

ORIGINAL ARTICLE

ANAPHYLAXIS

IgE to recombinant allergens Api m 1, Ves v 1, and Ves v 5 distinguish double sensitization from crossreaction in venom allergy

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Abstract

Background: Diagnostic tests in patients with Hymenoptera venom allergy are frequently positive to venoms of both honey bee and wasp (*Vespula*). Component-resolved analysis with recombinant species-specific major allergens (rSSMA) may help to distinguish true double sensitization from crossreactivity.

Methods: Included were 121 patients with systemic allergic reactions to Hymenoptera stings, 76 with double positivity of serum-specific IgE (sIgE) to both venoms, 45 with single positivity to bee or wasp venom, and 32 controls without history of systemic reactions to Hymenoptera stings and no sIgE to whole venoms. In venom-allergic patients and controls, sIgE to rSSMA Api m 1 of bee venom and to Ves v 1 and Ves v 5 of wasp venom were tested by ImmunoCAP.

Results: Only 47% of 76 patients with double positivity to whole venoms reacted also to rSSMA of both species. Specificity of sIgE to the 3 rSSMA was very high, with no sIgE to rSSMA of the other species in single-positive venom-allergic patients and only one control with low sIgE to Ves v 1. All wasp-allergic single-positive patients had sIgE to Ves v 5 and/or Ves v 1, and 78.3% of single-positive bee venom-allergic patients had sIgE to Api m 1.

Conclusion: Specificity of sIgE to rSSMA of both species is excellent. Sensitivity of sIgE to rSSMA was optimal for wasp venom. Sensitivity of bee venom Api m 1 could be increased by adding rSSMA of other important bee venom allergens.

Of patients with systemic allergic reactions (SAR) to Hymenoptera stings, more than 50% have serum-specific IgE antibodies (sIgE) to venoms of honey bee (*Apis mellifera*) and wasp, in USA, called yellow jackets (*Vespula* spp) (1). This double positivity is because of crossreactivity or true double sensitization (2,3). Distinction between these two possibilities is important for the selection of venoms for immunotherapy (VIT) (4): Treatment with both venoms is only indicated in true double sensitization, induced by stings of both species inducing sensitization to species-specific allergens of both bee venom (BV) and *Vespula* venom (VV). Crossreactivity may be due to partial amino acid sequence identity between allergens of BV and VV (5,6) or to crossreacting carbohydrate determinants (CCDs).

Using the ADVIA Centaur test (1), we showed in a recent study that sIgE to recombinant, nonglycosylated,

species-specific major allergens Api m 1 of BV and Ves v 5 of VV had an excellent sensitivity and an optimal specificity, and distinguished between crossreactions and true double sensitization in most patients with double positivity of sIgE to both whole venoms, thus reducing the need of VIT with both venoms and the costs of VIT considerably.

The ADVIA Centaur is no longer available, but since recently the ImmunoCAP with three recombinant species-specific major allergens (rSSMA) without CCDs, Api m1 from BV and Ves v 1 and Ves v 5 from VV, is commercially available from Phadia, now Thermo Fisher Scientific. In our study, we analyzed the value of these new tests with rSSMA from both BV and VV in patients with Hymenoptera venom allergy (HVA) with and without double positivity of sIgE to BV and VV.

Methods

Patients and controls

In this retrospective study, we included 121 patients with HVA documented by a history of SAR to Hymenoptera stings and positive sIgE to whole BV and/or VV: 76 showed double positivity of sIgE to both whole venoms; 45 were single positive, 23 to BV and 22 to VV. Severity of SAR was estimated based on the classification by Mueller (7,8). All patients were questioned with regard to the culprit insect. None of 32 controls had a history of SAR or large local reactions to Hymenoptera stings, and none had sIgE to BV or VV. All patients and controls consented to diagnostic laboratory tests.

A co-existing atopic constitution was assumed by a history of seasonal or perennial allergic rhinitis or asthma, by positive skin prick tests to common inhalant allergens, and/or by the presence of atopic dermatitis.

Diagnostic tests

Sera for diagnostic tests were obtained in 95.3% of double-positive and 83.3% of single-positive patients within 12 months after the last systemic sting reaction. Specific IgE to BV, VV, crossreacting carbohydrate determinants (CCDs) (MUXF3), and the rSSMA phospholipase A₂ (Api m 1), phospholipase A₁ (Ves v 1), and antigen 5 (Ves v5) were estimated by ImmunoCAP (Phadia, Uppsala, Sweden). All three rSSMA are produced in a form free of CCDs. A value of ≥ 0.35 kUa/l was considered as positive. Intracutaneous skin tests with BV and VV (ALK-Abello, Horsholm, Denmark) were available in 106 of the 121 patients with HVA. A wheal diameter of ≥ 5 mm with erythema at a venom concentration of ≤ 1 μ g/l was defined as a positive result.

