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From basic science to diagnostics - History of a patent on a honeybee venom allergen

1 | COMMENTARY

Hymenoptera venom can induce severe IgE-mediated systemic and even fatal allergic reactions. Fortunately, venom-specific immunotherapy (VIT) is one of the most effective disease-modifying curative treatments in the field of clinical allergology, but choosing the correct venom for VIT represents a crucial prerequisite for effective protection. In the past, therapeutic decisions based on specific IgE (sIgE) levels to whole venom extracts were not always straightforward. The presence of cross-reactive allergens and cross-reactive carbohydrate determinants (CCDs) in the extracts complicated the discrimination between cross-reactivity and true allergy to more than one venom.[1] In the last years, the increasing knowledge of the composition of relevant venom allergens and the availability of recombinant CCD-free marker allergens resulted in the development of an advanced component-resolved diagnostics (CRD) of venom allergy.[1] CRD has increased the sensitivity of sIgE detection and enabled the discrimination between primary sensitization and cross-reactivity, particularly in patients double-sensitized to honeybee and vespid venom.

The patent described herein covers the nucleic acid encoding the honeybee venom (HBV) allergen Api m 5 as well as the diagnostic and therapeutic use of the allergen.[2] When we started with our work, only few venom allergens had been identified on a molecular level and we, therefore, aimed to gain deeper insights into the allergen composition and molecular mechanisms of Hymenoptera venoms. Api m 5 was first described as 105 kDa allergen C (named allergen C as it was found in fraction 1C during size-exclusion chromatography of HBV) in 1977 and suggested to be an important allergen of HBV.[3] However, identity and function of allergen C remained elusive.

Although Api m 5 is a very potent allergen, HBV contains only minute amounts and strategies to purify it from whole venom had to be developed. However, no N-terminal sequence information could be obtained even from the purified allergen, most likely due to N-terminal protection. Finally, four internal peptides of the allergen were identified by tandem mass spectrometry and the use of these peptides for a search in the honeybee genome yielded a perfect match for a predicted gene. Despite the fact that internal and C-terminal parts of the coding region could be amplified and exactly matched the predicted sequence, all attempts to amplify the complete coding region from venom gland cDNA were unsuccessful. Careful reevaluation of the genomic locus showed that the predicted 5′-terminal sequence was erroneous; instead of the predicted 5′-terminal exon, the true 5′-end of the gene is built by two exons located further downstream of the predicted one. Intriguingly, the first exon only codes for the first two amino acids of the signal peptide of Api m 5. With this information, the full coding region of the allergen could be successfully cloned and Api m 5 produced as recombinant protein in an insect cell line. Nowadays, insect cells are the system of choice for recombinant production of venom allergens and are also used to produce most of the commercially available venom allergens for CRD. A major advantage of certain insect cell lines is that the recombinant allergens are free from interfering CCD-reactivity.[1]

The further characterization of Api m 5 demonstrated that it is a dipeptidyl peptidase IV (DPPIV), which cleaves dipeptides from the N-terminus of proteins and, hence, a homologue of human CD26.[4] Its putative function in HBV is the processing and activation of the membrane-toxic peptide melittin. The role of Api m 5 as major allergen of HBV was confirmed by its pronounced sIgE reactivity and the potent activation of basophils from HBV-allergic patients.[5-7]

Despite the allergenic potency of Api m 5, the path from identification to use in commercially available diagnostics took nearly 10 years. At the time when the advantages of CRD became obvious, recombinant HBV major allergen phospholipase A2 (Api m 1) was initially introduced as marker allergen for the diagnosis of HBV allergy. For years, Api m 1 was thought to be the most relevant and, perhaps, only major allergen in HBV. However, following studies demonstrated that using exclusively Api m 1 for diagnosis of HBV allergy and its discrimination from vespid venom allergy is insufficient.[8] The remaining diagnostic gap was clearly too large given a potentially life-threatening allergy. A subsequent study verified that HBV contains other relevant major allergens whose application can almost completely close the diagnostic cap.[6] These allergens were consequently developed for CRD in clinical routine.

