

Diagnosis of Hymenoptera venom sensitivity

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Purpose of review

The objective of this review is to highlight recent advances in the preparation, documentation and performance of reagents and methods used in the diagnosis of Hymenoptera-venom-induced immediate-type hypersensitivity.

Recent findings

The following potent allergens have been reported: (1) a low-molecular-weight honey-bee allergen (*Api m 6*) has been described; (2) venom allergens in the North American species of bumble-bee (*Bombus pennsylvanicus*) have been more fully characterized, with the focus on phospholipase A₂; (3) the vespid venom Ves v 5 allergen has been structurally mapped to identify immunoglobulin-E-binding epitopes; (4) the possible role of carbohydrate antigen epitopes as a cause of cross-reactivity among honey-bee and vespid venom proteins has been reported; and (5) the venom of *Pachycondyla chinensis*, an ant found commonly in the Far East, has been described. The most significant reports during this period have focused on the less-than-ideal performance of the intradermal venom skin-test reagents. The issue of the patient that is positive for venom allergy history but negative for an intradermal venom skin test is raised, and it is suggested that there is a need for caution and the use of serology as a supplementary diagnostic test.

Summary

The important issue this year is the reminder that intradermal skin tests may be negative in venom-allergic patients, possibly because of changes in the potency of the extracts. The clinical history should drive the diagnosis of insect-sting allergy. When negative confirmatory venom skin-test or serology results are considered to be inconsistent with a positive history, they should be repeated.

Keywords

Hymenoptera venom, diagnosis, skin test, venom-specific immunoglobulin E antibody, sting-challenge provocation test

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Abbreviation

Ig immunoglobulin

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Introduction

The diagnosis of Hymenoptera venom hypersensitivity rests on two criteria: a definitive clinical history that temporally associates an allergic reaction with an insect sting, and the detection of venom-specific immunoglobulin (Ig)E antibody in the skin and/or blood of the individual by using a 'confirmatory' skin test or serological assay [1]. Historically, the skin test has been considered the most diagnostically sensitive and specific confirmatory test. However, this dogma has been challenged recently with the observation that about 30% of adults with a positive history for systemic reactions to a Hymenoptera sting have negative results in intradermal skin tests. Moreover, 20–40% of these patients that are positive for a history of venom allergy but negative in skin tests exhibit positive venom-specific IgE-antibody serology results, and 10–20% experience a systemic reaction to a subsequent sting [2,3]. The current overview examines literature, published during the last year, that investigate this concern and other issues related to the characterization of Hymenoptera venom allergens and the performance of diagnostic tests.

Hymenoptera-venom allergens

The stinging insect responsible for an allergic reaction can sometimes be identified by the appearance and location of the insect and/or nest and/or by considering the circumstances of the sting. Some clinicians believe that venom only from that insect which induces a systemic reaction (if it can be identified) should be included in a venom-immunotherapy regimen [4]. It is the opinion of others that treatment should be performed with venoms from all the insects for which positive skin tests have been obtained [5]. In either case, accurate identification of the insect and the specificity of venom-specific IgE antibody during the diagnostic phase of the evaluation is critical to decisions about the venoms that are subsequently selected for use in treatment.

Honey bee

In a review of allergic and other reactions to insects, Moffitt and deShazo [6] summarize the work of others in relation to known peptides and proteins in the venoms of selected medically important Hymenoptera. Honey-bee venom, for instance, is known to contain at least six important allergenic proteins (phospholipase A₂, hyaluronidase, melittin, acid phosphatase, apamin, and peptide 401). Some of these have become available in recombinant form, and these have been proposed as possible alternative venom skin-testing reagents [7].

Kettner *et al.* [8] have characterized a low-molecular-weight IgE-binding honey-bee venom allergen (*Api m 6*) that exhibits both strong IgE antibody reactivity and T-cell proliferation responses. Using purification methods involving size exclusion and reverse-phase high-pressure liquid chromatography, the authors isolated an 8 kDa protein that reacted to 42% of honey-bee venom-specific IgE-positive sera. *Api m 6* is present in venoms in four isoforms, with a common central core of 67 residues and differences in both the amino-terminal and carboxy-terminal ends. Possibly the importance of such work is in the warning that it provides to manufacturers of diagnostic venom skin-test and serology reagents. Because exogenous amino acids are sometimes added to venoms during collection and processing (electrolytic stimulation, venom-sac dissection) to enhance stability, manufacturers of reagents are sometimes forced to dialyze out these small amines. In addition, there has been a suggestion that dialysis of venoms to remove small vasoactive amines may enhance the sensitivity of venom skin-testing reagents without compromising specificity [3]. Kettner's work teaches that caution should be exercised when venoms are dialyzed or chromatographed to remove small-molecular-weight components, because this process may lead to the loss of important low-molecular-weight allergens.