Statistics

The association of double positivity and single positivity to whole venoms and to rSSMA with sIgE to CCDs and the

association of double positivity and single positivity with atopy were analyzed by chi-squared test.

Results

Patients and controls

Table 1 summarizes the baseline data of patients and controls and shows that the groups of patients with HVA are comparable concerning age, gender, and severity of SAR. The majority of patients with HVA had SAR of grades III and IV, both patients with double positivity and single positivity for sIgE to whole venoms. Only two of the 121 patients with SAR did not remember previous Hymenoptera stings without SAR. Based on the history, 19 of the 76 double-positive patients had SAR to bee stings, 22 to *Vespula* stings, 10 to stings of both species, and 25 could not identify the culprit insect. The higher atopy rate in patients with double positivity (42.1%) than in patients with single positivity (28.8%) did not reach statistical significance ($\chi^2 = 2.11$; $P > 0.1$).

Specific IgE to whole venoms and recombinant major allergens

Results of sIgE to BV and VV, the rSSMA Api m 1, Ves v 1, Ves v 5, and CCDs in patients and controls are presented in Table 2. Only 47.4% of patients double positive to venoms were also double positive to rSSMA, while one of the 76 (1.3%) had no sIgE to the recombinants of BV and VV.

In all single-positive patients, the culprit insect matched also with history. All patients with single positivity to VV had sIgE to rSSMA of VV, while 78.3% with single positivity to BV had sIgE to Api m 1. None of the 45 patients with single positivity had sIgE to rSSMA of the other species, while one nonatopic control had low sIgE of 0.42 kUa/l to Ves v 1, but not to Ves v 5.

In patients with single positivity to VV, sensitivity of Ves v 5 was optimal, while only 33.3% had also sIgE to Ves v 1.

Table 1 Data of 121 patients with a history of SAR to Hymenoptera stings – 76 with double positivity to honey bee and *Vespula* venom, 45 with SP either to BV or to VV – and 32 controls with no history of SAR or large local sting reactions and negative specific IgE to the two venoms in ImmunoCAP

History/ImmunoCAP	SAR/Double positive	SAR/single positive to BV	SAR/single positive to VV	Controls
N	76	23	22	32
Age				
Mean age	36.27	37.74	40.9	45.9
SD	20.06	16.69	20.37	16.86
Range	7–72	12–65	7–73	20–81
Male/female	43/33	14/9	10/12	13/19
Severity of SAR				
Grade 1	7	4	3	No SAR
Grade 2	16	4	5	
Grade 3	34	8	8	
Grade 4	19	7	6	
Atopy	32/76 (42.1%)	6/23 (26.1%)*	7/22 (31.8%)*	10 (31.4%)

BV, bee venom; SAR, systemic allergic reactions; VV, *Vespula* venom.

*Of all 45 single-positive patients, 28.8% were atopic.

Table 2 Serum-specific IgE antibodies to whole venoms, recombinant species-specific major allergens, and without CCDs (MUXF3) in patients with SAR to Hymenoptera stings and controls

ImmunoCAP	Positive to BV and VV N = 76	Positive to BV only N = 23	Positive to VV only N = 22	Negative to BV and VV (controls) N = 32
Positive to Api m 1 and Ves v 1/Ves v 5	36 (47.4%)	0	0	0
Positive only to Api m 1	9 (11.8%)	18 (78.3%)	0	0
Positive only to Ves v 1/Ves v 5	30 (39.5%)	0	22 (100%)	1 (3.1%) pos to Ves v 1
All recombinants negative	1 (1.3%)	5 (21.7%)	0	31 (96.9%)
Positive to MUXF3 (CCDs)	41 (54%)	1 (4.4%)*	0*	0

BV, bee venom; CCDs, crossreacting carbohydrate determinants; SAR, systemic allergic reactions; VV, *Vespula* venom.

*Of all 45 SP patients, 2.2% had sIgE to MUXF3.

In patients with double positivity to BV and VV, the majority had sIgE to Ves v 5 (76.3%) and also to Ves v 1 (59.2%). Eight patients had only sIgE to Ves v 1 and 21 only to Ves v 5.

