

Asymptomatic Sensitization to Hymenoptera Venom Is Related to Total Immunoglobulin E Levels

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Key Words

Hymenoptera venom allergy • Bee venom • Wasp venom • Specific immunoglobulin E • Total immunoglobulin E

Abstract

Background: The detection of specific serum immunoglobulin E (sIgE) to Hymenoptera venoms is an established diagnostic tool to diagnose insect venom hypersensitivity. However, the specificity of sIgE detection is a debated issue.

Methods: In 145 subjects, total IgE (tIgE) and sIgE to Hymenoptera venoms as well as sIgE to rapeseed as a marker of cross-reactive carbohydrate determinants were measured. In addition, an atopy score was determined for each patient. We looked for a possible association between tIgE and the presence of sIgE in subjects with a negative history of large local or systemic reactions to insect stings. **Results:** Fifteen of 65 subjects (23.1%) with low levels of tIgE (<50 kU/l) had sIgE for bee or wasp venom, and 23 of 47 subjects (48.9%) with a tIgE from 50 to 250 kU/l showed sIgE. The highest rate of asymptomatic sensitization (22 of 33; 66.7%) was found in patients with tIgE levels higher than 250 kU/l. Median sIgE was approximately 4.8 times higher in subjects with tIgE levels above 250 kU/l than in those with tIgE levels <50 kU/l. Interestingly, a significant difference in median tIgE was re-

corded between individuals with and without sIgE to rapeseed [776.5 kU/l (25, 75% percentiles: 252.5, 2,000.0) vs. 50.5 kU/l (20.1, 172.0), respectively; $p < 0.001$]. **Conclusion:** Specific antibodies are frequently seen in individuals with high tIgE, but appear to be largely irrelevant in clinical terms. This might lead to misdiagnosis in persons with an inconclusive stinging history.

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Introduction

Skin testing, detection of allergen-specific immunoglobulin E (IgE) serum antibodies and the patient's medical history are the mainstays of diagnosis in cases of Hymenoptera venom hypersensitivity. Nevertheless, the criteria currently used to determine Hymenoptera venom allergy cannot accurately predict the occurrence or severity of anaphylactic symptoms after an insect sting [1]. Moreover, the specificity of serum IgE continues to be a debated issue [2].

The determination of allergen-specific IgE is also viewed from the aspect of time and the patient's comfort. Depending on the methods and venoms used, the specificity of IgE determination has been found to range be-

tween 60 and 94% [3]. Leading manufacturers of automated laboratory systems generally claim high sensitivities and specificities for their IgE tests. However, the studies leading to these results must be viewed critically; control subjects with high total IgE (tIgE) levels, positive skin tests and an atopic disposition are generally ruled out in order to obtain optimum specificities [4, 5].

In clinical practice, nonspecific sensitization to multiple allergens due to in vitro cross-reactions makes it difficult for the clinician to make an unequivocal diagnosis. In Hymenoptera allergy, cross-reactions due to cross-reactive carbohydrate determinants (CCDs) causing double-positive test results for honeybee and wasp venom have been frequently reported [6, 7]. In cases of double sensitization, a method-based problem also has to be considered; the frequency of double-positive test results ranges between 10% (Phadebas RAST) and 30% (CAP), depending on the test method used [8]. Some of the detected sensitizations might be explained by nonspecific in vitro cross-reactivity; during the IgE assay, serum IgE antibodies are free and can easily bind to all available epitopes. This binding is monovalent, and each IgE molecule bound to 1 genuine or cross-reacting epitope generates a signal in the assay cup, which is contrary to the in vivo situation, where the allergic response requires efficient cross-linking of at least 2 high-affinity IgE receptors on basophils or mast cells (bivalent interaction) in order to induce cell activation and mediator release.

In preliminary clinical observations at our outpatient clinic, subjects with high tIgE levels frequently showed multiple sensitizations to several allergens, especially to Hymenoptera venoms. Given this background, the influence of tIgE on the presence of specific IgE (sIgE) to Hymenoptera venoms in subjects with a negative history of large local or systemic reactions to insect stings was investigated.

Methods

Individuals

One hundred and forty-five individuals who had been admitted to our outpatient clinic for evaluation of allergic diseases were screened for IgE antibodies to bee and wasp venom as well as rapeseed, the latter being a marker of CCDs. A personal history was taken, and sIgE and tIgE were determined using the ImmunoCAP system. Additionally, a clinical atopy score was determined in order to distinguish between atopic and nonatopic individuals. All subjects had tolerated Hymenoptera stings in the past and had neither a history of a local nor systemic sting reaction.

Determination of sIgE and tIgE

sIgE and tIgE antibody levels in the subjects' serum were measured using the ImmunoCAP system (Phadia, Uppsala, Sweden) according to the manufacturer's instructions. Individuals were grouped according to their tIgE levels: group 1, <50 kU/l; group 2, 50–250 kU/l, and group 3, >250 kU/l.

