

Double Sensitization to Honeybee and Wasp Venom: Immunotherapy with One or with Both Venoms?

Value of FEIA Inhibition for the Identification of the Cross-Reacting IgE Antibodies in Double-Sensitized Patients to Honeybee and Wasp Venom

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Key Words

Cross-reactivity · Honeybee venom · Wasp venom · Yellow jacket venom · RAST inhibition · FEIA inhibition · Immunotherapy

Abstract

Background: Double sensitization to honeybee (*Apis mellifera*) and wasp venom (*Vespula* spp.) as determined by skin test and measurement of specific IgE is common in hymenoptera sting allergy. Double-sensitized patients have either distinct antibodies for each venom or cross-reacting antibodies that recognize similar or identical epitopes in both venoms. Unfortunately, patients often fail to identify the stinging insect which makes it difficult to distinguish cross-reactors from non cross-reactors. However, for economic reasons as well as for the benefit of the patients, it would be useful to identify complete cross-reactors. **Methods:** In this study we investigated 24 double-sensitized patients who were candidates for venom immunotherapy. Homologous and heterologous FEIA inhibition was carried out with honeybee (*Apis mellifera*) and wasp venom (*Vespula* spp.) preparations from two different providers. The inhibitor concentrations were ranging from 0 to 100 µg protein/ml. **Results:** Sera

of 4 patients were completely cross-reacting for one venom (3 honeybee, 1 wasp), 8 patients were partially cross-reacting and 10 patients were not cross-reacting. Two patients were excluded from the study due to insufficient homologous inhibition. Data from specific IgE measurements, skin test, and clinical history were not useful for the identification of cross-reacting patients.

Conclusion: FEIA inhibition is easy to perform and useful for the identification of patients with complete cross-reactivity. In these patients immunotherapy might be restricted to one venom which is beneficial for the patient and cost-effective.

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Introduction

Up to 5% of the population in Europe and North America show systemic reactions to hymenoptera stings [1]. The most prominent stinging insects are the honeybee (*Apis mellifera*) and wasps (*Vespula germanica* and *Vespula vulgaris*). Unfortunately, patients often fail to identify the stinging insect. In such cases, skin testing and in vitro measurement of venom-specific IgE antibodies are useful to detect the species involved in the allergic reac-

tion. In addition to clinical history, skin test results and IgE determination are also helpful for the selection of the appropriate venom for the immunotherapy [2]. About 30% of the hymenoptera-allergic patients show double sensitization to the honeybee and wasp in Pharmacia CAP [3]. Some of these patients, particularly those who were stung by just one insect, have a primary sensitization to only one insect venom with IgE antibodies reacting with similar epitopes in the other venom. This situation is termed 'cross-reactivity' and has been well investigated. The component hyaluronidase has been identified as the most prominent cross-reacting structure in both venoms [4–7]. In completely cross-reacting patients, immunotherapy with both venoms carries the risk of sensitization to other components in the non-cross-reacting insect venom. In addition, it is a source of unnecessary costs and inconvenience to the patient.

In our investigation, we have evaluated FEIA inhibition as a diagnostic tool to distinguish double-sensitized patients with cross-reactivity from patients with no or partial cross-reactivity.

Material and Methods

Sera

Serum samples from 24 patients (7 females, 17 males, median age 36 years, range 8–70, referred to the Allergy Unit in the period from August to December 1998) were collected and stored at –20 °C until use. All patients were candidates for venom immunotherapy and had double sensitization to honeybee and wasp venom as determined in Pharmacia CAP (i1 and i3, respectively). Since sera were diluted for inhibition experiments, specific IgE levels of the selected patients had to be above 1.0 kU/l.

Serum IgE

Specific serum IgE to honeybee venom (i1, venom from *A. mellifera*) and wasp venom (i3, a mixture of venoms from *V. vulgaris*, *V. germanica* and *V. maculifrons*) were measured with the immunoenzymatic CAP FEIA System (Pharmacia & Upjohn Diagnostics, Uppsala, Sweden), according to the manufacturer's recommendation.

FEIA Inhibition

Inhibition of specific IgE antibody measurements with venom preparations as inhibitors was carried out as follows. Patient serum (50 µl) and diluted venom (10 µl) were preincubated on a shaker at 4 °C overnight. Subsequently, the mixtures were entered as samples into the CAP assay (Pharmacia & Upjohn Diagnostics). We compared two different providers of honeybee and wasp venom. Inhibitions with Pharmedgen venom preparations (ALK, Hørsholm, Denmark) were done in serial dilutions from 0 to 100 µg/ml protein end concentration. For honeybee preparations venom from *A. mellifera* and for wasp preparations a mixture of venoms from six *Vespa* species (*V. germanica*, *V. vulgaris*, *V. maculifrons*, *V. flavopilosa*,

V. pensilvanica, *V. squamosa*) were used. The second venom for inhibition was provided by Pharmacia & Upjohn Diagnostics from their internal production. It was identical with venom immobilized on the CAPs. Inhibitions were carried out at 100 µg/ml protein end concentration.

