Component resolution reveals additional major allergens in patients with honeybee venom allergy☆

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Background: Detection of IgE to recombinant Hymenoptera venom allergens has been suggested to improve the diagnostic precision in Hymenoptera venom allergy. However, the frequency of sensitization to the only available recombinant honeybee venom (HBV) allergen, rApi m 1, in patients with HBV allergy is limited, suggesting that additional HBV allergens might be of relevance.

Objective: We performed an analysis of sensitization profiles of patients with HBV allergy to a panel of HBV allergens.

Methods: Diagnosis of HBV allergy (n = 144) was based on history, skin test results, and allergen-specific IgE levels to HBV. IgE reactivity to 6 HBV allergens devoid of cross-reactive carbohydrate determinants (CCD) was analyzed by ImmunoCAP.

Results: IgE reactivity to rApi m 1, rApi m 2, rApi m 3, nApi m 4, rApi m 5, and rApi m 10 was detected in 72.2%, 47.9%, 50.0%, 22.9%, 58.3%, and 61.8% of the patients with HBV allergy, respectively. Positive results to at least 1 HBV allergen were detected in 94.4%. IgE reactivity to Api m 3, Api m 10, or both was detected in 68.0% and represented the only HBV allergen–specific IgE in 5% of the patients. Limited inhibition of IgE binding by therapeutic HBV and limited induction of Api m 3– and Api m 10–specific IgG_4 in patients obtaining immunotherapy supports recent reports on the underrepresentation of these allergens in therapeutic HBV preparations.

Conclusion: Analysis of a panel of CCD-free HBV allergens improved diagnostic sensitivity compared with use of rApi m 1 alone, identified additional major allergens, and revealed sensitizations to allergens that have been reported to be absent or underrepresented in therapeutic HBV preparations.

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Key words: Apis mellifera*, cross-reactive carbohydrate determinant, Hymenoptera venom, insect venom allergy, honeybee venom allergy, recombinant allergen,* Vespula vulgaris

Diagnosis of Hymenoptera venom allergy is commonly based on a history of anaphylactic sting reactions, positive skin test results, and/or detection of specific IgE to venom of honeybee or *Vespula* species.[1] Positive results on skin and serologic tests with conventional venom preparations are frequently caused by antibodies cross-reactive to conserved structures found in venom allergens. These include homologous primary structures of protein allergens (eg, hyaluronidases, dipeptidyl peptidases IV, and vitellogenins) and cross-reactive carbohydrate determinants (CCD),[2,3] which are present on the majority of Hymenoptera venom allergens.[4] Double positivity to honeybee venom (HBV) and yellow jacket venom (YJV) in patients who have not been able to identify the culprit insect necessitates additional laboratory tests (eg, IgE inhibition assays or basophil activation tests)[5,6] that are expensive, time-consuming, difficult to interpret, and therefore rarely used in the clinical routine.

Recently, the diagnostic value of IgE detection to CCD-free, species-specific recombinant Hymenoptera venom allergens, such as HBV phospholipase A_2 (rApi m 1), YJV phospholipase A_1 (rVes v 1), and antigen 5 (rVes v 5), was demonstrated.[7-14] In contrast to the situation of YJV allergy,[7,9,14,15] the frequency of sensitization to rApi m 1, the only recombinant HBV allergen commercially available to date, in patients with HBV allergy ranges from 58% to 80%,[7,8,10,13,14,16] which is insufficient to support a definitive diagnosis of HBV allergy. This suggests that additional HBV allergens are of relevance for sensitization and hence the diagnosis of HBV allergy.

The best characterized HBV allergens are phospholipase A_2 (Api m 1), hyaluronidase (Api m 2), and the basic peptide melittin (Api m 4), which all constitute medium- to high-abundance proteins.[17,18] More recently, additional HBV allergens of lower abundance have been cloned and characterized, such as acid phosphatase (Api m 3),[19] dipeptidylpeptidase IV (Api m 5),[20] Api m 6,[21] major royal jelly proteins 8 and 9 (Api m 11.0101 and Api m 11.0201),[22] icarapin (Api m 10),[23,24] and vitellogenin (Api m 12).[25] Insect cell–based expression strategies allowed for

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Abbreviations used
CCD: Cross-reactive carbohydrate determinant
HBV: Honeybee venom
IQR25/75: 25% to 75% Interquartile range
sIgE: Allergen-specific IgE
YJV: Yellow jacket venom

detection of IgE reactivity of these allergens independent of the presence of CCDs.[12] The recombinant availability enabled analysis of different venom preparations, demonstrating that lower-abundance components, such as Api m 3 and Api m 10, although present in the crude HBV, are absent or underrepresented in preparations used for HBV immunotherapy.[23]

Here we analyzed the sensitization profile of patients with HBV allergy to a panel of CCD-free HBV allergens, including rApi m 1, rApi m 2, rApi m 3, nApi m 4, rApi m 5, and rApi m 10, by using the ImmunoCAP assay system (Thermo Fisher Scientific, Uppsala, Sweden). Inclusion of additional allergens improved the sensitivity of component-based diagnostics and demonstrated distinct sensitization profiles, some of which displayed prominent sensitizations to Api m 3 and Api m 10. In the same line, we observed a lack of Api m 3– and Api m 10–specific IgG_4 induction during HBV immunotherapy, suggesting that sensitization profiles to allergens that are not sufficiently present in therapeutic HBV preparations might be of relevance for the outcome of HBV immunotherapy.

