Insect anaphylaxis: addressing clinical challenges
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Current Opinion in Allergy and Clinical Immunology 2011, 11:332–336

Purpose of review
Few allergic reactions are as potentially life-threatening, or frightening to the patient, as anaphylaxis. Food, medications, and insect stings are the three most common triggers of anaphylaxis, but insect allergy provides the best opportunity to understand the biology of anaphylaxis. If the physician can establish a diagnosis of insect allergy, treatment with nearly 98% effectiveness can be initiated. However, sometimes patients have a compelling history of insect sting anaphylaxis, but negative skin and blood tests. This situation presents us with a fascinating opportunity to understand the biology of insect anaphylaxis.

Recent findings
Recent and ongoing work shows that occult mast cell disease may be critical in insect anaphylaxis. Mastocytosis, serum tryptase and basophil biology are key elements; genetic markers may potentially help us diagnose at-risk individuals and determine proper treatment. Understanding basophil activation may play an additional role both in diagnosis and knowing when therapy might be terminated.

Summary
Mast cell disease, serum tryptase and basophil biology are providing an opportunity to better understand and manage insect allergy. This evolving understanding should improve long-term management of insect anaphylaxis and help us to better understand the clinical dilemma of appropriate management of the history-positive patient in which testing is unable to detect venom-specific IgE. Furthermore,omalizumab’s immunomodulatory effects may play a role in difficult-to-treat insect allergy and mastocytosis. Finally, unrelated to these, but still important as an ongoing risk factor, is the continued underutilization of epinephrine for both acute and long-term management of insect anaphylaxis.

Keywords
anaphylaxis, basophil, epinephrine, hymenoptera, mast cell, mastocytosis, tryptase

Introduction
Of the three most common triggers of anaphylaxis – foods, medications, and insect allergies [1–4] – insect allergy provides us with unique opportunities to understand the biology of sensitivity and potentially fatal anaphylactic reactions. Insect allergy diagnosis is well established and can be treated with a 98% level of protection in sensitive individuals [5,6–8]. In most cases the sting event is obvious, the nature of the reaction is clear, and specific testing can be accomplished, when appropriate [5].

In clinical practice things are not always so straightforward. What if the physician believes that the patient has had a systemic reaction to an insect sting, but the highly reliable skin and blood tests are negative? [9–11] Since these tests are the basic criteria for initiating venom immunotherapy (VIT), without specific sensitivity established, VIT would not be started. As a result, this patient may remain at a greater risk for potentially fatal insect anaphylaxis [5]. It is this biological enigma that has forced allergists to re-examine management of this vexing clinical dilemma and as a result expand our understanding of the biology of anaphylaxis. Herein, we examine the recent literature in the areas of mastocytosis, utility of serum tryptase, basophil activation, genetic expression, risk factors and management tools as they relate to insect anaphylaxis.

Insect allergy: tryptase level and mastocytosis
The appropriate management of patients with a compelling history of insect-induced anaphylaxis, yet are skin and blood-test negative, remains a clinical challenge [9,11]. Mastocytosis has emerged as a surprising link in this clinical quandary. A significant percentage of patients with severe systemic reactions following insect sting, who are demonstrated to have an elevated baseline tryptase level, indeed, may have mastocytosis. Consequently, in...
patients with mastocytosis, the most common cause of anaphylaxis is insect sting [12].