Table 3 compares sIgE to whole venoms and to rSSMA in relation to the culprit insect according to the history. Double positivity of rSSMA was present in nine of 10 (90%) patients with a history of systemic reactions to both insects. One patient of this group was single positive to rSSMA of VV. He had developed a grade 4 reaction following a *Vespula* sting 3 months before testing and a mild grade I reaction to a bee sting 3 years before. sIgE to BV were of class 3, and sIgE to VV were of class 2.

Of 25 patients who were unable to identify the culprit insect, 18 (72%) were double positive to rSSMA. Of the 22 patients with a history of a *Vespula* as culprit insect, none was double positive to rSSMA, although five of 11 with available data remembered previous well-tolerated bee stings. One patient had no sIgE to rSSMA of either venom, while sIgE to both whole venoms were of class 2. The sting of the culprit insect had occurred 3 months before testing.

Of the 19 patients with a history of a bee as the culprit insect, 18 were positive to BV rSSMA, but nine also to VV rSSMA. Of nine patients with available data and double positivity of sIgE to rSSMA, eight remembered previous well-tolerated wasp stings. One patient, a 7-year-old boy, son of a beekeeper, had developed several mild SAR to bee stings, the last 9 months before testing. He had well-tolerated several *Vespula* stings before. Surprisingly, sIgE were positive only

to VV rSSMA, while sIgE to whole venoms were of class 2 for both species.

Specific IgE to CCDs in double- and single-positive patients with HVA

As shown in Table 2, sIgE to CCDs were present in 54% of patients with double positivity but only 2.2% of those with single positivity ($\chi^2 = 35.623$, $P < 0.0001$).

Of the 36 patients with double positivity for sIgE to rSSMA, 20 (55.6%) had sIgE to CCDs; of the 40 patients with single positivity to rSSMA, 21 (52%) had sIgE to CCDs. The difference was not significant.

Intracutaneous skin tests with whole venoms

In 61 of the 76 patients with double positivity to whole venoms in ImmunoCAP, i.e. skin tests with whole venoms were available. 51 (83.6%) were also double positive in i.c. skin tests.

Discussion

As shown in Table 3, identification of the culprit insect in patients with HVA is often difficult from history: One-third either did not see the stinging insect or was unable to identify it.

There is no doubt that sIgE to venom allergens are most important for SAR to Hymenoptera stings. The commonly used diagnostic tests based on sIgE to whole venoms are very

Table 3 Specific IgE to recombinant species-specific major allergens (rSSMA) of bee venom and *Vespula* venom in 76 patients with double positivity to both whole venoms: relation to the identification of the culprit insect from history

Identification of culprit insect from history	sIgE double positivity to BV and VV	sIgE double positivity to rSSMA (%)	sIgE single positivity to rSSMA BV (%)	sIgE single positivity to rSSMA VV (%)
Honey bee	19	9 (47.4)	9 (47.4)	1 (5.2)
<i>Vespula</i>	22	0	0	21 (95.5)*
Honey bee or <i>Vespula</i>	25	18 (72)	0	7
Honey bee and <i>Vespula</i>	10	9 (90)	0	1
Total	76	36 (47.4)	9 (11.8)	30 (39.5)*

BV, bee venom; VV, *Vespula* venom.

*One patient with sIgE double positivity to whole venoms had no sIgE to rSSMA.

sensitive but their specificity is suboptimal: Double positivity of sIgE to bee and VV is observed in 50–60% of patients with SAR to Hymenoptera stings, although most of them report a SAR only to one sting. According to the literature, double positivity based on positive i.c. skin tests at $\leq 1 \mu\text{g}/\text{ml}$ is observed in 40–50% of patients with HVA (8–10). This is confirmed by the results of our study. Moreover, sIgE and i.c. skin tests to whole venoms are positive in 10–20% of individuals with no history of SAR to Hymenoptera stings. As for double positivity, such results may be caused by sensitization to a previous well-tolerated sting or to crossreactivity.