Bumble-bee

Bumble-bee venoms contain phospholipase A₂, venom protease, hyaluronidase, and acid phosphatase. Less is known about the allergenic properties of these bumble-bee venom proteins than their honey-bee-venom counterparts, despite the fact that bumble-bees are increasingly used as pollinators of vegetables in commercial green houses. Two distinct categories of bumble-bee-sensitive patients have been identified. One group derives its primary sensitivity from field stings and often has IgE antibody that cross-reacts with honey-bee-venom proteins. A second group comprises the occupationally exposed individuals, who often have specific IgE antibody responses that are restricted to bumble-bee-venom proteins; their IgE antibody rarely cross-reacts with honey-bee-venom proteins [9].

In 2001, Hoffman *et al.* [10] used immunoassay inhibition, enzyme analysis, amino acid sequencing and three-dimensional computer modeling to characterize bumble-bee-venom proteins and study purified phospholipases from two bumble-bee species, namely *Bombus terrestris* and a North American species, *Bombus pennsylvanicus*. The authors showed that *B. terrestris* phospholipase A₂ and protease allergens differed in their IgE-binding epitopes from those in the venom of *B. pennsylvanicus*. Of diagnostic importance, bumble-bee phospholipase A₂ was shown to be only 53% identical to honey-bee phospholipase A₂, and thus the authors provide structur-

al evidence for cross-reactivity between honey-bee and bumble-bee venoms. They conclude that diagnosis of bumble-bee sensitivity in North American patients should be performed with both species of bumble-bee venom. Moreover, testing with honey-bee venom alone is now known to be insufficient for assessing a patient for hypersensitivity to bumble-bee venom, since at least *Apis* and *Bombus* phospholipases A₂ are known to be partially antigenically cross-reactive.

Vespid

Venoms from the vespids are known to contain phospholipase A₁, hyaluronidase, acid phosphatase, antigen 5, and two low-molecular-weight proteins: kinin and mastoparan [6]. Henriksen *et al.* [11] investigated vespid antigen 5 (*Ves v 5*), which they showed is related by a high level of amino acid sequence homology to a number of 'pathogenesis related' proteins found in mammals, reptiles, insects, fungi and plants. They refined the resolution of the crystalline structure of *Ves v 5* to 1.9 Å and showed that hydrogen bonding patterns and hydrophobic interactions of the α - β - α sandwich core structure are conserved among *Ves v 5* and its cross-reactive pathogenesis-related proteins. These structural data provide us with a structural explanation for the differences in IgE-binding epitopes in antigen 5 molecules from yellow jacket, hornet and paper-wasp venom. Their data also provide additional empirical support for Aalberse's earlier observation [12] that *Ves v 5* shares α -helix and β -sheet structural similarities with the dust mite group 1 allergens, lysozyme, lactalbumin, and grass group 4 allergens.

Bee and vespid venom cross-reactivity

Egner *et al.* [13] are among a number of groups that have observed that 30–50% of patients allergic to Hymenoptera venom exhibit skin and/or serological evidence for a so-called 'double positivity' to honey-bee and yellow-jacket venoms, in one case despite a patient's self-reported allergic reaction to only one of these two insects. While honey-bee and vespid venoms are generally thought to be largely antigenically distinct, both are known to contain immunologically cross-reactive hyaluronidase, a 42 000 MW protein that is believed to be one cause of this dual positivity [14]. Alternatively, these individuals are in fact truly sensitized to both honey-bee and yellow-jacket (vespid) venoms and they suffer from recall bias, only remembering being stung by one of the insects.