A multicenter, European study [13**] of 962 Hymenoptera venom-sensitive patients looked at predictors of severe systemic anaphylaxis following a sting. Of the 962 patients, 202 (26%) had severe anaphylaxis following a field sting. The reported severity was either grade 3 (anaphylactic shock, loss of consciousness) or grade 4 (cardiac arrest, apnea). The identified risk predictors of sting-related anaphylaxis included patients taking angiotensin converting enzyme inhibitors; patients with vespid allergy; patients of male sex; and most interestingly, patients with baseline serum mast cell tryptase levels above 5 ng/l. In a separate study, Bonadonna et al. [14**] also reported a correlation between systemic reaction to Hymenoptera sting and mast cell tryptase. Of 379 patients with a history of systemic insect sting reactions, 11.6% had serum mast cell tryptase levels exceeding 11.4 ng/ml. Of this group, the rate of systemic (Muller grade IV) anaphylaxis was 70.5%. Thirty-four of the patients with elevated mast cell tryptase level underwent bone marrow biopsy; of those, 61.8% were ultimately diagnosed with indolent systemic mastocytosis. The opposite was true of food and drug allergies. A subsequent study [15] looked at patients with a history of severe systemic anaphylaxis induced either by a food or a drug. Of 137 patients, only 9 (6.6%) had a basal tryptase above 11.4 ng/ml. Ultimately, only two (1.5%) were identified with mastocytosis. In comparison to the prior study, the patients with Hymenoptera-induced anaphylaxis had a higher rate (11.6%) of basal tryptase level above 11.4 ng/ml. In addition, of the 379 insect-allergic patients, 21 (5.5%) were subsequently diagnosed with indolent systemic mastocytosis compared with 2 of 137 (1.5%) patients with severe food or drug-related reactions. Blum et al. [16] confirmed this connection. This study conducted a 5-year retrospective analysis on 868 patients referred for systemic reaction to Hymenoptera sting. Of those, 758 had both total IgE and baseline tryptase levels drawn. Baseline tryptase level (>11.4 ng/ml) was associated with severe systemic reactions ($P=0.026$). In a German study [17], the correlation between severe systemic reaction to sting and elevated baseline tryptase ($P=0.003$) was further confirmed as was an association with increasing age ($P=0.001$). Because of the dramatic increase in severe sting-related anaphylaxis in patients with mastocytosis, physicians should consider clonal mast cell disease in anyone with unexplained anaphylaxis or sting-related anaphylaxis [18].

Not surprisingly, VIT in mastocytosis patients carries greater side effect risk than reported in the nonmastocytosis, venom-allergic patients. In a large multicenter, European study, Rueff et al. [19] studied 680 patients with established venom allergy to either honeybee or vespid. The objective was to determine if baseline tryptase level correlated with severe reactions occurring during the immunotherapy build-up phase. Fifty-seven patients, or 8.4%, experienced a significant enough adverse event as to warrant emergency intervention. The frequency of severe events correlated with higher basal tryptase levels with an odds ratio (OR) of 1.56 ($P<0.005$). Interestingly, bee venom also correlated with higher risk of systemic reaction. Contrary to the current recommendation of 3–5-year duration of VIT, patients with mastocytosis have lower probability of long-term protection and consequently a greater risk for recurrent severe, even fatal anaphylaxis [20]. A thorough review published in this journal by Bonadonna et al. [21] recommends VIT for life in patients with mastocytosis and venom allergy. Since the efficacy of venom immunotherapy is less than optimal in mastocytosis patients, they should continue to carry two epinephrine injectors [22**]. In addition, baseline tryptase levels should be considered in all patients with anaphylaxis related to Hymenoptera sting. In this review, we have discussed several studies that have reported a high correlation with mastocytosis when the baseline tryptase exceeds 11.4 ng/ml, suggesting that this diagnosis be entertained in patients with severe anaphylaxis from Hymenoptera.

**Key points**

- Mast cell disease is key in understanding anaphylaxis.
- Basophil activation may aid in the diagnosis of insect allergy.
- Epinephrine is underutilized for the acute management of insect anaphylaxis.

**Gene expression**

Indolent or occult mastocytosis continues to play a role in the presence and severity of anaphylaxis and insect venom anaphylaxis as well as gene expression. Niedoszytko et al. [23*] accessed the difference in gene expression between patients with indolent mastocytosis and a history of venom anaphylaxis compared to patients with indolent mastocytosis without anaphylaxis. Their preliminary results suggest a difference in gene profiling between the two groups using whole-genome gene expression analysis [23*]. The same group also suggested that the angiotensinogen AGT p.M235T gene polymorphism may be responsible for the development of severe anaphylactic reactions to insect venom and looked at methods to determine if analysis of gene expression might be predictive of the effectiveness of VIT. Forty-six patients with 48,071 genetic transcripts demonstrated a genetic pattern characteristic of successful VIT [24]. This finding suggests a role for genetic profiling for successful long-term VIT [25]. In other work, Karalkis et al. [26] looked at MCH associations between HLA class I and
HLA class II antigens. The purpose of the study was to look at broad HLA status as a predictor of those individuals who develop allergic reactions to insect stings, whereas others do not develop insect sting allergy. Twenty-one insect-venom-sensitive patients with life-threatening reactions were compared with 37 healthy individuals. Their finding indicated that HLA-B*18 and HLA-Cw*07 alleles may be associated with susceptibility to venom allergy [26].

