



Contents lists available at ScienceDirect

Allergy International

journal homepage: <http://www.elsevier.com/locate/alit>

Letter to the Editor

Sensitization to Api m 1, Api m 2, and Api m 4 in Japanese beekeepers who had experienced systemic reactions to honeybee stings

Dear Editor,

Honeybee (HB) venom contains 12 known allergens.¹ The major allergen components of HB venom are phospholipase A2 (Api m 1), hyaluronidase (Api m 2), and melittin (Api m 4).² Api m 1 and Api m 2, which are glycoproteins, are 13 kDa and 45 kDa in size, respectively.³ Api m 4 is a small 3-kDa peptide with no capacity for disulfide bond formation.⁴ Immunoglobulin (Ig)E antibodies specific to phospholipase A2, hyaluronidase, and melittin bind to recombinant (r)Api m 1, rApi m 2, and rApi m 4, respectively, and show increased test specificity due to the lack of carbohydrate determinants in the recombinant protein.⁵ The diagnostic sensitivity of the novel rApi m 1, rApi m 2, and rApi m 4 allergens was recently investigated in a routine clinical laboratory setting in a group of patients with HB venom allergy.¹ Api m 1 is by far the most important allergen in HB venom, but sensitization to Api m 2 and Api m 4 also occurs.¹ However, the significance of measuring the levels of sIgE to these allergen components is not known. In this study, we analyzed sensitization to Api m 1, Api m 2, and Api m 4 in Japanese beekeepers who had experienced systemic reactions (SRs) to HB stings.

The participants comprised 121 beekeepers (108 men, 13 women) who were almost all staff members of private apiaries based in Tochigi, Gunma, and Saitama prefectures, Japan. Of the beekeepers, 34 (31 men, 3 women) who had experienced an SR to a HB sting, excluding toxic reactions, were analyzed in this study (Table 1). Given that the main work of beekeepers is to collect honey and maintain breeding colonies, workers are at high risk of HB stings. SR severity was graded according to Mueller (from grade I to IV).⁶ All participants underwent a medical examination including an interview with an allergist and peripheral blood tests were performed on the day of the examination, between December 2015 and February 2016. Fifteen healthy subjects who had never experienced a honeybee sting were enrolled as controls. The study was approved by the Dokkyo Medical University Saitama Medical Center Research Ethics Committee, authorization numbers 1525 and 1526. Written informed consent was obtained from all participants prior to enrollment.

The interval between the last sting and peripheral blood collection differed among the participants. Levels of serum sIgE against HB venom, rApi m 1, rApi m 2, and synthetic source (s)Api m 4 were measured by AlaSTAT 3gAllergy (AlaSTAT), a chemiluminescent enzyme immunoassay (CLEIA) kit. sIgE detection by AlaSTAT is expressed as quantitative units (IU_A/mL) and has a working range

of 0.1–500 IU_A/mL. sIgE levels ≥ 0.1 IU_A/mL measured with AlaSTAT were considered positive.

Data are presented as the mean $\pm$ SD. All statistical analyses were performed using Microsoft Excel and JMP statistical software. The significance of the correlations between the severity of SRs and the sIgE levels or among the sIgE levels was analyzed by Pearson correlation coefficient. The chi-square test was used to assess the correlations between the severity of SRs and sIgE positivity. Wilcoxon rank sum test and Kruskal–Wallis test was used to determine if there are significant differences between the severity of SRs and double-positive or triple-positive sIgE results for the three allergen components. A *P* value < 0.05 was considered significant.

The results of sIgE to HB venom, rApi m 1, rApi m 2, and sApi m 4 in the 34 Japanese beekeepers are summarized in Table 1. The levels of sIgE to HB venom, rApi m 1, rApi m 2, and sApi m 4 were 3.69 ± 5.52 IU_A/mL (range, <0.1 –24.3), 3.67 ± 6.12 IU_A/mL (range, <0.1 –25.8), 5.56 ± 8.04 IU_A/mL (range, <0.1 –43.3), and 0.93 ± 2.90 IU_A/mL (range, <0.1 –15.4), respectively. Each of the 15 controls was negative for sIgE to HB venom, rApi m 1, rApi m 2, and sApi m 4. sIgE positivity to HB venom, rApi m 1, rApi m 2, and sApi m 4 was identified in 32 (94.1%), 31 (91.2%), 33 (97.1%), and 18 (52.9%) beekeepers, respectively. Double positivity to rApi m 1 and rApi m 2 was found in 30 individuals (88.3%) (Fig. 1a). Thus, in the Japanese beekeepers, Api m 1 and Api m 2 were by far the most important allergens in HB venom. In addition, some people were sensitized to Api m 4. Triple positivity to rApi m 1, rApi m 2, and sApi m 4 was found in 17 participants (50.0%). In the clinical significance, we found no evidence that the sensitization to sApi m 4 influence the severity or presentation of SRs. However, Ruiz *et al.* have reported that IgE–Api m 4 is useful for identifying a particular phenotype, such as selection of the most useful honeybee venom extracts for immunotherapy. It is anticipated that these could be used as biomarkers in the clinical course during maintenance immunotherapy of bee venom allergy.¹