Hemmer *et al.* [15] have recently investigated the role that carbohydrate determinants on venom proteins may play in inducing IgE antibodies that cross-react with honey-bee and yellow-jacket venoms. They used an IgE antibody inhibition CAP (a cellulose sponge matrix to which allergen is covalently coupled) assay to measure

IgE antibodies reactive with oilseed rape, which they used as a rough measure of carbohydrate-specific IgE antibody. They detected IgE anti-carbohydrate reactivity in two out of 14 sera positive for IgE anti-honey-bee venom only, two out of 16 sera positive for IgE anti-yellow-jacket venom only, and 33 out of 41 (80.5%) sera positive for IgE antibodies to both venom specificities ($P < 0.00001$). Moreover, they studied sera from 11 subjects with dual positivity that also displayed a strong IgE antibody positivity to carbohydrates in oilseed rape. IgE binding to venoms in nine of these 11 sera was completely inhibited by preincubation of the serum with either pollen extracts rich in α 1-3-fucosylated glycoproteins, a neoglycoprotein construct with defined glycans, or both. The authors concluded that carbohydrate-specific IgE may be another cause of the observed double positivity to honey-bee and yellow-jacket venoms that is seen in some patients allergic to Hymenoptera venom. Since carbohydrate-specific IgE antibodies are believed to have minimal clinical relevance, the authors express a concern that these carbohydrate-reactive IgE antibodies may confound the diagnostic skin test or serology result and thus interfere with accurate identification of the correct sensitizing insect.

Ants

The red and black fire ants (*Solenopsis invicta* and *Solenopsis richteri*, respectively) have been the most extensively studied members of the Formicidae family. Their venoms contain at least four potent allergens (Sol i 1–4), including a phospholipase and antigen 5 family protein, that can induce serious reactions in sensitized individuals [6]. Kim *et al.* [16] have recently described another ant (*Pachycondyla chinensis*) whose venom contains potent allergens that induce anaphylaxis in individuals living in South-East Asia. In their study, they prepared a 1:5 (w/v) *P. chinensis* ant whole-body extract and identified eight IgE-binding proteins ranging from 12 to 58 kDa by using sodium dodecyl sulfate/polyacrylamide gel electrophoresis and Western blot analysis. Only minimal enzyme-linked immunosorbent assay inhibition of IgE anti-*P. chinensis* binding to solid-phase *P. chinensis* antigens was achieved with heterologous imported fire-ant, wasp, honey-bee venoms, suggesting that these are immunologically distinct allergens. The authors showed no significant relationship between IgE anti-*P. chinensis* and IgG4 anti-*P. chinensis* levels in sera from their study subjects who exhibited anaphylaxis following ant bites.

Diagnostic confirmatory testing: skin testing, serology, and provocation

Venom skin testing by puncture and intradermal techniques is routinely performed as the diagnostic method of choice [1,15]. Intradermally administered

venom doses range from 0.001 to 1 μ g/ml, higher concentrations often producing false-positive skin reactions [3]. Venom-specific IgE antibody measurements made using a radioallergosorbent test or a more recent non-isotopic variant (e.g. the CAP System) are considered useful alternative diagnostic tests.

Large local reactors

Of those who experience a large local reaction induced by a Hymenoptera sting, 50–80% will have a positive skin test and/or venom-specific IgE blood test. However, because the incidence of systemic reactions among the large local reactor group is 5–10%, only 1–2% being considered life threatening, they are not viewed as candidates for venom immunotherapy under current practice guidelines [1]. Hamilton *et al.* [17] report on a large local reactor with positive venom skin and IgE-antibody blood tests who elected to receive immunotherapy and then undergo a sting-challenge provocation test to study its direct clinical benefit. Venom immunotherapy reduced skin reactivity 100-fold, with an unequivocal clinical benefit of reducing morbidity and ablating the large local reaction. This case report suggests that the clinical indication for venom immunotherapy should be re-examined and possibly extended to include large local reactors who work or play in locations where they experience frequent inadvertent stings.

History-positive, skin-test-negative subjects

It has been known for some time that patients who exhibit severe systemic reactions to a Hymenoptera sting may exhibit a negative intradermal skin test at 1 μ g venom/ml and yet have detectable IgE anti-venom in their blood [18].

In a recent study, Golden *et al.* [19] used a history, repeat intradermal skin test, and skin challenge prospectively to examine the prevalence of negative skin-test responses in patients with a history of systemic reactions to stings. They studied 307 patients with positive histories for sting-induced systemic reactions. Negative intradermal skin tests at 1 μ g/ml were observed in 99 (32%) patients from this group. A total of 43 of the 99 history-positive, skin-test-negative subjects had positive IgE anti-venom serology results, suggesting that IgE antibody is present in the blood at a level that is not detectable in the skin with currently available venom skin-testing reagents. Of the 43 history-positive, skin-test-negative, serology-positive subjects, 36 had low positive IgE anti-venom levels in the 1–3 ng/ml range, while the other seven had high venom-specific IgE antibody levels ranging from 4–243 ng/ml. Sting challenges performed on 51 of the 99 history-positive, skin-test-negative subjects (37 venom-specific IgE-positive; 14 venom-specific IgE-negative) identified 11 reactors. Nine of these 11 sting-challenge