Crossreactions between BV and VV are responsible for the majority of double positivity of sIgE to both venoms. They are attributable to partial peptide sequence identity of BV and VV allergens hyaluronidase and dipeptidyl peptidase (6,11), or to CCDs (3,12), which are present in most allergens of BV and also in three of VV (2) and are of little clinical relevance. Moreover, CCDs are contained in other insects and plants including pollen and fruits. The significantly higher prevalence of sIgE to CCDs in our patients with double than single positivity underlines the important role of crossreactions to CCDs for double positivity. The presence of sIgE to CCDs in patients with double positivity does, however, not exclude true sensitization to relevant peptide allergens of both venoms. A better solution to distinguish crossreactivity from true double sensitization is the inhibition test with CCDs and whole venoms in ImmunoCAP or immunoblot: Elimination of sIgE to one of the two venoms by inhibition with CCDs indicates true sensitization only to the other venom, while no elimination of sIgE by CCDs or the other venom proves true double sensitization (12). Inhibition tests are, however, expensive and not easy to interpret in patients with low sIgE (3).

The basophil activation test (BAT) is said to have a high sensitivity and specificity for patients with HVA (13–15). There are, however, publications indicating that IgE to CCDs may also activate basophils (16). The test is expensive and needs considerable experience with flow cytometric methods, and in 5–10% of patients, this method cannot be applied because their basophils fail to upregulate CD63 using anti-IgE antibodies as positive controls in BAT (17).

Estimation of sIgE to recombinant species-specific major allergens (rSSMA) of BV and VV is currently the most promising approach to resolve the problem of double positivity to both whole venoms. Several studies confirmed the high specificity of rSSMA in this situation, while sensitivity may still not be optimal (1,9,18–20). Excellent specificity of sIgE to rSSMA of both species was confirmed in this study in single-positive patients and controls. The low level of sIgE to Ves v 1 in one of 32 controls may be due to sensitization by a previous wasp sting without SAR. Sensitivity of sIgE to rSSMA of VV was also excellent.

The sensitivity of Api m 1 of 78.3% in single-positive BV-allergic patients confirmed Api m 1 as major BV allergen. It was, however, lower than in the ADVIA Centaur (1), where 97% of BV-allergic patients had sIgE to Api m 1. In a study with ELISA (9), 19 of 20 (95%) BV-allergic patients

had sIgE to Api m 1. In another publication with ImmunoCAP on 175 BV-allergic patients including large local reactions, sensitivity of sIgE to Api m 1 was only 57% (18). Besides different laboratory methods, different selection of patients with BV allergy may be responsible for these differences in sensitivities. A possibility to improve the component-resolved diagnosis for BV allergy could be the addition of other important BV rSSMA, like Api m 3 or Api m 10, expressed in Sf9 insect cells and thus not containing CCDs (21–25).

The data analyzing the relationship between culprit insect from history and positivity to rSSMA (Table 3) show double positivity of sIgE to rSSMA in 90% of patients with a history of systemic reactions to both insects. The only single-positive patient of this group had sIgE to rSSMA of VV. The interval of 3 years between a mild systemic bee sting reaction and the suboptimal sensitivity of BV Api m1 may explain this.

The single positivity of sIgE to rSSMA in 95.5% of patients with a history of *Vespula* as culprit insect indicates a high reliability of the correct identification of the *Vespula* in this group. The absence of sIgE to VV rSSMA in one patient may be explained by a misidentification and/or suboptimal sensitivity of Api m 1 or of VV rSSMA.

In the group with a history of a bee as the culprit insect, the sensitivity of BV rSSMA was also high with 94.7%. However, 9 (47.4%) patients had also sIgE to VV rSSMA that may be due to sensitization by previous well-tolerated wasp stings.

Based on our observations, we recommend, for patients with double positivity of sIgE to whole venoms, that the presence of sIgE to rSSMA of both species indicates VIT with both venoms. Considering the excellent sensitivity of rSSMA to VV, sIgE only to Api m1 indicates VIT only with BV. In patients with sIgE only to VV rSSMA, VIT with VV is certainly indicated; the addition of BV for VIT should be considered in the presence of strongly positive sIgE, i.c. skin tests, or basophil activation tests to both whole venoms.

In conclusion, our study confirms the estimation of sIgE to rSSMA of both venoms as a valuable approach to distinguish between true double sensitization, indicating VIT with both venoms, and crossreactions in patients with double positivity to BV and VV. The component-resolved diagnosis for BV allergy could be improved by the addition of other important BV rSSMA.

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Author contributions

AH, PS, OH, and UM were involved in the selection of patients and controls as well as of diagnostic tests included

in this study. UM analyzed the data and wrote the manuscript. All authors read and approved the final form of the manuscript.

Conflicts of interest

None of the authors has any conflict of interest with respect to the contents of this article.

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