In order to calculate the precision of the inhibition assay, 1 patient was measured in 10 replicates at three different inhibitor concentrations (0, 10 and 100 µg/ml protein end concentration). The coefficient of variation ranged from 3.38 to 4.29% and was within the range of the standard CAP assay without inhibition (10% according to the manufacturer).

The extent of homologous and heterologous venom inhibition was computed individually with the following formula:

$$\text{Inhibition (\%)} = 100\% \cdot \frac{\text{buffer control (kU/l)} - \text{inhibited sample (kU/l)}}{\text{buffer control (kU/l)} - \text{background}}$$

Inhibition values below zero were equalized to 0%. Serum samples that produced a homologous inhibition of less than 70% were excluded from the evaluation. If the homologous inhibition was greater than 70%, the ratio of heterologous to homologous inhibition was computed. Values above 85% were considered as completely cross-reactive, values from 85 down to 30% were defined as partially cross-reactive, and below 30% patients were not cross-reactive at all [8].

Skin Tests

Intracutaneous skin tests were performed with honeybee and wasp venom (*Vespa* spp., Pharmedgen by ALK) at serial dilutions from 0.00001 to 1.0 µg/ml. In 1 patient a prick test was used to determine the skin reaction (*Vespa* spp., Pharmedgen, ALK, concentrations from 1 to 100 µg/ml). The results of the skin tests were evaluated according to the guidelines of the European Academy of Allergy and Clinical Immunology [9].

Clinical Evaluation

The patients were evaluated on the basis of their case history, particularly allergy grade [10], stinging insect according to the patient and reactions to former stings.

Statistics

Statistical comparisons of the data were made with the Mann-Whitney U test for unpaired data and with the Wilcoxon signed rank test for paired data. The significance level chosen was $p < 0.05$.

Results

Using FEIA inhibition with ALK venom we could divide our patients into four groups of cross-reactivity (table 1, fig. 1). A first group comprised 4 patients with a primary sensitization to either honeybee or wasp venom and complete cross-reaction to the other venom. The second group included 8 patients with primary sensitization to one species and partial cross-reactivity to the other. The 10 patients in the third group were double-sensitized without any cross-reactivity. In the last group we have found 2 patients who showed insufficient homologous

Table 1. Results of FEIA inhibition in 24 patients with sensitization to both honeybee and wasp venom

	Inhibition with ALK venom, %				Inhibition with Pharmacia venom, %			
	homologous inhibition		heterologous inhibition		homologous inhibition		heterologous inhibition	
	bee (i1)	wasp (i3)	bee (i1)	wasp (i3)	bee (i1)	wasp (i3)	bee (i1)	wasp (i3)
<i>Complete cross-reactivity</i>								
Hi1	70	91	0	89	94	100	0	100
Gr2	100	100	0	100	100	100	0	89
St3	93	100	21	87	97	100	11	97
Kr4	100	97	100	6	100	100	100	20
<i>Partial cross-reactivity</i>								
Ny5	74	84	53	3	96	95	47	32
Hi6	78	100	39	7	100	100	45	11
Sc7	78	98	54	12	94	99	72	34
Ob8	87	98	56	6	ND	ND	ND	ND
Zi9	100	100	0	60	100	100	26	68
Er10	86	100	0	44	100	100	0	65
Ve11	91	100	35	26	ND	ND	ND	ND
Ba12	85	99	39	20	99	100	48	26
<i>No cross-reactivity</i>								
Di13	95	97	0	19	100	100	13	12
Sc14	70	93	0	5	100	98	3	3
St15	90	100	1	15	100	100	7	30
Su16	100	100	15	4	100	100	36	18
Wü17	100	100	4	14	100	100	17	25
Zu18	100	100	10	0	100	100	15	0
Bi19	100	100	18	18	100	100	7	50
Ba20	93	98	3	0	97	100	6	0
Gy21	95	91	17	26	98	99	0	24
Mü22	100	100	27	3	100	100	13	0
<i>Insufficient homologous inhibition</i>								
Ei23	46	58	21	16	ND	ND	ND	ND
Fr24	81	18	32	44	ND	ND	ND	ND

The patients are grouped according to the pattern of cross-reactivity. Inhibitions were carried out with venom preparations from ALK as well as Pharmacia & Upjohn. ND = Not determined.

inhibition with the ALK venom preparations. These patients were excluded from further analysis.