**Basophils/basophil activation test**

Mast cells and their mediators have been well studied. Our understanding of the role of the basophil also continues to expand. Since VIT is the principal management tool in insect anaphylaxis, an accurate diagnosis of venom hypersensitivity is crucial for its effective use. Current recommendations in the US dictate that skin prick testing, followed by serial increasing intradermal concentrations of specific venom and/or specific IgE in vitro testing, be used to determine venom hypersensitivity. However, there remains a subset of patients with a history of systemic reaction after insect sting with negative testing in a range of 6–20% [27]. The basophil activation test (BAT) may provide an additional window for accurate diagnosis of insect venom hypersensitivity in the history-positive but venom test-negative patient. Furthermore, there is the issue of double positive results that may be secondary to true multiple venom allergies or cross-reactivity occurring in up to 50% of individuals [28].

In the case of cross-reactivity, knowing the true culprit is important when choosing components to use in VIT, since including components to which the patient is not truly allergic could lead to development of desensitization to other epitopes [27]. Lastly, there is currently no recommended testing to determine when and if patients should stop VIT [5]. Testing to determine which patients are at an increased risk of reaction to a sting after VIT has been stopped would help clinicians determine which patients would benefit from continued lifelong VIT. The BAT has been studied as a surrogate test to assist in answering these questions.

The BAT measures CD63 activation on basophils when stimulated in vitro with specific IgE via flow cytometry. A large cohort study [29] of more than 1000 patients found 4% of patients with a history of systemic reaction after insect sting had negative skin testing (both skin prick and intradermal testing) and IgE testing. Sixty percentage of these negative patients were subsequently positive by BAT. There was only one patient reported as negative to BAT and positive via intradermal testing [29]. Testing using specific IgE is limited when low IgE levels causing results in a false-negative test. Skin testing is limited to intradermal testing concentrations maximum of 1 μg/ml due to irritant effects present at higher concentrations.

BAT can be accomplished when low IgE is present and has wider concentration limits – again revealing the utility of this test. In a study [27] of BAT use in children, it was found to be less sensitive compared with adult studies (67–75 vs. 87–100%, respectively). The availability of the test decreases its utilization since there are only a few specialized laboratories where BAT is standardized and can be reliably performed. Furthermore, approximately 15–20% of the general population are considered BAT nonresponders or nonreleasers. However, this number can be decreased to 5% if the basophils are incubated with IL-3 [30**]. This test remains a viable option for patients who are otherwise negative via standard venom hypersensitivity testing. The limitations need to be further studied. BAT in patients with double positive venom hypersensitivity testing has recently been studied. Peternelj et al. [30**] classified 17% of double positive patients via IgE testing as positive for only a single venom on BAT. Kosnik et al. [28] were able to find 1/3 of patients in their cohort as single positive after BAT [29]. These studies reveal the potential benefit of BAT in more specifically characterizing patients with otherwise unclear testing thereby allowing more accurate VIT in these patients.

Next we examine the use of BAT in predicting a patient’s response to future insect stings after completion of VIT. Literature is conflicting on this topic. Patients who had completed VIT for bee venom hypersensitivity were studied and sting challenge was performed as a gold standard method of determining reactivity. BAT in patients treated for at least 3.1 years of VIT correlated well with sting challenge. When tested with concentrations of 100 ng/ml of bee venom, 80% of sting challenge reactors had a positive BAT and 87.5% of non-reactors had a negative BAT [31]. Other authors have confirmed or rejected these findings in their studies, so further work is needed before recommendations for BAT and VIT can be applied [32–34].

**The role of omalizumab in mast cell disease**

The management of insect allergic individuals can be complicated by comorbid occult or indolent mastocytosis. Omalizumab has been reported to be a useful adjunctive therapy in individuals with severe anaphylaxis to bee venom immunotherapy [35,36]. Several recent cases report the usefulness of omalizumab in the management of insect anaphylaxis and indolent or occult mastocytosis. Kontou-Fill et al. [37*] reported the successful use of omalizumab mono-therapy as an effective strategy in a 48-year-old with occult mastocytosis who was venom-specific IgE negative and suffered three near-fatal bee sting reactions. Douglass et al. [38] reported the successful use of omalizumab in treating systemic mastocytosis in an atopic patent. In both Kontou-Fill et al.’s work and...
that of Douglass et al., the clinical effectiveness as measured by serum tryptase was directly attributed to omalizumab. This suggests that the effect of omalizumab may reside in its impact on mast cell stability. Further investigation is warranted before drawing long-range conclusions, but the observations are important.