reactors had a positive venom-specific IgE antibody level in a particle-based radioallergosorbent test at 1 ng/ml or more. The authors concluded that venom skin tests should be repeated in patients that are positive for a history of venom allergy when the skin test is negative. Moreover, a concomitant serological IgE anti-venom test should be performed, since the currently available diagnostic skin-test reagents appear to lack the necessary diagnostic sensitivity. The group estimates that a patient with a positive history of a sting reaction has a 5–10% reaction rate if both the venom skin test and the serology result are negative. This increases to a 20% reaction rate if they have a negative skin test but a positive venom-specific IgE-antibody serology result.

In an editorial, Reisman [20] examined this dilemma of the negative skin-test reactor. He reminded readers of published data indicating that the presence of a positive skin test or serum venom-specific IgE in a person with a history of venom anaphylaxis does not always indicate that a re-sting reaction will occur. In fact, only 60% or fewer patients considered at risk because of a positive history, venom skin test and/or venom-specific IgE antibody in the serum will react to subsequent intentional or field stings [21–23]. The age of the subject and the severity of the initial skin reaction are cited as primary risk factors, with adults and those with more severe initial reactions being more likely to react than children or individuals with mild initial reactions. Earlier studies have suggested that the IgE anti-venom radioallergosorbent test has a lower diagnostic sensitivity than the intradermal skin test, about 20% of skin-test-positive subjects exhibiting a negative serology result. However, the work of Golden *et al.* [19] suggests that certain venom-specific IgE-antibody assays display a better diagnostic sensitivity than currently available commercial venom skin-testing reagents. Reisman raises the possibility that the potency of currently available venom skin-testing reagents may have changed over time. Standardization of different venom batches is based on the hyaluronidase content, and it is possible that the concentration of other important venom allergens may have changed in newer lots. For patients with severe initial skin reactions and negative skin tests, Reisman recommends a venom-specific IgE-antibody serology test and a repeat skin test at periodic intervals, the initial repeat test being at 3 months.

In a 2001 editorial, Hamilton opines that the ultimate responsibility for insuring the quality of a serum based venom-specific IgE antibody result rests with the referring physician who selects the clinical laboratory to perform the serology [24]. There are at least 15 different IgE antibody assay formats manufactured by seven different companies that can be used by clinical laboratories to measure venom specific IgE antibody.

All are not equivalent in their analytical performance (sensitivity, specificity and reproducibility). Moreover, the requesting physician should expect substantial variation in the quality of the test results among laboratories due to differences in daily quality assurance programs. At the minimum, the laboratory that is selected for testing should perform (a) at a level of testing that insures quality and permits certification based on criteria such as those defined by the United States Clinical Laboratory Improvement Act of 1988 (CLIA-88) and (b) within the 95% confidence limit for its peer group in a national diagnostic allergy (SE) proficiency survey such as the one conducted by the College of American Pathologists [25].

Alternative diagnostic test

Zollner *et al.* [26] revisited the Western blot as an possible alternative diagnostic test for the detection of wasp-venom-specific IgE antibody in human serum. They used serum from 30 patients with positive histories for wasp allergy to compare the diagnostic performance of Western blots with that of intradermal skin tests and a single commercial serology assay. While the Western blot is reported to exhibit a highly favorable diagnostic sensitivity, its specificity was less than ideal unless positive phospholipase A₁ and hyaluronidase bands were specifically identified. Concern about its slower turnaround time, more complex technology and more difficult interpretation will continue to relegate the Western blot analysis for venom-specific IgE antibody to the same research assay status as the venom-induced basophil histamine-release assay.

Conclusion

A number of advances have been made in the characterization of bee and vespid venom allergens, including the report of a highly sensitizing new ant venom from *P. chinensis* found in the Far East. The major news during the last year, however, has been the concern raised about the apparent loss of sensitivity of the commercially available Hymenoptera venoms used for intradermal skin testing. Care should be exercised when the negative results of a diagnostic test are not consistent with the patient's positive clinical history for sting-induced reactions. In these cases, a repeat skin test and a serology test for venom-specific IgE antibody may be prudent.

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