

Letter to the Editor

Low sensitivity of commercially available rApi m 1 for diagnosis of honeybee venom allergy

To the Editor:

Several studies have shown that the major recombinant allergen in honeybee venom,¹ phospholipase A₂ allergen (Api m 1), might be useful for *in vitro* and *in vivo* diagnosis of honeybee venom allergy.²⁻⁵ Correctly refolded² rApi m 1 has demonstrated *in vivo* diagnostic sensitivity up to 95%.³ In *in vitro* determinations of specific IgE (sIgE) against rApi m 1, sensitivity between 78%³ and 97%⁴ and even up to 100%⁵ has been reported. Analytic specificity of recombinant allergens was evaluated by using IgE immunoblotting, ELISA, or both,^{3,5} and one report used a commercially available liquid-phase detection system (ADVIA Centaur; Siemens Medical Solution Diagnostics, Deerfield, Ill).⁴

Venom recombinants are produced in the bacterial expression systems as nonglycosylated proteins and in eukaryotic systems as glycosylated allergens. Glycosylated allergens expressed in eukaryotic cells, such as baculovirus-infected insect cells, have an IgE-binding capacity comparable with that of natural allergens.⁶ However, IgE reactivity to carbohydrate epitopes can lead to false-positive results, which decrease the diagnostic specificity of glycosylated recombinants.^{4,5} In late 2009, the first nonglycosylated recombinant venom allergen component was available for the widely used ImmunoCAP solid-phase assay (CAP-FEIA; Phadia, Uppsala, Sweden). This allergen was a novel *Escherichia coli*-expressed nonglycosylated Api m 1, which was a substitute for the previously used nApi m 1. Because this recombinant is potentially very useful and commercially available, we wanted to evaluate its diagnostic utility and compare it with the nApi m 1 allergen in a routine clinical laboratory setting by analyzing a large group of patients with well-defined honeybee venom allergy before adopting its use in our clinical practices.

In total, 184 subjects (mean age, 43 years; age range, 16-75 years; 101 women) with established honeybee venom allergy (20 with large local reactions and 15 with Mueller grade I, 32 with grade II, 74 with grade III, and 34 with grade IV reactions) were recruited during a 5-year period. In all subjects honeybee and wasp (*Vespula* species) venom-specific IgE and total IgE levels were prospectively measured with CAP-FEIA. Tests for CAP-FEIA recombinant *E. coli*-expressed nonglycosylated Api m 1 (i208) and nApi m 1 (k203) were performed in early winter 2010 from stored (at -40°C) samples. CAP-FEIA to oilseed rape (OSR; canola, *Brassica napus*; f316) was used as an indicator of cross-reacting carbohydrate determinants (CCDs).⁷ Fifteen samples were missed for nApi m 1 (we included 169 subjects), 9 samples were missed for rApi m 1 (we included 175 subjects), and 10 samples were missed for CCD measurements (we included 174 subjects). Thus we tested 168 sera for all allergens. In 2010, the production of nApi m 1 (k203) was discontinued.

In all study subjects we demonstrated a positive specific IgE response to honeybee venom (2.1 kU/L; range, 0.41->100 kU/L) and a negative specific IgE response (<0.35 kU/L) to wasp venom (*Vespula* species, Fig 1). Next, we examined the subjects for the presence of specific IgE antibodies to rApi m 1 and nApi m 1, as detected by using the CAP-FEIA system (Fig 1). Surprisingly, we found that more subjects had positive specific IgE responses

for nApi m 1 (153/169 [91%]) than for rApi m 1 (100/175 [57%]; $P < .0001$, Fisher exact test). These marked differences were even more prominent in subjects who had experienced severe anaphylactic reactions. After a honeybee sting, rApi m 1 recognized only 51% and 63% of subjects in the Mueller grade III and IV subgroups, respectively. On the other hand, nApi m 1 recognized 87% of subjects in the Mueller grade III subgroup and almost all subjects (97%) in the Mueller grade IV group ($P < .0001$, Fisher exact test). The median concentration of rApi m 1 in 100 subjects with positive responses was 4.4 kU/L (range, 0.36->100 kU/L), which was comparable with the median concentration of nApi m 1 (4.8 kU/L; range, 0.36->100 kU/L) in 153 subjects with positive responses. The major finding was that 53 subjects who had clearly positive responses for nApi m 1 (3.4 kU/L; range, 0.37->100 kU/L) had negative IgE reactivity for rApi m 1. Nevertheless, nApi m 1 had still missed 9% of subjects with established honeybee venom allergy.