Risk factors
Since venom allergy is one of the top three causes of anaphylaxis, it is necessary to have proper prevention and management strategies in place for these patients [39**]. It has been well established that patients are under-treated with epinephrine during anaphylaxis and strategies for prevention are overlooked at discharge [40,41]. A recent study [38] on epinephrine administration in the emergency department for stinging insect hypersensitivity found that only 18% of adult patients presenting with anaphylaxis received epinephrine. However, the same study reported 92% of children, defined as below 17 years of age, received epinephrine. Literature supports the use of epinephrine in cases of anaphylaxis as the first-line therapy with increased risk of death in patients who did not receive epinephrine or in cases when it was delayed in patient management. For patients presenting with systemic reactions, only 11% were referred to an allergist, only 3% had documentation of insect avoidance, and 68% received a prescription for self-injectable epinephrine at discharge [38]. Another study [42] in beekeepers found that only 66% of beekeepers who attended the emergency department for systemic reactions were reviewed by an allergist for further management. These findings may represent a lack of understanding among healthcare providers about the lifesaving benefits of VIT in insect venom hypersensitivity. It also stresses that education is needed since prevention and treatment are essential in preventing further reactions. VIT has been repeatedly shown to decrease risk of further reactions if re-stung and is now standard practice in individuals that have insect hypersensitivity [38].

The need for a second dose of epinephrine has been noted in patients with anaphylaxis based on limited data in food-induced anaphylaxis [43]. Risk factors associated with requiring a second epinephrine dose were observed in a recent study by Manivannan et al. [44]. This study included all causes of anaphylaxis (not only those specifically due to venom). Overall 13% of patients presenting with anaphylaxis received two doses of epinephrine. These patients were statistically significantly younger and presented more likely with respiratory, cardiac and gastrointestinal symptoms and were less likely to have urticaria. A history of asthma was not predictive of repeating epinephrine doses. Although 71.4% of patients who required a second dose of epinephrine were prescribed self-injectable epinephrine, the overall prescription rate was still low. Only 39.3% of patients treated with a single dose of epinephrine were discharged with a prescription for self-injectable epinephrine. Allergy referral was given for 51.9% of patients requiring repeated epinephrine doses and 40.3% of patients given single epinephrine dose [44].

Another important aspect focuses on who is at risk of anaphylaxis. A recent questionnaire was evaluated in a population of British beekeepers. It revealed some novel associations with the development of systemic reactions in this distinct population. Factors that were found to be associated with increased risk of systemic reactions included having a family member with bee venom allergy, having practiced beekeeping for more than 2 years, having premedication with antihistamine prior to sting, and being female [39**]. This study was performed on a selected group of individuals and may not be applied universally. In a large European work, Rueff et al. [13**] reported that, of 962 insect-allergic patients, male sex was considered a risk factor for future anaphylaxis. Clearly, these are areas that need to be further studied prospectively before recommendations can be made.

Conclusion
Insect allergy is a common trigger for anaphylaxis and treatment provides a high level of protection. Improved understanding of the biology of various effector cells and their mediators will improve diagnosis, thereby enabling appropriate long-term treatment. Novel therapies such as omalizumab may also have a therapeutic role in difficult-to-manage insect-allergic individuals. Unfortunately, despite our recent observations in diagnosis management, the proper utilization of epinephrine in the outpatient arena and appropriate specialty referral remain as obstacles to effective long-term management of insect allergy and anaphylaxis.

Acknowledgements
The authors would like to acknowledge the support of Ms Barbara Dineen and Dr Gregory Roper for their assistance in research and editorial support. The authors further acknowledge that they receive no grant or direct financial support.

Disclosure: J.M. Tracy is an invited contributor for UpToDate. None of the other authors has any conflicts of interests, financial or otherwise with any of the topics discussed.

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 (p. 398).

Anaphylaxis and insect allergy


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