Component-Based Assessment of the Main Allergens in Honeybee Venom in a Spanish Allergic Population

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To the Editor:

Hymenoptera stings may prove life-threatening owing to the anaphylactic reaction that can occur after the sting. Identifying the culprit insect and the molecules involved in the reaction is essential for diagnosing and treating allergic patients [1].

The diagnosis of Hymenoptera venom allergy (HVA), which includes honeybee venom (HBV) allergy, consists in the collection of the clinical data observed after the sting, followed by confirmation of IgE-mediated sensitization by skin testing and/or the detection of the IgE antibodies in serum samples [2]. To this end, accurate diagnosis and effective treatment of HVA depend on the identification of specific allergens in venoms.

While Api m 1 is considered the major allergen in bee venom, other allergens, such as Api m 2, Api m 3, Api m 4, Api m 5, and Api m 10, also play a significant role depending on the population studied. In fact, identifying these additional allergens enables the diagnosis of 94% of HBV-allergic patients [3]. Api m 6 is a widely recognized allergen among Spanish HVA patients and has therefore been proposed for inclusion in the panel of allergens used in the molecular diagnosis [4].

The role of sensitization to Api m 10 in the effectiveness of immunotherapy for HBV allergy has been addressed [5]. No prospective studies have investigated the extent to which sensitization to this component is involved in HBV allergy or the extent to which its absence in therapeutic HBV preparations reduces the efficacy of specific immunotherapy. Nevertheless, since Api m 10 is very unstable in aqueous solution, its presence and stability in therapeutic preparations may affect the success of immunotherapy. The use of a diluent containing human serum albumin and phenol for reconstitution of HBV seems to have a stabilizing effect on the allergen [6].

In the Allergy Department of Lucus Augusti Hospital, Lugo, Spain, we performed a study based on data from 53 patients who had experienced a systemic reaction after being stung by *Apis mellifera*. Our objective was to provide more data to support and/or clarify the relevance of the different allergens in bee venom, including Api m 10 and the allergens not incorporated in molecular platforms (Api m 6, Api m 7, Api m 8, Api m 9, Api m 11, and Api m 12). This study was approved by the local ethics committee. An HBV extract was prepared with all known allergens described to date for this source (according to the WHO/IUIS Allergen Nomenclature Subcommittee). The extract was analyzed using liquid chromatography with tandem mass spectrometry to confirm its allergenic components (Supplementary data table 1) and compared with a commercial crude HBV (Latoxan) [7] (Supplementary data figure 1). The stabilizing effect of human serum albumin in aqueous bee venom extracts on Api m 10 was also analyzed. To this end, sera from 6 patients with sIgE to Api m 10 (≥3.5 kU_A/L) were pooled and used in a Western blot analysis of HBV with and without human serum albumin (Supplementary data figure 2). The results confirmed the stability of Api m 10 for at least 3 months.

The patients were diagnosed with HBV allergy by intradermal testing using the HBV extract and by the analysis of specific IgE in serum to whole *A mellifera* venom and the recombinant allergens Api m 1, Api m 2, Api m 3, Api m 4, Api m 5, and Api m 10 (Supplementary data table 2). The remaining allergens described were not measured owing to the lack of the recombinant allergens in the specific platforms. Tryptase levels were also measured. Nine of the 53 patients (16.9%) had grade I reactions according to the Mueller scale [8], 11 (20.7%) had grade II reactions, 24 (45.2%) had grade III reactions, and 9 (16.9%) had grade IV reactions.

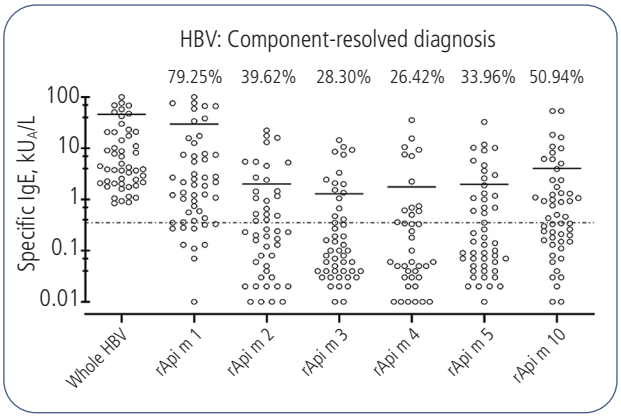

Figure. IgE-mediated immunoreactivity of individual patient sera with recombinant allergens. The lower-end cutoff of the CAP-FEIA (≥0.35 kU_A/L) is represented as a dotted line. HBV indicates honeybee venom.

The serological analysis with the patients' sera revealed the following values: median total IgE, 69.2 (10-1903) kU/L; specific IgE to HVB whole extract, 4.99 (0.84-1151) kU_A/L; and tryptase, 4.71 (1.72-11.8) ng/mL. In terms of specific IgE (median value) to individual allergens (component-resolved diagnosis), 79.25% of patients recognized Api m 1 (2.17 kU_A/L), 50.94% Api m 10 (0.9 kU_A/L), 39.62% Api m 2 (0.57 kU_A/L), 33.96% Api m 5 (1.06 kU_A/L), 28.30% Api m 3 (2.17 kU_A/L), and 26.42% Api m 4 (0.61 kU_A/L) (Figure).

Western blot assays were also developed with the HBV to analyze sIgE to other relevant allergens, such as Api m 6, as identified in the group of patients studied by Vega-Castro et al [4].

Our findings underscore the complexity and variability of HBV allergy, thus highlighting the importance of a detailed allergen profile for accurate diagnosis and effective treatment. The predominance of sensitization to Api m 1 and Api m 10 reinforces the need to include these allergens in diagnostic panels. The identification and confirmation of additional allergens, such as Api m 6, and the stability of Api m 10 in therapeutic preparations further inform management strategies for HBV allergy. The use of human serum albumin as a stabilizing agent appears promising for maintaining the efficacy of immunotherapy over time. These findings advocate for the continued refinement of diagnostic and therapeutic approaches, ensuring that they encompass the full spectrum of relevant allergens and thus optimize patient outcomes in the management of HBV allergy.

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Conflicts of Interest

FP works for Inmunotek laboratories. Inmunotek provided the material for the study to be carried out.

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