In most patients a protein concentration of 100 µg/ml was necessary to obtain a homologous inhibition of more than 70% (table 2). Only in some patients could sufficient inhibition values be obtained with lower concentrations.

Since homologous inhibition with ALK venom was as low as 70% in some patients, we repeated the inhibition experiments with Pharmacia venom at a protein concentration of 100 µg/ml (table 1). As expected, higher values for homologous inhibition were obtained with the Pharmacia venom (94–100%). In all cases, the homologous inhibition was equal or higher for the extract from Phar-

maciac compared to ALK. Analysis with the Wilcoxon signed rank test showed statistical significance for this finding ($p = 0.002$ for honeybee and $p = 0.008$ for wasp in homologous inhibition). Surprisingly, heterologous inhibition did not show significant change over all patients ($p = 0.371$ and $p = 0.096$ for honeybee and wasp heterologous inhibition, respectively). For honeybee inhibited with wasp venom, only 2 patients showed higher inhibitions for Pharmacia venom, whereas for heterologous inhibitions of wasp venom, 2 patients even showed higher inhibitions with ALK preparations. None of the patients changed the cross-reactivity group due to measurements with venom provided by Pharmacia.

Fig. 1. Inhibition patterns in double-sensitized patients. **a** Complete cross-reactivity. **b** Partial cross-reactivity. **c** No cross-reactivity. For each group one characteristic patient is shown. ■ = Honeybee venom; □ = wasp venom.

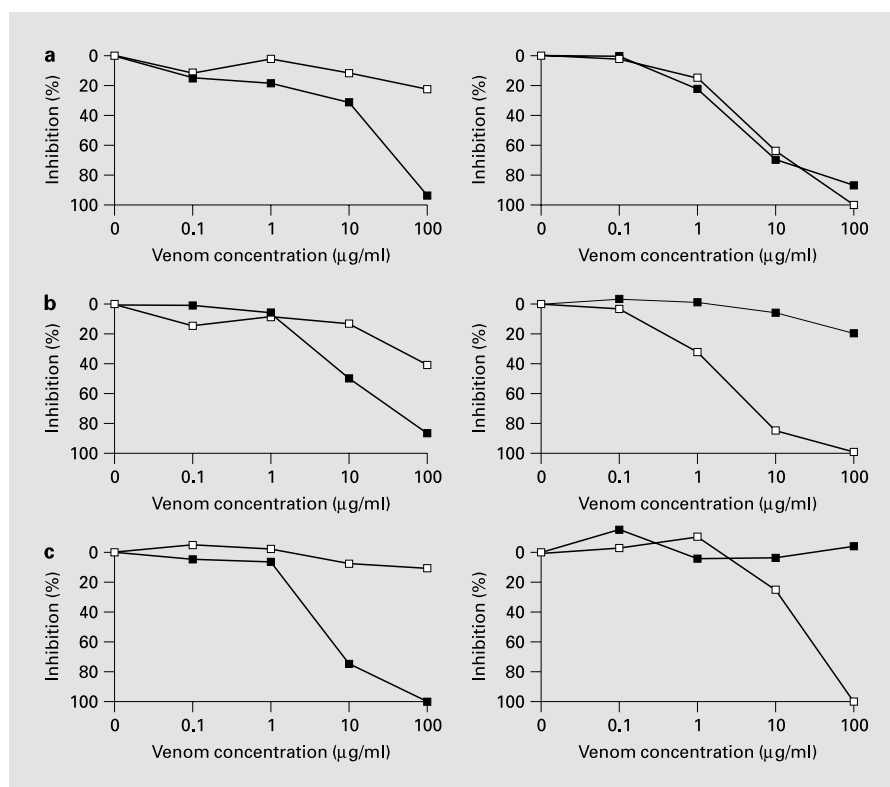


Table 2. Number of patients with inhibitions higher than the cutoff values (≥ 70 , ≥ 30 and $\geq 80\%$) at inhibitor protein concentrations ranging from 0 to 100 $\mu\text{g/ml}$ (venom preparations from ALK)

	Homologous inhibition		Heterologous inhibition			
	$\geq 70\%$		$\geq 30\%$		$\geq 80\%$	
	n	%	n	%	n	%
<i>Honeybee (i1)</i>						
0.1 $\mu\text{g/ml}$	0	0	0	0	0	0
1.0 $\mu\text{g/ml}$	0	0	0	0	0	0
10 $\mu\text{g/ml}$	6	27	4	18	1	5
100 $\mu\text{g/ml}$	22	100	7	32	1	5
<i>Wasp (i3)</i>						
0.1 $\mu\text{g/ml}$	0	0	0	0	0	0
1.0 $\mu\text{g/ml}$	1	5	0	0	0	0
10 $\mu\text{g/ml}$	10	46	3	14	1	5
100 $\mu\text{g/ml}$	22	100	5	23	3	14

The 2 patients with incomplete homologous inhibition are excluded.