Nowadays, these allergens, which include Api m 5, are available for routine CRD and allow the elucidation of sensitization profiles of the patients (Figure 1). CRD demonstrated to provide additional value for the discrimination between honeybee and vespid venom allergy. Hence, CRD is able to support the choice of the correct venom for VIT and to prevent unnecessary treatment with more than one venom.[1]

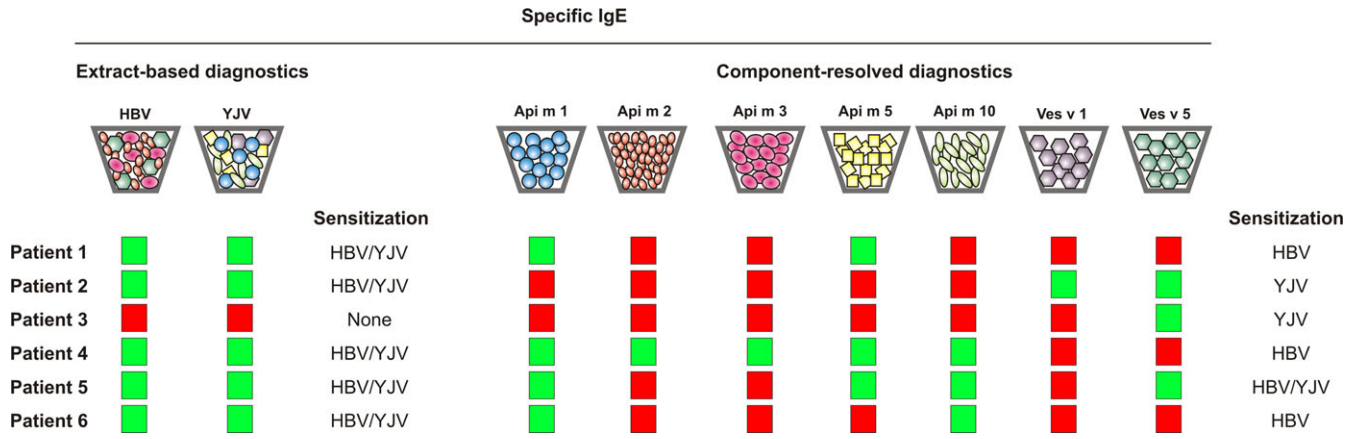

FIGURE 1 Component-resolved diagnostics (CRD) of venom allergy. Venom extract-based diagnostics provide the information if a patient is sensitized to the whole venom or not. CRD is based on the molecular knowledge of the composition of relevant venom allergens and their availability as recombinant CCD-free proteins. CRD gives detailed information about the sensitization profiles of patients. Hence, in many cases, it allows discrimination between cross-reactivity and primary sensitization as well as might increase sensitivity of sIgE detection. green, positive sIgE result; HBV, honeybee venom; red, negative sIgE result; YJV, yellow jacket venom

Subsequently, the Api m 5 homologues from *Vespula vulgaris* and *Polistes dominula* venom Ves v 3 and Pol d 3 were identified and characterized.[5,7] Due to the extent of homology, both major allergens are protected by the same patent. Although all three allergens show a significant degree of cross-reactivity on protein level (38% and 32% of Api m 5-reactive sera show also sIgE reactivity with Ves v 3 and Pol d 3, respectively[5,7]), Api m 5 already proved to be a relevant tool for CRD. So far, the two homologous vespid allergens are not available for routine diagnosis. However, it could be speculated that they might also be able to contribute to better discrimination between allergies to different types of vespid species, since it has been shown that measuring sIgE levels to homologous major allergens is able to identify the primary sensitizing venom.[9]

2 | CONCLUSION

In recent years, knowledge of the composition of Hymenoptera venoms regarding relevant allergens has rapidly increased. Analyses using recombinant CCD-free allergens allowed their detailed characterization and the identification of novel major venom allergens. Today, these allergens, including Api m 5, build the pillars of component-resolved diagnostics of venom allergy which has demonstrated high potential for better patient care. In the future, sensitization profiles of patients might even more direct therapeutic decisions in a personalized and patient-tailored manner.

CONFLICTS OF INTEREST

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