Significantly positive correlations were noted between the levels of HB venom–sIgE and rApi m 1–sIgE ($R = 0.98$, $P < 0.0001$) (Fig. 1b). There were correlations between the levels of HB venom–sIgE and rApi m 2–sIgE ($R = 0.42$, $P = 0.014$) (Fig. 1b) or sApi m 4–sIgE ($R = 0.68$, $P < 0.0001$) (Fig. 1b). These results indicated differences in antigenicity to allergen components in patients with HB venom allergy. No significant correlations were noted between the severities of SRs and sIgE levels against HB venom, rApi m 1, or rApi m 2. There were no correlations between the severities of SRs and positive results for the highest levels of sIgE for the three allergen components. In addition, there were no significant differences between the severity of SRs and

Peer review under responsibility of Japanese Society of Allergy.

<https://doi.org/10.1016/j.alit.2018.08.008>1323–8930/Copyright © 2018, Japanese Society of Allergy. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Table 1
Sensitization to HB venom, rApi m 1–, rApi m 2–, and sApi m 4–sIgE in Japanese beekeepers who had experienced SRs to HB stings.

Subjects	Age [†]	Sex	SRs	HB venom sIgE (class)	rApi m 1 sIgE (class)	rApi m 2 sIgE (class)	sApi m 4 sIgE (class)
1	4	M	4	0.54 (1)	0.20 (0+)	0.80 (2)	<0.10 (0)
2	5	M	4	2.12 (2)	1.34 (2)	1.96 (2)	0.29 (0+)
3	4	M	1	1.61 (2)	0.95 (2)	4.10 (3)	0.23 (0+)
4	4	M	1	0.45 (1)	0.25 (0+)	3.17 (2)	<0.10 (0)
5	4	M	3	1.25 (2)	0.48 (1)	4.39 (3)	0.16 (0+)
6	1	M	4	22.6 (4)	23.30 (4)	1.74 (2)	15.40 (3)
7	3	M	3	0.33 (0+)	0.15 (0+)	1.58 (2)	<0.10 (0)
8	5	M	4	1.16 (2)	0.90 (2)	0.97 (2)	<0.10 (0)
9	4	M	4	1.14 (2)	0.73 (2)	7.34 (3)	<0.10 (0)
10	1	F	4	9.43 (3)	16.10 (3)	8.82 (3)	1.39 (2)
11	5	M	1	3.35 (2)	3.58 (3)	0.22 (0+)	<0.10 (0)
12	4	F	1	0.13 (0+)	<0.10 (0)	0.35 (1)	<0.10 (0)
13	1	M	1	5.48 (3)	4.15 (3)	9.04 (3)	<0.10 (0)
14	5	M	1	6.22 (3)	4.04 (3)	10.20 (3)	<0.10 (0)
15	4	M	2	0.74 (2)	0.63 (1)	0.73 (2)	0.14 (0+)
16	5	M	1	1.67 (2)	2.65 (2)	<0.10 (0)	1.32 (2)
17	1	M	4	24.30 (4)	25.80 (4)	19.80 (4)	1.44 (2)
18	5	M	4	3.65 (3)	1.85 (2)	1.08 (2)	0.15 (0+)
19	4	M	4	1.07 (2)	0.29 (0+)	4.07 (3)	0.46 (1)
20	1	M	4	7.47 (3)	7.10 (3)	43.30 (4)	0.79 (2)
21	3	F	1	5.31 (3)	5.08 (3)	8.12 (3)	0.74 (2)
22	5	M	4	8.40 (3)	7.80 (3)	8.66 (3)	7.85 (3)
23	4	M	1	0.60 (1)	0.49 (1)	0.97 (2)	<0.10 (0)
24	5	M	1	1.34 (2)	1.62 (2)	0.74 (2)	<0.10 (0)
25	4	M	4	1.98 (2)	1.23 (2)	6.99 (3)	0.31 (0+)
26	2	M	4	1.36 (2)	0.69 (1)	0.63 (1)	<0.10 (0)
27	3	M	1	0.71 (2)	0.68 (1)	0.90 (2)	0.23 (0+)
28	4	M	3	1.84 (2)	1.60 (2)	6.64 (3)	0.22 (0+)
29	4	M	1	1.18 (2)	1.25 (2)	1.37 (2)	<0.10 (0)
30	5	M	4	0.79 (2)	0.80 (2)	6.97 (3)	<0.10 (0)
31	5	M	4	2.19 (2)	1.71 (2)	7.18 (3)	0.16 (0+)
32	5	M	1	5.00 (3)	7.22 (3)	15.80 (3)	0.23 (0+)
33	4	M	2	<0.10 (0)	<0.10 (0)	0.24 (0+)	<0.10 (0)
34	5	M	4	<0.10 (0)	<0.10 (0)	0.18 (0+)	<0.10 (0)

M, male; F, female; SRs, systemic reactions; HB, honeybee.