There was no difference in skin tests and IgE measurements between the patients with cross-reactivity and the ones with partial or no cross-reactivity (table 3). Even though the CAP values due to cross-reactivity were all low compared to the primary sensitization (patients 1–4), this

finding was not significant ($p = 0.106$, Mann-Whitney test of the relative differences between bee and wasp) since some patients with no cross-reactivity showed large differences between honeybee and wasp as well. The same was true for skin tests ($p = 0.182$).

Table 3. Clinical characteristics, skin tests and specific IgE in 24 patients with double sensitization to honeybee and wasp venom

Patient	Sex	Age	Mueller grade	Stinging insect according to the patient	Previous stings according to the patient	CAP honeybee i1 kU/l	CAP wasp i3 kU/l	Intracutaneous skin test		Responsible insect according to FEIA inhibition
								with honeybee venom	with wasp venom	
Complete cross-reactivity										
Hi1	f	57	3	NI	?	13.8	2.28	0.1	1	honeybee
Gr2	m	21	4	honeybee	honeybee (LR)	4.91	1.22	0.1	0.1	honeybee
St3	m	31	3–4	hornet	wasp (SR)	28.4	4.43	0.1	1	honeybee
Kr4	m	63	4	wasp	wasp (SR)	1.61	17.7	1	1	wasp
Partial cross-reactivity										
Ny5	m	70	4	wasp	wasp (NR), honeybee (SR)	12	62.5	0.1	0.1	
Hi6	f	36	3	NI	NI (SR)	2.22	10.6	1	0.01	
Sc7	m	52	4	wasp	wasp (SR)	18.3	144	1	0.00001	
Ob8	m	19	3	wasp	wasp (LR), honeybee (LR)	3.95	15.4	0.1	0.01	
Zi9	f	34	strong local	honeybee	?	5.75	2.63	0.01	0.1	
Er10	m	37	3	NI	NI (LR)	16.2	1.61	0.01	1	
Ve11	m	23	3	NI	NI (LR)	6.62	7.88	0.1	0.1	
Ba12	m	36	4	NI	NI (LR)	13.2	15.1	0.01	0.01	
No cross-reactivity										
Di13	f	37	3–4	NI	wasp (LR), honeybee (LR)	5.84	24.7	0.01	0.01	
Sc14	m	65	2–3	NI	wasp (LR), honeybee (LR)	15	32.3	1	0.01	
ST15	m	51	4	NI	NI (SR)	25.8	2.62	0.01	0.1	
Su16	f	27	3–4	honeybee	NI (LR)	1.03	2.9	1	0.001	
Wü17	f	50	2	wasp	wasp (LR), honeybee (LR)	3.34	4.31	1	0.1	
Zu18	m	32	4	NI	honeybee (LR)	3.19	5.02	1	0.1	
Bi19	f	44	3–4	NI	VIT wasp and honeybee	1.8	1.11	0.1	0.1	
Ba20	m	33	2	wasp	wasp (LR), honeybee (LR)	24	21.7	0.1	0.01	
Gy21	m	22	3	honeybee	NI (NR)	58.6	9.25	0.01	1	
Mü22	m	66	4	wasp	wasp (SR), honeybee (NR)	1.6	12.5	NR	0.01	
Insufficient homologous inhibition										
Ei23	m	35	2–3	honeybee	wasp (LR)	17.3	4.5	1	1	
Fr24	m	8	3	wasp	wasp (SR), honeybee (NR)	4.4	1.89	prick test –	prick test +	

NI = Not identified; LR = local reaction; SR = systemic reaction; NR = no reaction; ? = patient does not remember.

