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rApi m 3 and rApi m 10 improve detection of honey bee sensitization in Hymenoptera venom allergic patients with double sensitization to honey bee and yellow jacket venom

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Conflict of Interest:

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Author's Contributions

Marcel Frick:	coordinated patient recruitment, collected and analyzed data, provided critical revision of the manuscript
Sabine Müller:	coordinated patient recruitment, collected and analyzed data, provided critical revision of the manuscript
Frank Bantleon:	produced and provided recombinant allergens and critical revision of the manuscript
Johannes Huss-Marp:	analysed the data, designed the figures and provided critical revision of the manuscript
Jonas Lidholm:	generated experimental ImmunoCAP tests, provided critical revision of the manuscript
Edzard Spillner:	produced and provided recombinant allergens and critical revision of the manuscript
Thilo Jakob:	initiated and supervised the study, analyzed data, wrote the manuscript

Abstract

Recombinant allergens improve the diagnostic precision in Hymenoptera venom allergy (HVA), in particular in patients with double sensitization to both honey bee (HBV) and yellow jacket venom (YJV). While currently available vespid allergens allow detection of >95% of YJV allergic patients, the sensitization frequency to the only available HBV allergen rApi m 1 in HBV allergic patients is lower. Here we demonstrate that sIgE to additional HBV marker allergens rApi m 3 and rApi m 10 allows detection of genuine HBV sensitization in 46 - 65 % of Api m 1 negative sera. This is of particular relevance in patients with double sensitization to HBV and YJV that did not identify the culprit insect. Addition of sIgE to rApi m 3 and rApi m 10 provides evidence of HBV sensitization in a large proportion of rApi m 1 negative patients and thus provides a diagnostic marker and rationale for VIT treatment with HBV, that otherwise would have been missing.

Background:

Double positive IgE test results to honey bee (HBV) and yellow jacket venom (YJV) do not always reflect genuine double sensitization, but are frequently caused by antibodies crossreactive to homologous peptide sequences in protein allergens that are present in both venoms (e.g. the hyaluronidases Api m 2 and Ves v 2, the dipeptidyl peptidases, Api m 5 and Ves v 3, the vitellogenins Api m 12 and Ves v 6) or to crossreactive carbohydrate determinants (CCD) commonly present on Hymenoptera venom glycoproteins (reviewed in 1). The introduction of CCD-free recombinant marker allergens that are specific for either HBV or YJV has improved the diagnostic precision in Hymenoptera venom allergy (HVA), since they allow unequivocal discrimination between genuine HBV and YJV sensitization (1-3).

Currently available marker allergens for YJV (rVes v 1, rVes v 5) allow detection of > 95% of YJV allergic patients (4, 5). In contrast the frequency of sensitization to the currently available major HBV allergen rApi m 1 in HBV allergic patients is lower. Initial use of rApi m 1 in the IgE diagnostics of HVA reported a sensitivity of 97 % in a group of monosensitized HBV allergic patients using a liquid-phase detection system (ADVIA Centaur; Siemens Medical Solution Diagnostics, Deerfield, Ill) which in the meantime has been discontinued by the manufacturer (6). Subsequent studies using a solid phase system (ImmunoCAP, Thermo Fisher, Uppsala, Sweden) reported lower frequencies of Api m 1 sensitizations among HBV allergic patients ranging from 58 – 80% (2, 3, 7, 8) leaving a considerable diagnostic gap in identifying HBV sensitization. We recently reported the

relevance of additional allergens such as rApi m 2, rApi m 3, nApi m 4, rApi m 5 and rApi m 10 in patients with confirmed HBV allergy (8) that can serve to close this gap. Here we address the use of these HBV allergens in patients with HVA that did not identify the culprit insect and displayed double sensitization to HBV and YJV.

Methods:

Sera from patients with a convincing history of HVA seen at our allergy clinic between 2010 and 2014 were included in the study. Diagnosis of HVA was based on a history of anaphylactic sting reaction, skin test and measurement of IgE to HBV (i1) and YJV (i3). IgE reactivity to rVes v 1, rVes v 5, rApi m 1, rApi m 2, rApi m 3, nApi m 4, rApi m 5 and rApi m 10 was analyzed using commercially available and experimental ImmunoCAP tests (Thermo Fisher Scientific, Uppsala, Sweden). Among 755 patients with a convincing history of HVA, 45.2% (341) displayed double sensitization (≥ 0.35 kU_A/L) as determined by HBV and YJV ImmunoCAP. Among double sensitized patients, 37.0 % (126/341) reported not to have identified the culprit insect while 24.3 % (83/341) reported honey bee and 38.7 % (132/341) vespids (yellow jacket, hornet) as culprit insect.

Results and Discussion:

To assess the potential benefit of additional HBV allergens, we analyzed IgE reactivities in particular in HBV/YJV double positive patients that had not identified the culprit insect (group A). As a second group we included double positive patients that had reported honey bee as the culprit insect (group B).