[†] Class 1, 30–39 years; 2, 40–49 years; 3, 50–59 years; 4, 60–69 years, 5, over 70 years. Class: 0, <0.1; 0+, 0.10–0.34; 1, 0.35–0.69; 2, 0.70–3.49; 3, 3.50–17.4; 4, 17.5–52.4; 5, 52.5–99.9; 6, ≥100.

double-positive or triple-positive sIgE results for the three allergen components. sIgE levels to allergen components decline over several years if a person does not experience a subsequent HB sting, and sIgE levels are thought to begin decreasing soon after stings.⁷ Furthermore, our data showed that there was no relationship between occurrence of SRs and the amount of sIgE to HB venom or the three allergen components (data not shown). A report has shown that the strength of sIgE is related to the possibility of occurrence of anaphylaxis in children with food allergy.⁸ In order to compare between that report and our data, it is necessary to analyze the age class and number of HB stings experienced, and to keep the period from the latest HB sting to blood sampling constant.

Measurements of sIgE to HB venom are known to have inadequate sensitivity in patients with HB venom allergy. We also measured sIgE to HB venom in Japanese beekeepers who had experienced SRs to HB stings, but the sensitivity was not satisfactory. However, the combination of rApi m 1 and rApi m 2 resulted in the sensitivity increasing from 94.1% (32/34) to 100% (34/34). sIgE measurement by conventional testing in Japanese beekeepers with sIgE against HB venom using allergen extracts “spiked” (supplemented) with allergen components may improve the sensitivity for detecting HB venom allergy.⁹ Köhler *et al.* reported that IgE reactivity to rApi m 1, rApi m 2, and native (n)Api m 4 was detected by ImmunoCAP methods in 72.2%, 47.9%, and 22.9% of patients with HB venom allergy, respectively.¹⁰ Additionally, positive results to at least one HB venom allergen were detected in 94.4%. Although the levels of sIgE to HB venom were similar in our data, the levels of sIgE to the three allergen components were higher in our data than in the recently reported data. These results are attributed to differences in the sIgE measurement methods, race, and/or species of HB.

In conclusion, combination measurement of sIgE to rApi m 1 and rApi m 2 may improve the sensitivity for HB venom allergy detection. Measurement of sIgE to allergen components spiked into allergen extracts by conventional testing in Japanese beekeepers has potential for serologic elucidation of HB venom allergy.

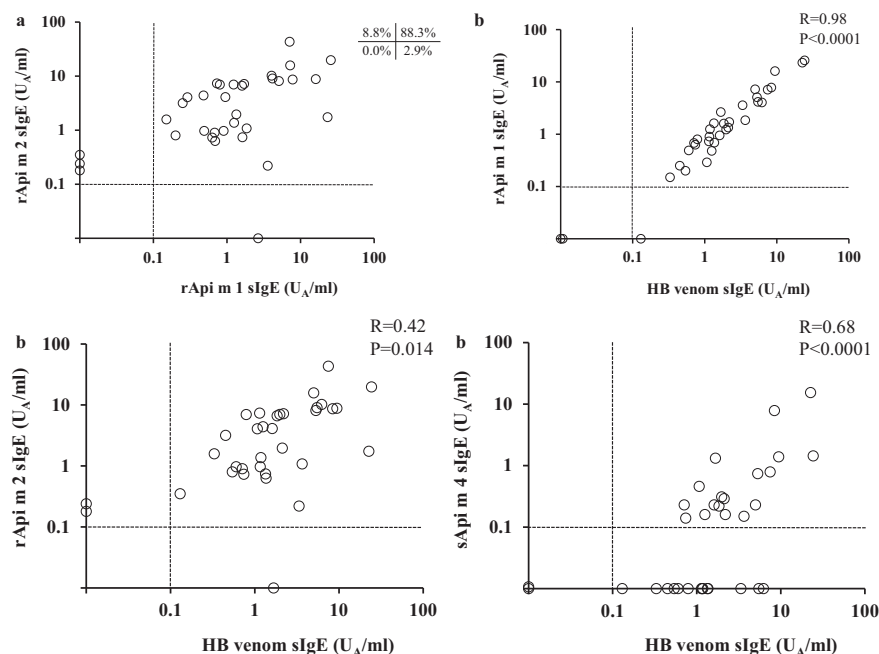


Fig. 1. (a) Correlations between levels of sIgE to rApi m 1 and levels of sIgE to rApi m 2 in Japanese beekeepers who had experienced SRs to HB stings ($n = 34$). (b) Relationship between HB venom-specific (s)IgE levels and rApi m 1–, rApi m 2–, and sApi m 4–sIgE antibody in Japanese beekeepers who had experienced SRs to HB stings ($n = 34$). HB, honeybee; SRs, systemic reactions.