Also, clinical data did not help the diagnosis of cross-reactivity (table 3). In almost half of the patients (10/23) either the last sting or previous stings were by unidentified hymenoptera. Under this condition the interpretation of the double sensitization was not possible without additional information. Four patients claimed to be stung by only one hymenoptera group, e.g. either honeybee or wasp and hornet (Gr2, St3, Kr4, Sc7). Indeed 3 patients out of this group showed complete cross-reactivity for one venom. But in patient St3 the cross-reacting venom was opposite to the claimed. The 4th patient (Sc7) showed only partial cross-reactivity for the claimed insect. Eight patients, including 1 patient with preceding immunotherapy, reported previous stings by both honeybee and wasp. None of these patients were totally cross-reactive. In such cases absence of complete cross-reactivity can be assumed without inhibition experiments, if the patient's statement is reliable.

Allergy grade according to Mueller [10] was not significantly different in patients with complete cross-reactivity as compared to patients with no or partial cross-reactivity (Mann-Whitney test, $p = 0.331$).

Discussion

We found that 18% of double-sensitized patients being candidates for immunotherapy were completely cross-reactive for one venom. This number is in accordance with a previous study by Schlenvoigt et al. [8], who found a proportion of 14%. Clinical data as well as skin tests and specific IgE determination were not sufficient for the identification of complete cross-reactors. To choose the right venom for immunotherapy, additional information like inhibition data were needed.

The main problem of FEIA inhibition is the choice of the inhibitor. In our study, we tested venom preparations from two different providers: First we took Pharmalgen venom by ALK which we also use for induction of immunotherapy according to rush and ultrarush protocols. The other venom was provided by Pharmacia and was identical with the venom linked to the CAPs in the FEIA assay. As expected, homologous inhibition was clearly better with the latter venom. Surprisingly however, the results of heterologous inhibition did not change significantly. This indicates that Pharmalgen venom preparations contain all epitopes involved in cross-reaction. Its use is therefore sufficient for the diagnosis of complete cross-reactivity. The advantage of this finding lies in the fact that the extract tested in FEIA inhibition is identical with the ones used for the induction of immunotherapy and for skin testing in our allergy unit. The 2 patients excluded from the study due to insufficient homologous inhibitions had probably IgE antibodies which recognized epitopes on the Pharmacia venom preparations which were not present in the Pharmalgen extract. In such cases FEIA inhibition is not suitable for the detection of cross-reactivity.

The analysis of inhibition over different inhibitor concentrations showed that inhibitor concentrations of 100 µg/ml protein are needed for determination of cross-reactivity. Higher concentrations are problematic for technical reasons: either serum has to be more diluted with allergen solution, which excludes patients with low specific IgE, or too concentrated venom stock solutions crystallize when stored for several days. Concentrations below 100 µg/ml are not sufficient for the detection of cross-reactivity in most of the patients.

Our data suggest that routine inhibition assays can be done at an inhibitor concentration of 100 µg/ml only. Since we have found a low coefficient of variation there is no need to perform measurements in duplicate. Therefore, a number of six CAP incubations per patient is sufficient for a cross-reactivity determination (homologous inhibition, heterologous inhibition and buffer control with both honeybee and wasp CAPs). For practical reasons, the patient's specific IgE levels to honeybee and wasp have to be above 1.0 kU/l.

We believe that FEIA inhibition should be reserved to patients who are candidates for immunotherapy and in whom the clinical history and the laboratory results do not enable to determine whether only honeybee or only wasp venom or both venoms should be used for immunotherapy. Our calculations using prices for medical treatment in the Zürich area (Switzerland) show that the ex-

Table 4. Cost-effectiveness of the FEIA inhibition assay

Charges for VIT with both venoms	SFr. 6,770.-/patient
Charges for VIT with one venom	SFr. 3,920.-/patient
Difference	SFr. 2,850.-/patient
Charges saved by the 18% complete cross-reactive patients	SFr. 513.-/patient
Charges for FEIA inhibitions (6 CAPs and venom)	SFr. 270.-/patient
Average charges saved	SFr. 243.-/patient

Calculations were done with charges to patients covered by the compulsory health insurance plan in the Zürich area. We chose the ambulant venom immunotherapy (VIT) for our calculations since it was cheaper than rush and ultrarush VIT.

penses saved by 18% of the patients who had immunotherapy with only one venom exceed additional costs for FEIA inhibition in all patients (table 4). This is in accordance with a previous investigation with other hymenoptera species that found inhibition measurements to be cost-effective [11].

In addition to the economic benefit of FEIA inhibition, inconvenience to the patients with complete cross-reactivity is reduced due to a lower number of visits and injections. Also, the risk of sensitization to new components can be eliminated.

In conclusion, our data strongly suggest that FEIA inhibition is a cost-effective and helpful tool to select venom for immunotherapy in double-sensitized patients. Prospective studies are needed to confirm the value and reliability of FEIA inhibition.

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