individuals with dual YJV and HBV specific IgE positivity.
- 6 Seppala U, Selby D, Monsaive R, *et al.* Structural and immunological characterization of N-glycans from the major yellow jacket allergen Ves v 2: the N glycan structures are needed for the human antibody recognition. *Mol Immunol* 2009; 46:2014–2021.
- Demonstration of the differential reactivity of carbohydrate epitopes on the Ves v 2 allergen.

- 7 Seismann H, Blank S, Braren I, *et al*. Dissecting cross-reactivity in hymenoptera venom allergy by circumvention of α -1,3 core fucosylation. *Mol Immunol* 2010; 47:799–808.
- Detailed description of hyaluronidase glycosylation and its clinical significance in the diagnosis of true dual honeybee and yellow jacket sensitization.
- 8 Muller UR, Johansen N, Petersen AB, *et al*. Hymenoptera venom allergy: analysis of double positivity to honeybee and vespula venom by estimation of IgE antibodies to specific major allergens Api-m-1 and Ves-v-5. *Allergy* 2009; 64:543–548.
- 9 Erzen R, Korosec P, Silar M, *et al*. Carbohydrate epitopes as a cause of cross-reactivity in patients allergic to Hymenoptera venom. *Wien Klin Wochenschr* 2009; 121:349–352.
- Use of carbohydrate containing molecules to discriminate true dual from cross-reactive sensitization to yellow jacket and honeybee venoms or yellow jacket and Polistes wasp venoms.
- 10 Padavattan S, Schmidt M, Hoffman DR, *et al*. Crystal structure of the major allergen from fire ant venom, Sol i 3. *J Mol Biol* 2008; 383:178–185.
- 11 Xin Y, Choo YM, Hu Z, *et al*. Molecular cloning and characterization of a venom phospholipase A2 from the Bumblebee *Bombus ignitus*. *Comp Biochem Physiol B Biochem Mol Biol* 2009; 154:195–202.
- 12 Gajski G, Garaj-Vrhovac V. Genotoxic potential of bee venom (*Apis mellifera*) on human peripheral blood lymphocytes in vitro using single cell gel electrophoresis assay. *J Environ Sci Health A Tox Hazard Subst Environ Eng* 2008; 43:1279–1287.
- 13 Francese S, Lambardi D, Mastrobuoni G, *et al*. Detection of honeybee venom in envenomed issues by direct MALDI MSI. *J Am Soc Mass Spectrom* 2009; 20:112–123.
- Study of venom diffusion in tissue using mass spectrometry imaging.
- 14 Armentia A. Adverse reactions to wine: think outside the bottle. *Curr Opin Allergy Clin Immunol* 2008; 8:266–269.
- 15 Scherer K, Bircher AJ, Heijnen IA. Diagnosis of stinging insect allergy: utility of cellular in vitro tests. *Curr Opin Allergy Clin Immunol* 2009; 9:343–350.
- 16 Korosec P, Erzen R, Silar M, *et al*. Basophil responsiveness in patients with insect sting allergies and negative venom specific IgE and skin prick test results. *Clin Exp Allergy* 2009; 39:1730–1737.
- 17 Meiler F, Zumkehr J, Klunker S, *et al*. In vivo switch to IL-10 secreting T regulatory cells in high dose allergen exposure. *J Exp med* 2008; 205:2887–2898.
- 18 Varga EM, Francis JN, Zach MS, *et al*. Time course of serum inhibitory activity for facilitated allergen-IgE binding during bee venom immunotherapy in children. *Clin Exp Allergy* 2009; 39:1353–1357.
- 19 Sturm GJ, Schuster C, Kranzelbinder B, *et al*. Asymptomatic sensitization to hymenoptera venom is related to total immunoglobulin E levels. *Int Arch Allergy Immunol* 2009; 149:261–264.
- 20 Hamilton RG, Macglashan DW Jr, Saini SS. IgE antibody-specific activity in human allergic disease. *Immunol Res* 2010. [Epub ahead of print]
- Discussion of how IgE specific activity (specific IgE to total IgE) can impact on the extent of effector cell release of mediators.
- 21 Johansson SG, Nopp A, Oman H, *et al*. The size of the disease relevant IgE antibody fraction in relation to 'total-IgE' predicts the efficacy of anti-IgE (Xolair) treatment. *Allergy* 2009; 64:1472–1477.
- 22 Finegold I. Issues in stinging insect allergy immunotherapy: a review. *Curr Opin Allergy Clin Immunol* 2008; 8:343–347.
- 23 Golden DB, Kelly D, Hamilton RG, *et al*. Venom immunotherapy reduces large local reactions to insect stings. *J Allergy Clin Immunol* 2009; 123:1371–1375.
- Supportive data for expanding the clinical indications for venom immunotherapy to include large local reactions who are occupationally or recreationally exposed to hymenoptera stings.
- 24 Oude Elberink JN, van der Heide S, Guyatt GH, *et al*. Immunotherapy improves health related quality of life of adult patients with dermal reactions following yellow jacket stings. *Clin Exp Allergy* 2009; 39:774–776.
- 25 Severino M, Bonadonna P, Passalacqua G. Large local reactions from stinging insects: from epidemiology to management. *Curr Opin Allergy Clin Immunol* 2009; 9:334–337.
- 26 Ruëff F, Przybilla B, Biló MB, *et al*. Predictors of severe systemic anaphylactic reactions in patients with Hymenoptera venom allergy: importance of baseline serum tryptase: a study of the European Academy of Allergology and Clinical Immunology Interest Group on Insect Venom Hypersensitivity. *J Allergy Clin Immunol* 2009; 124:1047–1054.
- Multicenter investigation of the utility of baseline tryptase as a predictor for severe allergic reactions following a subsequent Hymenoptera sting.
- 27 Dugas-Breit S, Przybilla B, Dugas M, *et al*. Serum concentration of baseline mast cell tryptase: evidence for a decline during long term immunotherapy for hymenoptera venom allergy. *Clin Exp Allergy* 2010. [Epub ahead of print]
- 28 Guenova E, Volz T, Eichner M. Basal serum tryptase as risk assessment for severe Hymenoptera sting reactions in elderly. *Allergy* 2010. [Epub ahead of print]