Most likely, carbohydrate epitopes of the native allergen did not influence these results because only 9% (16/174) of our subjects showed IgE reactivity to CCDs (Fig 2). The proportion of IgE positivity for CCDs found in this study was comparable with results found in other studies of venom-mono-sensitized subjects (ie, 14.3% OSR positive responses in honeybee venom-sensitized patients and 12.5% in wasp venom-sensitized patients).⁷ On the other hand, in venom double-positive sera a much higher proportion of CCD positivity was reported.⁷ There were no significant differences between CCD positivity according to the severity of sting reactions and no matching between IgE reactivity to OSR and reactivity to rApi m 1 and nApi m 1. Namely, 10 of 16 CCD-positive subjects had positive responses for both rApi m 1 and nApi m 1, and only 6 CCD-positive subjects had positive responses just for nApi m 1. It is important to note that OSR was used as a measure for carbohydrate-specific IgE because OSR pollen, but not Gramineae pollen, contains MMF glycans, which are characteristic for honeybee Api m 1.⁷ The discrepancy between nApi m 1 and rApi m 1 reactivity might be influenced also by the other bee venom epitopes, such as Api m 2, Api m 3, Api m 4, or Api m 10.⁵ Nevertheless, the notion that Api m 1 represents a major allergen has been shown in numerous studies,¹⁻⁵ including this one with native CAP-FEIA Api m 1 allergen.

Recently, an initial report about the diagnostic value of CAP-FEIA i208 rApi m 1 was published.⁸ Unfortunately, the strength of this communication was limited by the low number of subjects studied. Only 34 subjects with a history of honeybee venom allergy (only 18 of whom were honeybee mono-sensitized) limited the clinical relevance and prevented a comprehensive and statistically sound analysis. The diagnostic sensitivity of rApi m 1 reported in this communication was 79%.⁷

Previous reports also suggest that total serum IgE is a potential risk factor of the severity of systemic venom-induced anaphylactic reactions.^{9,10} The median concentration of total IgE was 37.8 kU/L (range, 3.1-169 kU/L), 36 kU/L (range, 3.6-223 kU/L), 38.9 kU/L (range, 8.9-1000 kU/L), 47.7 kU/L (range, 4.6-947 kU/L), and 40 kU/L (range, 6.6-685 kU/L) in the large local reaction and Mueller grade I, II, III, or IV subgroups, respectively (Fig 2). Similar honeybee venom-specific IgE rank was highly comparable between subjects with different severity grades (Fig 1). Therefore an influence of total and specific IgE on the severity

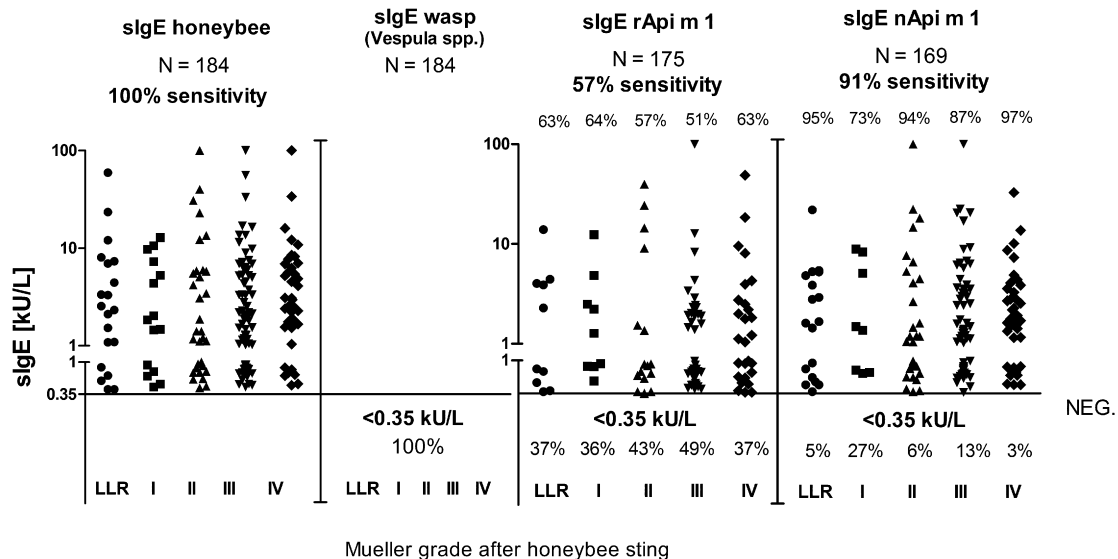


FIG 1. Honeybee venom, wasp venom, rApi m 1, and nApi m 1 CAP-FEIA sIgE measurements in patients with honeybee venom allergy. LLR, Large local reaction.