In group A (culprit insect not identified, n=126), 54.0 % (68/126) of sera displayed positive IgE reactivity (≥ 0.35 kU_A/L) to rApi m 1, demonstrating a genuine HBV sensitization. Of the rApi m 1 negative sera (n=58), 15 (25.8 %) displayed positive results for rApi m 3, 3 (5.2 %) for nApi m 4 and 27 (46.6 %) for rApi m 10 (fig. 1A). Combination of rApi m 3 and rApi m 10 data allowed detection of HBV sensitization in 31 (65.4 %) of the rApi m 1 negative sera. No additional case of HBV sensitization was identified by nApi m 4. The combination of rApi m 1, rApi m 3 and rApi m 10 resulted in detection of HBV sensitization in 78.6 % of the patients in group A.

In group B (culprit insect: honey bee, n=83), sIgE reactivity (≥ 0.35 kU_A/L) to rApi m 1 was detected in 66.3 % (55/83). Of the 28 rApi m 1 negative sera, 8 (28.6 %) displayed IgE reactivity to rApi m 3, 4 (14 %) to nApi m 4 and 12 (43 %) to rApi m 10 (fig. 1B).

Combination of rApi m 3 and rApi m 10 data allowed detection of HBV-specific IgE in 13

(46.4 %) of the rApi m 1 negative sera. nApi m 4 identified two additional sera that were not already detected by rApi m 3 or rApi m 10. Combination of rApi m 1, rApi m 3, nApi m 4, and rApi m 10 resulted in detection of HBV sensitization in 84.3 % of the 83 patients in group B.

We additionally analyzed sIgE to rApi m 2 and rApi m 5 in rApi m 1 negative sera of both groups. In group B (culprit insect: honey bee), IgE reactivity (≥ 0.35 kU_A/L) to rApi m 2 was detected in 35.7 % (10/28) and to rApi m 5 in 46.4 % (13/28). Combination of data for all HBV allergens (rApi m 1, rApi m 2, rApi m 3, nApi m 4, rApi m 5 and rApi m 10) resulted in the detection of sIgE in 92.7 % of the patients, broadly confirming our previous data on IgE reactivities in HBV allergic patients (8). In group A, IgE reactivity to rApi m 2 was detected in 29.3 % (17/58) and to rApi m 5 in 34.5 % (20/58) of the rApi m 1 negative sera. The combination of all HBV allergens resulted in the detection of sIgE in 87.6 % (110/126) of the patients who had not identified the culprit insect. However, while sIgE to rApi m 3, rApi m 4 and rApi m 10 are specific markers for HBV sensitization, sIgE to rApi m 2 and rApi m 5 may result from cross-reactivity to rVes v 2 and rVes v 3 (9, 10), the corresponding homologous allergens in YJV. Thus, for the purpose of unambiguously detecting a genuine HBV sensitization, only positive test results to rApi m 3, nApi m 4 and rApi m 10 should be considered, in particular in patients that did not identify the culprit insect (fig. 2A).

Finally, we compared results obtained with the currently available venom allergens (rApi m 1, rVes v 5/rVes v 1) to results obtained after including additional HBV marker allergens. The added value is best exemplified in patients of group A (culprit insect not identified). Available molecular diagnostics demonstrated double sensitization (rApi m 1 and rVes v 5 and/or rVes v 1 positive) in 43.7%, single positivity for rApi m 1 in 10.3% and single positivity for rVes v 5/rVes v 1 in 43.6%. Three patients (2.4%) tested negative to all three recombinant allergens. This profile changed considerably when sIgE detection to rApi m 3 and rApi m 10 was included (fig 2 B): the percentage of confirmed double sensitizations to HBV and YJV allergens increased from 43.7% (55/126) to 65.9 % (83/126), single positives for HBV allergens increased from 10.3 % (13/126) to 12.7 % (16/126), single positives for YJV allergens rVes v 5/rVes v 1 were reduced from 43.7 % (55/126) to 21.4% (27/126) and the number of double negative sera dropped from 3 to 0. Similar changes were observed in group B (culprit insect honey bee) when sIgE detection to rApi m 3 and rApi m 10 was included: the percentage of confirmed double sensitizations to HBV and YJV allergens increased from 43.4% (36/83) to 54.2 % (45/83), single positives for HBV allergens increased from 22.8 % (19/83) to 27.7 % (23/83), single positives for YJV allergens rVes v 5/rVes v 1 were reduced

from 26.5 % (22/83) to 15.7 % (13/83) and the number of double negative sera dropped from 6 to 2. Finally, we also analyzed double positive patients that had reported yellow jacket as the culprit insect (n=110). Again similar changes were observed: the percentage of confirmed double sensitization to HBV and YJV allergens increased from 27.3 % (30/110) to 46.4 % (51/110), single positives for YJV allergens rVes v 5/rVes v 1 were reduced from 70.9 % (78/110) to 51.8 % (57/110), single positives for HBV allergens increased from 0 to 1 and the number of double negative sera dropped from 2 to 1.

