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REVIEW

Component-resolved diagnosis in hymenoptera allergy

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Abstract Component-resolved diagnosis based on the use of well-defined, properly characterised and purified natural and recombinant allergens constitutes a new approach in the diagnosis of venom allergy. Prospective readers may benefit from an up-to-date review on the allergens. The best characterised venom is that of *Apis mellifera*, whose main allergens are phospholipase A2 (Api m1), hyaluronidase (Api m2) and melittin (Api m4). Additionally, in recent years, new allergens of *Vespula vulgaris* have been identified and include phospholipase A1 (Ves v1), hyaluronidase (Ves v2) and antigen 5 (Ves v5). *Polistes* species are becoming an increasing cause of allergy in Europe, although only few allergens have been identified in this venom.

In this review, we evaluate the current knowledge about molecular diagnosis in hymenoptera venom allergy.

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Introduction

Stings by hymenoptera, namely bees, wasps, yellow jackets, hornets and ants, usually cause just local reactions. However, in some cases, they can induce systemic symptoms, and even fatal reactions.[1,2]

Reactions to hymenoptera venom are also responsible for decreased quality of life and significant anxiety about future stings.[1,3] The results of the quality-of-life questionnaire demonstrated that a well-tolerated sting challenge test improves the quality of life of venom-allergic patients by reducing the anticipatory anxiety associated with the fear of being stung.[4,5]

Diagnosis of hymenoptera venom allergy forms the basis of treatment.[6] Venom immunotherapy is the only treatment that addresses the cause of the anaphylactic reaction. It has proven very effective in inducing tolerance, with a protection rate ranging from 75% to 98%.[7]

Diagnosis of hymenoptera allergy is based on a systemic reaction after a sting, a positive skin test result and detection of specific IgE antibodies. Both skin test and specific IgE frequently reveal multiple sensitisations, which complicates the choice of the venom for immunotherapy (VIT).[8] Double-positive results are a common issue when diagnosing Hymenoptera venom allergy based on crude venom extracts, since up to 59% of patients react to both honey bee venom and yellow jacket venom. Moreover, there is an increasing pattern of double sensitisation to *Vespula* and *Polistes* in southern European countries.[9,10] On the other hand, cases of double positivity of IgE to bee and *Vespula* venom are often caused by clinically irrelevant cross-reactive antibodies against cross-reacting carbohydrate residues or by homologue allergens expressed in different Hymenoptera venoms.[10,11]

Component-resolved diagnosis (CRD) based on the use of well-defined, properly characterised and purified natural and recombinant allergens constitutes a new approach in venom allergy diagnosis.[12–14]

The best characterised venom is that of *Apis mellifera*, whose main allergens are phospholipase A2 (Api m1), hyaluronidase (Api m2) and melittin (Api m4).[2,15] Additionally, allergens of *Vespula vulgaris* have been identified, as phospholipase A1 (Ves v1), hyaluronidase (Ves v2) and antigen 5 (Ves v5).[16] *Polistes* species are becoming an increasing cause of allergy in Europe, although only few allergens have been identified to date.

In this paper, we analyse the current knowledge about molecular diagnosis in hymenoptera venom allergy.

Apis mellifera

Bee venom is a complex mixture of allergenic proteins with enzymatic function, together with other pharmacologically-active molecules, such as biogenic amines and basic peptides.

The complete genome sequencing of the bee has allowed the study of the composition of its venom, making it a model for the study of these insects. The most recent proteomic analysis of bee venom reveals that there must be more than 100 different components. Currently, 12 allergens have been identified in the bee, most of them in the venom[17,18] (Table 1).

The best-known bee venom allergens so far are phospholipase A2 (Api m 1), hyaluronidase (Api m 2) and melittin peptide (Api m 4), which constitute the majority of the dry weight of venom.

Phospholipase A2 (Api m 1) has been considered the most important and potent allergen of bee venom since 1976.[19] It is the most abundant enzyme capable of sensitising the vast majority of patients allergic to bee venom.[10,20] Its enzymatic activity causes the hydrolysis of membrane phospholipids, producing a significant increase in arachidonic acid and leukotrienes; these are responsible for bronchoconstriction, mucus production and vascular permeability.[21] Phospholipase A2 was cloned in 1989.[22] In 1992, it was recombinantly expressed through a prokaryotic system using the *E. coli* bacteria, achieving an enzymatic activity and skin reactivity similar to the natural purified form.[23,24]

In the bee venom compound-based diagnosis, it has been observed that use of allergen Api m 1 has different sensitivity according to the technique and sources used.[25–28]

Technique sensitivities vary ranging from 95.6 to 97% of ADVIA Centaur,[10,29] to 61.8–91% of ImmunoCAP,[8,20,25–28] being the highest observed prevalence of sensitisation using native Api m 1. In contrast, Immulite technique has shown a sensitivity of 83.1% with the recombinant form of Api m 1.[30]

Hyaluronidase (Api m 2) is a glycosylated enzyme considered a major allergen of bee venom.[20,29] It hydrolyses the hyaluronic acid in the target tissue, enhancing the penetration of the other components of the venom.[21]

Bee hyaluronidase shares 55% sequence identity with vespid hyaluronidase,[32] which may explain the cross-reactivity between them, together with carbohydrate determinants.[33]

Hyaluronidase was isolated in 1984[34] and cloned in 1993.[35] It is recombinantly produced in prokaryotic (*E. coli*) and eukaryotic (Baculovirus) systems, the latter having an enzymatic activity and IgE binding capacity similar to that of the natural purified allergen.[31]

The most abundant peptide of the venom is **melittin (Api m 4)**; with a very low molecular weight it represents the major toxic component of bee venom. Schröder et al. achieved its synthesis in 1971[36] and in recent years it has been confirmed that melittin is an allergen in highly purified preparations, through the detection of specific IgE antibody by radioallergosorbent test (RAST).[37] It is currently available in native and synthetic form.

It is considered a minor allergen with sensitisation prevalence ranging around 22.9%–29%.[20,37] However, it has recently been described as a major allergen in a population (69 patients) in the south of Spain with allergy to *A. mellifera* venom whose prevalence of sensitisation to Api m 4 was of 53.6%. Sensitisation to Api m 4 has behaved as a biomarker in patients with poor tolerance to the start of the bee venom immunotherapy with very low s-IgE values.[29]

Use of IgE-Api m 4 as the single discrimination criterion demonstrated different ways of being allergic to bee venom. Patients with sIgE-Api m 4 $\geq$0.98 kU/L had more severe reactions with stings, higher skin sensitivity, and

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