

## Letter to the Editor

**Identification of Hymenoptera venom-allergic patients with negative specific IgE to venom extract by using recombinant allergens**

To the Editor:

Epidemiologic studies indicate that 0.05% to 2% of the European and North American population develop systemic reactions after stings from honeybee or yellow jacket species.<sup>1</sup> The diagnosis of Hymenoptera venom allergy is based on a combination of a clinical history of anaphylaxis to Hymenoptera sting, positive skin test response, and specific IgE (sIgE) antibodies. Because of the insufficient specificity and sensitivity of intradermal skin testing, ranging, respectively, between 54% and 100% and 26% and 89% depending on extracts and concentrations used, the measurement of sIgE against native extracts of Hymenoptera is an additional but not substitutive *in vitro* test.<sup>2</sup>

Thus, the group of patients with a clinical history of anaphylaxis but negative sIgE measurement against commercial venom extracts represents a major diagnostic challenge. Over the years, the origin of commercial extracts used and the technology employed have continuously been modified. In addition, recombinant venom allergens have been recently used for improving the diagnostic specificity of *in vitro* allergen tests.<sup>3,4</sup> Nevertheless, 10% to 20% of the patients with positive serum sIgE against venom extract have negative sIgE measurements against any of the major recombinant antigens (Api m 1 and Ves v 5), suggesting the possibility that a percentage of patients may be sensitized against other clinically relevant venom allergens.<sup>5</sup>

The first aim of this study was to characterize IgE reactivity to recombinant allergens of *honeybee* venom (Api m 1, Api m 2, Api m 3, and Api m 5) and *yellow jacket* venom (Ves v 1, Ves v 2, Ves v 3, and Ves v 5)<sup>6</sup> and in particular, to analyze the diagnostic relevance of previously untested allergens rVes v 3, rApi m 3, and rApi m 5. Our second aim was to investigate the improvement in honeybee and yellow jacket venom natural extracts, analyzing sera collected from 2000 to 2003 with commercially available native extract during the years 2000 to 2003 and the current commercially available Hymenoptera venom extract.

To this end, sera from 86 patients with a history of anaphylactic reaction grade II after Hymenoptera insect sting with positive skin test result to native venom extracts to the culprit insect were collected. Patients were classified either as serologically positive if sIgE could be identified by at least 1 standard test (UniCAP250, Thermo Fischer or Immulite2000, Siemens Healthcare Diagnostics, Eschborn, Germany) or as serologically negative when specific sIgE levels were less than 0.35 kU/L. The sIgE reactivity against Api m 1, Ves v 1, and Ves v 5 was analyzed with ImmunoCAP, whereas the sIgE reactivity against Api m 2, Api m 3, Api m 5, Ves v 2, and Ves v 3 was analyzed with ELISA as already described.<sup>7</sup> Ten subjects having been stung without adverse reaction and without a history of atopic disposition (total IgE level < 100 kU/L) were included as controls.

To demonstrate the improvement in sIgE level measurement in recent years, sera from 28 patients with previously undetected sIgE against venom extracts (19 yellow jacket and 9 honeybee venom) analyzed between 2000 and 2003 were recharacterized with current technologies (ImmunoCAP 250).

**TABLE I.** Negative sera tested with Hymenoptera extracts during the years 2000 to 2001 were newly tested with current extracts and recombinant allergens

| Allergen                             | No. of positive/no. of total sera that were negative in 2000-2003 |
|--------------------------------------|-------------------------------------------------------------------|
| Current extract yellow jacket venom  | 8 of 19 (42.1%)                                                   |
| rVes v 1                             | 0 of 18 (0%)                                                      |
| rVes v 2                             | 3 of 18 (16.6%)                                                   |
| rVes v 3                             | 8 of 19 (42.1%)                                                   |
| rVes v 5                             | 15 of 19 (78.9%)                                                  |
| Positive to any recombinant allergen | 16 of 19 (84.2%)                                                  |
| Current extract honeybee venom       | 3 of 8 (37.5%)                                                    |
| rApi m 1                             | 0 of 8 (0%)                                                       |
| rApi m 2                             | 3 of 8 (37.5%)                                                    |
| rApi m 3                             | 7 of 8 (87.5%)                                                    |
| rApi m 5                             | 3 of 8 (37.5%)                                                    |
| Positive to any recombinant allergen | 8 of 8 (100%)                                                     |

Three (37.5%) of the 8 patients negative to the old venom extracts had positive sIgE against the current honeybee venom, and 8 (42.1%) of the 19 patients negative to the old venom extracts had positive sIgE against the current yellow jacket venom (Table I). Thus, although current venom extracts improved the sensitivity of *in vitro* tests compared with venom extracts used between 2000 and 2003, the analysis with current venom extracts did not identify the patients who had negative serum sIgE in the period 2000 to 2003. Therefore, with the up-to-date technology, there still remain patients with negative sIgE.

The nonreactive sera were further analyzed with different specific recombinant allergens together with nonreactive sera collected between 2006 and 2011. In line with previous reports, most of the sIgE-positive yellow jacket venom-allergic patients (n = 43) and the sIgE-positive honeybee venom-allergic patients (n = 15) recognized the respective “major” recombinant allergens rVes v 5 (39 [90.7%] of 43) and rApi m 1 (9 [60%] of 15). Interestingly, the antigens rVes v 3 and rVes v 2 were also detected in this group, suggesting that allergic subjects may suffer from sensitization to multiple allergens (Table II).