Importantly, for patients in group A (culprit insect not identified), the addition of sIgE measurements to Api m 3 and Api m 10 allowed objective evidence of HBV sensitization to be obtained in 31 additional patients and thus provided a diagnostic marker and rationale for VIT treatment with HBV extract, that otherwise would have been missing.

For the management of patients of group A, the addition of rApi m 3 and rApi m 10 resulted in an increased number of patients that would receive VIT to both venoms (43.9 % to 65.9 %). A question that remains is how to manage those patients of group A that display negative results for all tested HBV allergens. Currently, the combination of Api m 1, Api 3 and Api m 10 allows the detection of HBV sensitization in patients with anaphylactic bee sting reactions with a sensitivity of 81.9 % (group B, present study) to 87.5% (8). This still leaves a relevant diagnostic gap that needs to be reduced. Additional factors (like circumstances of sting situation, level of sIgE, development of sIgE response) may have to be considered to decide on an individual level, if double positive patients of group A that are positive for Ves v 5 and or Ves v 1 but negative to Api m 1, Api m 3 and Api m 10 should also receive VIT with HBV or only with YJV.

Summary and outlook:

Expanding the repertoire of HBV allergens for the molecular characterization of sensitization profiles improves the diagnostic precision in HVA. This is of particular relevance in patients that did not identify the culprit insect and display double positive test results for HBV and YJV. In this study, the additional use of marker allergens such as rApi m 3 and rApi m 10 (and to a lesser extent nApi m 4) allowed unambiguous detection of HBV sensitization in approximately two thirds of rApi m 1 negative sera. In the absence of the additional IgE measurements, those patients would have been regarded as having a primary sensitization only to YJV and not to HBV. Thus, addition of these allergens to the battery of diagnostic reagents represents a significant improvement towards closing the diagnostic gap in identification of patients with primary HBV sensitization (fig. 2A). Potentially cross-reactive

allergens such as rApi m 2 and rApi m 5 provide less useful information with respect to discrimination between primary HBV and vespid sensitization. However, if the vespid counterparts rVes v 2 and rVes v 3 become available for testing, comparison of IgE levels to these homologous HBV and JYV allergens could be helpful to identify the primary sensitizer and thus allow us to reduce the diagnostic gap even further.

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Figure legend

Figure 1

sIgE levels to rApi m 3, nApi m 4 and rApi m 10 in patients with hymenoptera venom allergy that were double positive for YJV (i3) and HBV (i1) and displayed negative IgE results for rApi m 1 (< 0.35 kU_A/L): **A**, patients that did not identify the culprit insect; **B**, patients that reported honey bee as culprit insect.

Figure 2

A, Added value of rApi m 3, and rApi m 10 for the detection of genuine HBV sensitization in patients that were double positive for YJV (i3) and HBV (i1) and had not identified the culprit insect (group A, n=126). Percentage of patients detected by different combinations of sIgE to Api m 1, rApi m 3, nApi m 4 and rApi m 10. **B**, Comparison of test outcome with currently available Hymenoptera venom allergens (Api m 1, Ves v 5/Ves v 1) alone (grey bars) or with additional HBV allergens rApi m 3 and rApi m 10 (black bars) in patients that were double positive for YJV (i3) and HBV (i1) and had not identified the culprit insect. DP, double positive for HBV and YJV allergens; YJV⁺ HBV⁻ single positive for YJV allergens; HBV⁺, YJV⁻ single positive for HBV allergens; DN, double negative for HBV and YJV allergens.

Figure 1

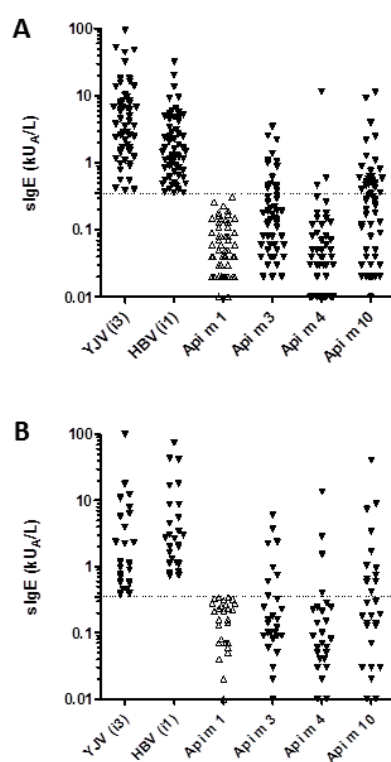


Figure 2