Acknowledgement

The authors would like to thank S. Sekiguchi, N. Suzuki, S. Ohno. Department of Respiratory Medicine and Clinical Immunology, Dokkyo Medical University, Saitama Medical Center, for their excellent technical assistance.

Conflict of interest

TF and DS are fulltime employees of Siemens Healthcare Diagnostics and Pfizer Japan, respectively. The rest of the authors have no conflict of interest.

Hirokuni Hirata ^{a,*}, Kozo Sato ^a, Takeshi Ogasawara ^b, Tatsuo Funakoshi ^c, Daisuke Shima ^d, Masamitsu Tatewaki ^a, Sadaaki Shiromori ^a, Ryosuke Souma ^a, Hideyuki Satoh ^a, Kumiya Sugiyama ^a, Masafumi Arima ^e, Kazuhiro Kurasawa ^e, Takeshi Fukuda ^f, Yasutsugu Fukushima ^a

^a Department of Respiratory Medicine and Clinical Immunology, Dokkyo Saitama Medical Center, Saitama, Japan

^b Department of Mathematics and Statistics, Premedical Sciences, Dokkyo Medical University, Tochigi, Japan

^c Siemens Healthcare Diagnostics, Tokyo, Japan

^d Medical Affairs, Global Established Pharma Business, Pfizer Japan, Tokyo, Japan

^e Department of Rheumatology, Dokkyo Medical University, Tochigi, Japan

^f Tochigi Medical Center Shimotsuga, Tochigi, Japan

* Corresponding author. Department of Respiratory Medicine and Clinical Immunology, Dokkyo Medical University Saitama Medical Center, 2-1-50 Minamikoshigaya, Koshigaya-shi, Saitama 343-8555, Japan.
E-mail address: hirokuni@dokkyomed.ac.jp (H. Hirata).

References

1. Ruiz B, Serrano P, Moreno C. IgE-Api m 4 is useful for identifying a particular phenotype of bee venom allergy. *J Invest Allergol Clin Immunol* 2016;**26**: 355–61.
2. Ruiz B, Serrano P, Verdú M, Moreno C. Sensitization to Api m 1, Api m 2, and Api m 4: association with safety of bee venom immunotherapy. *Ann Allergy Asthma Immunol* 2015;**114**:350–2.
3. King TP, Spangfort MD. Structure and biology of stinging insect venom allergens. *Int Arch Allergy Immunol* 2000;**123**:99–106.
4. King TP. Molecular approaches to the study of allergens. *Monogr Allergy* 1990;**28**:84–100.
5. Eberlein B, Krischan L, Darsow U, Ollert M, Ring J. Double positivity to bee and wasp venom: improved diagnostic procedure by recombinant allergen-based IgE testing and basophil activation test including data about cross-reactive carbohydrate determinants. *J Allergy Clin Immunol* 2012;**130**:155–61.
6. Mueller HL. Diagnosis and treatment of insect sensitivity. *J Asthma Res* 1966;**3**: 331–3.
7. Golden DB, Tracy JM, Freeman TM, Hoffman DR. Negative venom skin test results in patients with histories of systemic reaction to a sting. *J Allergy Clin Immunol* 2003;**112**:495–8.
8. Nomura T, Kanda Y, Kato T, Sobajima T, Morishita T, Sugiura S, et al. Probability curves focusing on symptom severity during an oral food challenge. *Ann Allergy Asthma Immunol* 2014;**112**:556–7.
9. Yoshida N, Hirata H, Watanabe M, Sugiyama K, Arima M, Fukushima Y, et al. Improved sensitivity to venom specific-immunoglobulin E by spiking with the allergen component in Japanese patients suspected of Hymenoptera venom allergy. *Allergol Int* 2015;**64**:248–52.
10. Köhler J, Blank S, Müller S, Bantleon F, Frick M, Huss-Marp J, et al. Component resolution reveals additional major allergens in patients with honeybee venom allergy. *J Allergy Clin Immunol* 2014;**133**:1383–9.

Received 16 May 2018

Received in revised form 2 August 2018

Accepted 19 August 2018

Available online xxx