METHODS

Patients

Sera from 184 patients with anaphylactic reactions to either honeybee (n = 144) or yellow jacket (n = 40) stings (as identified by the patient) and 40 HBV-nonallergic control subjects were analyzed. Diagnosis of HBV allergy was based on a combination of the patient's history of an anaphylactic sting reaction, a positive skin test result, and positive IgE levels to HBV (ImmunoCAP i1), as recently described.[14] As defined by the inclusion criteria, all patients with HBV allergy displayed IgE to HBV (≥0.35 kU_A/L), and 90 (62.5%) also had positive test results to YJV (ImmunoCAP i3). Diagnosis of YJV allergy was based on a combination of the patient's history of yellow jacket sting anaphylaxis, a positive skin test result, and positive IgE results for YJV (ImmunoCAP i3) and negative results for HBV (ImmunoCAP i1). The HBV-nonallergic control subjects had all experienced a bee sting, although without an anaphylactic or large local reaction. All patients and control subjects had provided informed written consent, and the study was approved by the local ethics committee.

Allergens and IgE antibody measurements

rApi m 2, rApi m 3, rApi m 5, and rApi m 10 were expressed as secreted full-length proteins by *Spodoptera frugiperda* (Sf9) insect cells, as recently described.[12,19,20,23,26,27] In brief, Sf9 cells were grown in suspension at 27°C in serum-free medium (Lonza, Verviers, Belgium) containing 10 μg/mL gentamicin (Invitrogen, Carlsbad, Calif) to a density of 1.5×10^6 cells per milliliter and then infected with a high-titer stock of recombinant baculovirus containing the allergen gene to be expressed. For protein production, the cells were incubated at 27°C and 110 rpm for 72 hours. The recombinant proteins were then purified from culture medium by using a nickel-chelating affinity matrix (NTA-agarose; Qiagen, Hilden, Germany). The purity of each recombinant protein was assessed by using SDS-PAGE (see Fig E1 in this article's Online Repository at www.jacionline.org).

Api m 4 was purified from HBV by means of sequential steps of ion exchange and size exclusion chromatography. The purity of the preparation was assessed immunologically and by using SDS-PAGE (not shown).

Experimental ImmunoCAP tests (Thermo Fisher Scientific) containing the purified HBV allergens were prepared, as previously described.[28] All IgE antibody measurements were performed with a Phadia 250 instrument, according to the manufacturer's instructions (Thermo Fischer Scientific).

Immunoreactivity of patient sera

Serum IgE reactivity was analyzed on a CAP-FEIA platform (Phadia 250) using commercially available ImmunoCAP tests for HBV (ImmunoCAP i1), YJV (ImmunoCAP i3), rApi m 1 (ImmunoCAP i208), rVes v 5 (ImmunoCAP i209), rVes v 1 (ImmunoCAP i211), and the CCD marker MUXF3 (ImmunoCAP i213) and experimental ImmunoCAP tests for rApi m 2, rApi m 3, nApi m 4, rApi m 5, and rApi m 10. Selected sera were also analyzed for IgE reactivity to major royal jelly protein 8 and 9 (Api m 11.0101 and Api m 11.0201) and 3 additional HBV proteins (not been assigned as allergens) by using ELISA, as recently described.[22] Allergen-specific IgG_4 reactivity to rApi m 1, nApi m 4, rApi m 3, and rApi m 10 in selected sera was analyzed by using a Phadia 250 instrument and 1:100 or 1:20 serum dilutions.

CAP-FEIA inhibition

Inhibition of allergen-specific IgE (sIgE) binding to HBV (ImmunoCAP i1) by nApi m 1 (Latoxan, Valence, France), rApi m 3, or rApi m 10 was performed by means of preincubation of patient sera and inhibitors at the indicated concentrations for 2 hours at room temperature before the CAP-FEIA analysis. Alternatively, sera were preincubated with a crude HBV preparation (Latoxan) or solubilized freeze-dried therapeutic HBV preparations (ie, not absorbed to alum) at 300 μg/mL.

RESULTS

IgE reactivity to HBV allergens in patients with HBV allergy, patients with YJV allergy, and HBV-nonallergic control subjects

IgE reactivity (≥0.35 kU_A/L) to the commercially available rApi m 1 (i208) was detected in 72.2%, to rApi m 2 in 47.9%, to rApi m 3 in 50.0%, to nApi m 4 in 22.9%, to rApi m 5 in 58.3%, and to rApi m 10 in 61.8% of patients with HBV allergy (Fig 1). In patients with YJV allergy, no relevant IgE reactivity was detected, except to rApi m 5 (3/40, Fig 1), the cross-reactive dipeptidylpeptidase also present in YJV as Ves v 3. Of the 40 HBV-nonallergic control subjects, 6 (15%) displayed IgE reactivity of 0.35 kU_A/L or greater to HBV (ImmunoCAP i1), which is in line with previous reports.[29] In this subgroup of 6 control subjects, IgE reactivity to rApi m 1 was detected in 3, to rApi m 5 in 2, and to rApi m 10 in 1 subjects. No IgE reactivity to any of the tested HBV allergens was detected in the ImmunoCAP i1 negative control sera (Fig 1). Among the patients with HBV allergy, positive results to at least 1 HBV allergen were detected in 94.4%, and positive results to at least 1 of the HBV-specific allergens Api m 1, 3, 4, or 10 were detected in 89.6% (Fig 2). The majority of patients with HBV allergy were sensitized to more than 1 allergen (74.3%), and a minority (9.7%) were sensitized to all allergens tested. Interestingly, HBV-monosensitized patients (n = 54) had lower total IgE levels, lower levels of sIgE to HBV (ImmunoCAP i1), and lower levels of sIgE to all HBV allergens tested when compared with patients with HBV allergy who were also sensitized to YJV (ImmunoCAP i3, n = 90; see Table E1 in this article's Online Repository at www.jacionline.org).