Atopy Score

The Erlangen Atopy Score proposed by Diepgen et al. [9] is a diagnostic scoring system based on medical history, clinical criteria of atopic symptoms and skin symptoms. Individuals with more than 10 points were classified as being atopic.

Data Analysis

All data are expressed as medians (25, 75% percentiles) unless otherwise stated. For comparison of groups, continuous variables (e.g. age) were analyzed by the Kruskal-Wallis test. Proportions of groups were compared by χ^2 analysis or Fisher's exact test. Correlations between continuous variables were determined using Spearman's rank correlation coefficients as measures of the direction and strength of associations. A stepwise binary logistic regression analysis was performed to assess influencing factors on the occurrence of sensitization. A test result consistent with $p < 0.05$ was considered significant. The SPSS 15.0 software (SPSS Inc., Chicago, Ill., USA) was used for statistical analysis.

Results

Of 145 subjects, 36 (24.8%) had experienced stings in the preceding 12 months. Of these, 13 (36.1%) reported bee stings and 23 (63.9%) wasp stings. Of these 145 subjects, 59 (40.7%) revealed sIgE either for 1 or 2 Hymenoptera venoms. Eighteen of these 59 (30.5%) were sensitized to honeybee venom, 16 (27.1%) to wasp venom and 25 (42.4%) to both. After determination of the atopy score, 51 subjects (35.2%) were classified as atopic. The median sIgE to Hymenoptera venoms was approximately 4.8 times higher in subjects with a tIgE above 250 kU/l as compared to those with tIgE levels <50 kU/l (table 1). The frequency of atopic disposition and rapeseed antibodies was significantly higher in subjects with tIgE levels >250 kU/l, whereas data concerning stings experienced in the preceding 12 months and the subject's lifetime were inconclusive (table 2). tIgE, Hymenoptera stings in the last year, stings in the subject's lifetime and the presence of antibodies to rapeseed were considered to influence sensitization. On stepwise binary logistic regression, only tIgE ($p = 0.002$) and stings in the last year ($p = 0.033$) were found to have significantly influenced the presence of sIgE.

With regard to asymptomatic sensitization, 15 of 65 subjects with low tIgE (<50 kU/l) had sIgE to Hymenop-

Table 1. Demographic and clinical data of our subjects

	Total IgE, kU/l			Total	p value
	<50	50–250	>250		
Subjects, n	65	47	33	145	
Female, n (%)	38 (58.5)	28 (59.6)	16 (48.5)	82 (56.6)	0.564
Age, years	36.0 (26.0, 57.0)	33.5 (25.0, 56.0)	38.0 (25.0, 51.5)	36.0 (25.0, 55.0)	0.681
tIgE, kU/l	20.0 (8.0, 34.4)	122.0 (68.7, 173.0)	754.0 (385.5, 1,847.5)	64.3 (21.7, 217.5)	<0.001
sIgE Hymenoptera venom, kU/l	0.60 (0.43, 1.01)	1.04 (0.46, 3.33)	2.85 (0.96, 5.68)	1.06 (0.57, 4.34)	0.004
sIgE bee venom, kU/l	0.44 (0.37, 1.01)	1.64 (0.59, 3.18)	2.60 (0.68, 7.25)	1.64 (0.54, 3.90)	0.004
sIgE wasp venom, kU/l	0.91 (0.58, 1.27)	1.0 (0.46, 3.39)	2.93 (0.96, 5.11)	1.14 (0.59, 3.70)	0.108

Values for age, tIgE and sIgE are medians (25, 75% percentiles in parentheses). No statistical difference was found between the tIgE groups with respect to age and sex distribution. Median tIgE and sIgE to Hymenoptera venoms were calculated in sensitized individuals only.

Table 2. Potential factors influencing sensitization

	Total IgE, kU/l			Total	p value
	<50	50–250	>250		
Subjects	65	47	33	145	
Atopy	8 (12.3)	18 (38.3)	25 (75.8)	51 (35.2)	<0.001
CCD antibodies	1 (1.5)	3 (6.4)	12 (36.4)	16 (11.0)	<0.001
Sting last year	13 (20.0)	17 (36.2)	6 (18.2)	36 (24.8)	0.089
Stings per lifetime					
≤5	22 (33.8)	15 (32.7)	11 (33.3)	48 (33.1)	0.664
6–10	17 (26.2)	12 (25.5)	15 (45.4)	44 (30.3)	0.545
>10	22 (33.8)	18 (38.3)	4 (12.1)	44 (30.3)	0.045

Values shown are numbers of subjects, with percentages in parentheses.

tera venoms, and 23 of 47 with tIgE levels from 50 to 250 kU/l were sensitized. The highest rate of asymptomatic sensitizations was found in subjects with tIgE levels above 250 kU/l: 22 of 33 were found to be positive (fig. 1). Remarkably, IgE to rapeseed was only found in subjects with double sensitization to honeybee and wasp venom; 13 of 28 double-sensitized subjects (46.4%) had sIgE to rapeseed, whereas no monosensitized subjects had sIgE to rapeseed.