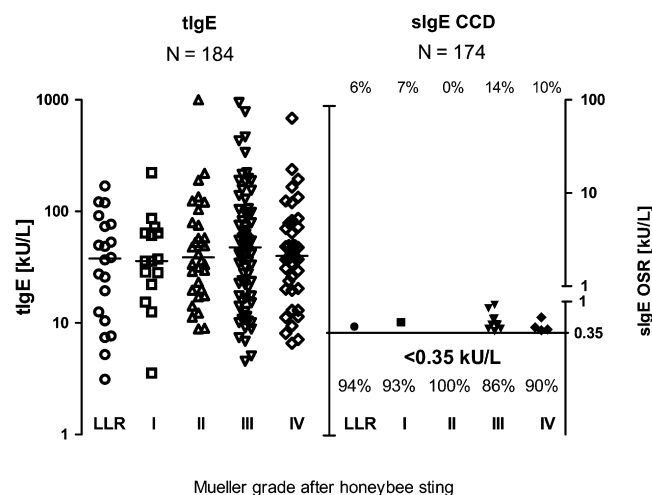


FIG 2. Total IgE (tIgE) and sIgE reactivity for CCDs measured with OSR CAP-FEIA in patients with honeybee venom allergy. The horizontal solid bars indicate the median value for each subgroup. LLR, Large local reaction.

of systemic reactions after a previous honeybee field sting could not be shown.

In conclusion, our results suggest that the current CAP-FEIA rApi m 1 has limited clinical usefulness for the detection of honeybee venom allergy because of its low diagnostic sensitivity. Thus improved diagnostic tests are needed.

Peter Korošec, PhD^a
 Rudolf Valenta, MD^b
 Irene Mittermann, PhD^b
 Nina Čelesnik, BS^a
 Renato Eržen, MD^a
 Mihaela Zidarn, MD^a
 Mitja Košnik, PhD, MD^a

From ^athe University Clinic of Respiratory and Allergic Diseases, Golnik, Slovenia, and
^bthe Christian Doppler Laboratory for Allergy Research, Center for Physiology and

Pathophysiology, Department of Pathophysiology/Division of Immunopathology, Medical University of Vienna, Vienna, Austria. E-mail: peter.korosec@klinika-golnik.si.
 Disclosure of potential conflict of interest: R. Valenta receives research support from the Austrian Science Fund, the Christian Doppler Research Association, and Biomay and has consulted for Biomay and Phadia. The rest of the authors have declared that they have no conflict of interest.

REFERENCES

- Sobotka AK, Franklin RM, Adkinson NF Jr, Valentine M, Baer H, Lichtenstein LM, et al. Allergy to insect stings. II. Phospholipase A: the major allergen in honey bee venom. *J Allergy Clin Immunol* 1976;57:29-40.
- Müller UR, Dudler T, Schneider T, Cramer R, Fischer H, Skrbic D, et al. Type I skin reactivity to native and recombinant phospholipase A2 from honeybee venom is similar. *J Allergy Clin Immunol* 1995;96:395-402.
- Müller U, Fricker M, Wyman D, Blaser K, Cramer R. Increased specificity of diagnostic tests with recombinant major honeybee venom allergen phospholipase A2. *Clin Exp Allergy* 1997;27:915-20.
- Müller U, Johansen N, Petersen AB, Fromberg-Nielsen J, Haeberli G. Hymenoptera venom allergy: analysis of double positivity to honey bee and *Vespula* venom by estimation of IgE antibodies to species-specific major allergens Api m 1 and Ves v 5. *Allergy* 2009;64:543-8.
- Mittermann I, Zidarn M, Silar M, Markovic-Housley Z, Aberer W, Korošec P, et al. Recombinant allergen-based IgE testing to distinguish honeybee and wasp allergy. *J Allergy Clin Immunol* 2010;125:1300-7.
- Soldatova LN, Cramer R, Gmachl M, Kemeny DM, Schmidt M, Weber M, et al. Superior biologic activity of the recombinant honeybee venom allergen hyaluronidase expressed in baculovirus-infected insect cells as compared with *Escherichia coli*. *J Allergy Clin Immunol* 1998;101:691-8.
- Hemmer W, Focke M, Kolarich D, Wilson IB, Altmann F, Wöhrl S, et al. Antibody binding to venom carbohydrates is a frequent cause for double positivity to honeybee and yellow jacket venom in patients with stinging-insect allergy. *J Allergy Clin Immunol* 2001;108:1045-52.
- Hofmann SC, Pfender N, Weckesser S, Huss-Marp J, Jakob T. Added value of IgE detection to rApi m 1 and rVes v 5 in patients with Hymenoptera venom allergy. *J Allergy Clin Immunol* 2011;127:265-7.
- Sturm GJ, Heinemann A, Schuster C, Wiednig M, Groselj-Strele A, Sturm EM, et al. Influence of total IgE levels on the severity of sting reactions in Hymenoptera venom allergy. *Allergy* 2007;62:884-9.
- Blum S, Gunzinger A, Müller UR, Helbling A. Influence of total and specific IgE, serum tryptase, and age on severity of allergic reactions to Hymenoptera stings. *Allergy* 2011;66:222-8.

doi:10.1016/j.jaci.2011.03.012