Most of the patients allergic to yellow jacket venom who had been serologically nonreactive to yellow jacket venom in standard sIgE tests were reactive to the allergen rVes v 5 (13 [76.5%] of 17) and to the antigen rVes v 3 (4 [21.0%] of 19), while they were less responsive to rVes v 2 (2 [11.1%] of 18) and rVes v 1 (1 [5.5%] of 18), with an overall reactivity of 14 out of 17 (82.3%). In contrast, the largest fraction of patients allergic to honeybee venom with negative sIgE against native extract were reactive to the antigen rApi m 3 (6 [66.7%] of 9) while low sIgE reactivity was found with the “major” allergens rApi m 1 (2 [22.2%] of 9), rApi m 2 (2 [22.2%] of 9), and rApi m 5 (3 [33.3%] of 9), with an overall reactivity of 7 (77.7%) of 9 (Table II).

Here, we show, for the first time, that the measurement of the combination of recombinant antigens may increase the sensitivity of routine Immuno-CAP to venom extract.

In line with previous reports, most of the patients allergic to yellow jacket venom and honeybee venom with positive sIgE against commercially available venom extract were shown to be positive to “major” allergens.<sup>5</sup> This study additionally

**TABLE II.** Clinical and serologic data of patients with Hymenoptera venom allergy\*

| Native extract    | History of vespula allergy |                 |                 |                 |                          | History of honeybee allergy |                |                 |               |                          |
|-------------------|----------------------------|-----------------|-----------------|-----------------|--------------------------|-----------------------------|----------------|-----------------|---------------|--------------------------|
|                   | rVes v 1 (%)               | rVes v 2 (%)    | rVes v 3 (%)    | rVes v 5 (%)    | Any or all allergens (%) | rApi m 1 (%)                | rApi m 2 (%)   | rApi m 3 (%)    | rApi m 5 (%)  | Any or all allergens (%) |
| sIgE < 0.35       | 1 of 18 (5.5)              | 2 of 18 (11.1)  | 4 of 19 (21.0)  | 13 of 17 (76.5) | 14 of 17 (82.3)          | 2 of 9 (22.2)               | 2 of 9 (22.2)  | 6 of 9 (66.7)   | 3 of 9 (33.3) | 7 of 9 (77.7)            |
| sIgE > 0.35       | 23 of 43 (53.5)            | 15 of 42 (35.7) | 27 of 43 (62.8) | 39 of 43 (90.7) | 43 of 43 (100)           | 9 of 15 (60)                | 8 of 15 (53.3) | 13 of 15 (86.7) | 9 of 15 (60)  | 15 of 15 (100)           |
| Nonallergic group | 0 of 10 (0)                | 0 of 10 (0)     | 0 of 10 (0)     | 0 of 10 (0)     | 0 of 10 (0)              | 2 of 10 (20)                | 0 of 10 (0)    | 1 of 10 (10)    | 0 of 10 (0)   | 2 of 10 (0)              |

\*This table includes patients of Table I who were either positive or negative to the current commercially available native extracts.

demonstrates a high rate of sensitization against recently discovered allergens in patients allergic to Hymenoptera venom and suggests an important role of other allergens in the diagnosis of allergic diseases.

The analysis of a panel of recombinant allergens from yellow jacket venom and honeybee venom in the group with negative serological results against venom extracts increased the sensitivity in the diagnosis of both yellow jacket venom and honeybee venom allergies. Interestingly, rVes v 5 and rApi m 3 further increase the sensitivity of the *in vitro* test, highlighting the relevance of “major” recombinant allergens in the improvement of *in vitro* diagnosis of yellow jacket venom and honeybee venom allergies.

In conclusion, the technologies used for sIgE measurement against culprit venom improved in the last years. Moreover, the detection of sIgE to a panel of recombinant venom allergens further improves allergic diagnostic tests.

We thank Beate Heuser and Anita Berger for technical assistance.

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This work was supported by the “Hochschulwissenschaftsprogramm” (HWP), “Kommission Klinische Forschung” (KKF) of the Technical University Munich, and the

Christine Kühne – Center for Allergy Research and Education (CK-CARE). L.C. and D.P. were supported by a scholarship of Bayerische Forschungsförderung.

Disclosure of potential conflict of interest: U. Darsow has consultant arrangements with Bencard and Leo and has received payment for lectures from Bencard and Novartis. R. Bredehorst is cofounder of PLS-Design GmbH, which owns patent rights for Api m 3, Api m 5, and Ves v 3. J. Ring has received research support from ALK-Abelló, Allergopharma, Almirall-Hermal, Astellas, Bencard, Biogen-Idec, Galderma, GlaxoSmithKline, Leo, Merck Sharp Dohme, Novartis, Phadia, PLS Design, and Stallergenes. E. Spillner is cofounder of PLS-Design GmbH. M. W. Ollert has consultant arrangements with Siemens Healthcare Diagnostics and Hitachi Chemical Diagnostics, has received payment for lectures from Phadia/Thermo Fisher, has received payment for education presentations from Siemens Healthcare Diagnostics, and is the scientific cofounder of the University of Hamburg biotech spin-off PLS-Design GmbH (Hamburg, Germany). The rest of the authors declare that they have no relevant conflicts of interest.

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<http://dx.doi.org/10.1016/j.jaci.2013.09.047>