Additionally, there was a clear positive correlation between tIgE and sIgE, with a shared variance of 12.3% ($r = 0.350$, $r^2 = 0.123$, $p = 0.006$). Interestingly, we observed a significant difference in median tIgE between individuals with and without sIgE to rapeseed [776.5 kU/l (252.5, 2,000.0) vs. 50.5 kU/l (20.1, 172.0); $p < 0.001$].

Discussion

We found that clinically irrelevant (double) sensitization was dependent on tIgE levels and was mainly observed in subjects with high tIgE levels. Stings in the last year influenced the presence of sIgE, but the frequency of stings was comparable between the 3 tIgE groups. Interestingly, the frequency of bee and wasp sensitization was nearly the same, although approximately two thirds of the subjects had been stung by wasps. This is in contrast to the general population of Hymenoptera venom-allergic patients in Austria, who are mainly sensitized to wasp venom (73%) [10]. Thus, one might speculate that these asymptomatic sensitizations might originate from other sources than a Hymenoptera sting. As we found sIgE to rapeseed only in double-sensitized and not in monosensitized subjects, it appears likely that this nonspecific in

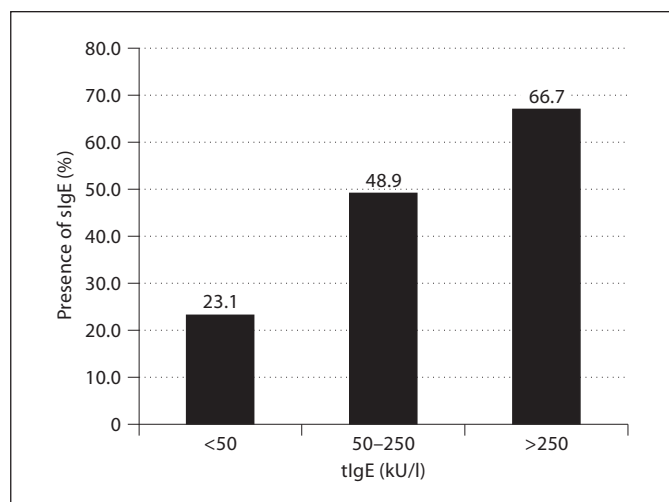


Fig. 1. Frequency of asymptomatic sensitization to Hymenoptera venoms according to tIgE levels. The groups with tIgE <50 kU/l, 50–250 kU/l and >250 kU/l consisted of 65, 47 and 33 subjects, respectively. The frequency of asymptomatic sensitization was increased in patients with elevated IgE levels ($p < 0.001$).

vitro (cross-)reactivity due to CCDs only plays a role in subjects with double-positive test results for honeybee and wasp venom. However, these observations are limited by the fact that we investigated CCDs only indirectly by measuring sIgE to rapeseed, and we did not perform inhibition tests. Nevertheless, the reasons for double and monosensitization without identifiable CCD antibodies

are not entirely obvious. As mentioned earlier, a method-based problem or nonspecific in vitro cross-reactivity due to the monovalent IgE binding in these assays might be potential factors. As the presence of sIgE to rapeseed was associated with approximately 15.5-fold higher tIgE levels, it remains unclear whether antibodies to CCDs cause higher levels of tIgE or whether individuals with high tIgE levels more easily generate antibodies to CCD. Of the subjects with low tIgE (<50 kU/l), 23.1% had detectable sIgE to Hymenoptera venoms; this concurs well with the claims of test manufacturers. However, in subjects with tIgE levels from 50 to 250 kU/l and >250 kU/l, 48.9 and 66.7% showed sIgE, respectively. Subjects with high tIgE levels were probably excluded from company studies in which test specificities are evaluated.

It remains unclear whether these sensitizations in individuals with no history of a systemic sting reaction signify an absence of risk for all tested subjects in the future. But it should be noted that specific antibodies are frequently registered in individuals with high tIgE and appear to be largely irrelevant in clinical terms; this might lead to misdiagnosis in subjects with an inconclusive sting history. Therefore, determination of sIgE antibodies as specified in the guidelines of the European Academy of Allergology and Clinical Immunology is mandatory only in patients with a clear history of a systemic sting reaction [11]. With regard to technology, it would be desirable to improve the quality of the source materials used for testing; in particular, the inclusion of recombinant allergens would be meaningful.

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