Mass Spectrometry Detects Api m 10 in Venom Immunotherapy Products With Suspected Absence of Api m 10

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Honeybee venom (HBV) allergy is potentially life-threatening. The only disease-modifying treatment is venom immunotherapy (VIT) [1,2]. Twelve HBV proteins (Api m 1 to Api m 12) have been characterized as allergens according to the WHO/IUIS Allergen Nomenclature (www.allergen.org). Absence and underrepresentation of some allergenic components in authorized aqueous VIT products have been reported [3,4]. Based on a retrospective analysis of sensitization profiles in HBV-allergic patients whose VIT failed [5] and component-resolved evaluation of the allergen content in VIT products [6], lack of Api m 10 has been hypothesized to be responsible for failure of VIT. However, none of these studies included a prospective patient analysis of molecular sIgE-binding to HBV allergens before initiation of VIT or an assessment of the respective VIT product applied [1]. The authors focused on Api m 10, a major allergen that is recognized by up to 74.5% of HBV-allergic patients [4,7,8]. Based on IgG immunoblot examinations (using polyclonal anti–Api m 10 antibodies) of a limited number of VIT product batches, Api m 10 was reported to be present in crude HBV but absent or quantitatively underrepresented in several authorized HBV VIT products [3,5,6]. The question of whether this phenomenon is product-specific or varies from batch to batch of the same product remains controversial [5,6]. However, negative test results are always open to alternative interpretations, namely, the analyte is absent, the detection limit of the method is insufficient, or, for other methodological reasons, the measurement method does not detect the target or only detects it partially. The application of an orthogonal method can compensate for the limitation of a method with regard to points 2 and 3. If this method also yields negative results, the detection limit of at least 1 method must be reliably determined. On this point, major concerns exist among allergists about whether the limited published data are robust, have implications for diagnosis and treatment of HBV-allergy in general, and specifically apply to VIT product selection in Api m 10–sensitized individuals. Clarification of these questions is of the utmost therapeutic and regulatory relevance. The confirmed absence or underrepresentation of clinically relevant components in VIT leading to a reduction in or lack of product efficacy for specific patient subgroups may have implications for postmarketing regulatory measures.

Given that unjustified doubts and quality deficiencies in allergen products might impact patients' supply of effective and safe VIT, the Paul-Ehrlich-Institut has initiated independent experimental investigations. Here, we report data on Api m 10 in VIT products recorded using orthogonal analytical methods. In Germany, 4 aqueous VIT products made by 3 different manufacturers are authorized (https://www.pei.de/EN/medicinal-products/allergens/therapy-subcutaneous/subcutaneous-therapy-node.html). In total, 31 different batches of these 4 products (A, B, D, n=8 each; C, n=7) were analyzed for the presence of Api m 10 using 2 orthogonal techniques, ie, techniques that rely on fundamentally different principles to ensure maximum reliability when combined. The first involves IgG-immunoblotting using a polyclonal rabbit anti–Api m 10 antibody raised against splice variant 1 (UniProtKB Q5EF78), as reported elsewhere [3,5,6]. The second uses high-definition mass spectrometry (HDMS^E), which has unequivocally proven the presence of allergens [9,10].

Our IgG-immunoblotting analyses detected Api m 10 in 8/8 batches of product A, 5/8 batches of product B, 0/7 batches of product C, and 8/8 batches of product D (Table; Figure E1). Although the results were not identical to those of published studies for all batches, their core finding was reproduced, namely, some batches yield a negative result when analyzed with immunoblot (Table; Figure E1). However, orthogonal analysis with mass spectrometry (MS) unambiguously revealed that Api m 10 was present in all 31 batches studied. HBV preparations were trypsin-digested, and the resulting peptides originating from Api m 10 were readily and unequivocally detected by HDMS^E (Table; Table E1). Compared to immunoblot analyses, MS analysis may have been more sensitive or may have also detected non–IgG-binding (eg, unfolded) Api m 10. At this point, our qualitative MS data confirmed the presence of Api m 10 in all 4 HBV products for VIT, even when immunoblot experiments suggested its absence.

Our data indicate that the antibody-based approaches used in our study and in previous studies to assess therapeutic

Table. Qualitative Detection of Api m 10 in 31 Batches of Honeybee Venom Immunotherapy Products.[a]

Manufacturer	Product	Sample	HDMS^E	IgG immunoblot results		
				PEI	Ref. 6[b]	Ref. 5[b]
1	A	Batch 1	+	+	ND	-
		Batch 2	+	+	+	-
		Batch 3	+	+	ND	-
		Batch 4	+	+	+	ND
		Batch 5	+	+	+	ND
		Batch 6	+	+	+	ND
		Batch 7	+	+	+	ND
		Batch 8	+	+	+	ND
2	B	Batch 1	+	-	ND	-
		Batch 2	+	-	ND	ND
		Batch 3	+	-	ND	-
		Batch 4	+	+	+	ND
		Batch 5	+	+	+	ND
		Batch 6	+	+	ND	ND
		Batch 7	+	+	ND	ND
		Batch 8	+	+	ND	ND
3	C	Batch 1	+	-	ND	-
		Batch 2	+	-	-	-
		Batch 3	+	-	ND	-
		Batch 4	+	-	-	ND
		Batch 5	+	-	ND	ND
		Batch 6	+	-	ND	ND
		Batch 7	+	-	ND	ND
	D	Batch 1	+	+	+	+
		Batch 2	+	+	+	+
		Batch 3	+	+	ND	ND
		Batch 4	+	+	ND	ND
		Batch 5	+	+	ND	ND
		Batch 6	+	+	ND	ND
		Batch 7	+	+	ND	ND
		Batch 8	+	+	ND	ND

Abbreviations: HDMS, high-definition mass spectrometry; ND, not determined; PEI, Paul-Ehrlich-Institut.
[a]The presence of Api m 10 was assessed using HDMS^E and IgG-immunoblotting.
[b]IgG immunoblot results previously published by Frick et al [5] and Blank et al [6] are displayed in the column.

HBV products cannot confirm the absence of Api m 10 in VIT products. However, our study does not provide quantitative analyses. Even though Api m 10 was unambiguously present in all batches, it remains to be clarified whether or not Api m 10 is underrepresented (ie, its quantity is too low to induce a protective immune response), as previously suggested [3,5,6]. To answer this question, 2 advances would have to be made, namely, methods need to be developed and validated to detect Api m 10 with sufficient and confirmed sensitivity and specificity, and the amount of Api m 10 required to induce a protective immune response must be determined. Despite its presence at the molecular level, as detected by HDMS^E, Api m 10 may not be immunologically active and/or present in sufficient amounts to induce a protective immune response, or mechanisms other than venom-specific IgG_4 may play a role in the protective immune response in sensitization to Api m 10 [11].

Manufacturer information on the component-specific composition contained in HBV products is very limited, and quantitative data are lacking. Moreover, there are no large prospective component-specific clinical trials, and well-characterized case reports and case series have presented contradictory results [11-13], generating concerns over HBV immunotherapy [14]. Currently, it is not possible to prospectively assess the relevance of our qualitative MS findings with detection of Api m 10 in all the 31 batches examined for the treatment course. In particular, the individual allergen quantities required for the induction of an allergen-specific protective immune response in vivo remain undetermined.

Based on our findings and limitations, Paul-Ehrlich-Institut has initiated further studies with qualitative and quantitative methods [15] to help clarify outstanding issues that may have regulatory implications.

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Conflicts of Interest

JL is an employee of Thermo Fisher Scientific. The remaining authors declare that they have no conflicts of interest.

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