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Wasp Venom is Appropriate for Immunotherapy of Patients with Allergic Reaction to the European Hornet Sting

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Aim. To identify whether it is the yellow jacket (*Vespula germanica)* or European hornet *(Vespa crabro)* venom that induces sensitization in patients with IgE-mediated allergic reaction to the venom from the sting of a European hornet. Since these patients usually have positive skin tests and specific IgE to all vespid venoms, it would be useful to distinguish cross-reactors from non-cross-reactors to perform immunotherapy with the venom that induced the sensitization.

Methods. We performed inhibition tests in 24 patients who had experienced anaphylactic reaction after being stung by a European hornet.

Results. Of 24 patients with allergic reaction after *Vespa crabro* sting, 17 were sensitized only to epitopes of *Vespula germanica* venom. Only 4 out of 24 patients were sensitized to epitopes completely cross-reactive with *Dolichovespula arenaria* venom.

Conclusion. In Slovenia, the vast majority of patients with anaphylactic reaction to *Vespa crabro* sting seem to be sensitized to *Vespula germanica* venom. We consider wasp venom an appropriate immunotherapeutic agent for such patients, except for those with proven primary sensitization to specific epitopes of *Vespa crabro* venom. Fluorescence enzyme immunoassay inhibition should be considered a convenient tool for the identification of primary sensitization in patients allergic to vespid venoms.

Key words: *cross reactions; Hymenoptera; hypersensitivity, immediate; IgE; immunotherapy; insect bites and stings; venoms, Hymenoptera; wasp venoms*

Hymenoptera order of insects consists from Apidae and Vespoidea families. Honeybees and bumblebees are members of Apidae family, whereas the German yellow jacket *(Vespula germanica),* the European hornet *(Vespa crabro*), the yellow hornet *(Dolichovespula arenaria),* and paper wasps *(Polistes)* are members of Vespoidea family. About 0.1% of population have anaphylactic reaction to Hymenoptera insects. For patients who experienced a severe systemic IgE-mediated reaction to Hymenoptera insect sting, specific immunotherapy is the therapy of choice (1). Specific immunotherapy has to be performed with casual allergen after the sensitization is confirmed by positive skin test or increased specific IgE. Patients who experienced IgE-mediated reaction following the sting of a European hornet usually have positive skin tests and specific IgE to all vespid venoms. These patients have either specific antibodies to particular venom or cross-reacting antibodies that recognize similar or identical epitops in venoms of the insects in Vespoidea family (2). For the benefit of the patient, as well as for economic reasons, it would be useful to distinguish cross-reactors from non-cross-reactors and thereby perform immunotherapy with the venom that induced sensitization. To distinguish between primary sensitization and cross-reactivity, we performed cross-inhibition tests. Preincubation of a patient's serum with an antigen results in binding of specific IgE to the added antigen. As only free specific IgE is able to react in the antibody assay, binding of specific IgE antibodies to the antigens results in a sharp decrease or even disappearance of specific IgE antibodies. When the allergen that elicited the synthesis of specific IgE is added, no residual specific IgE antibodies are detected. Inhibition is complete. When cross-reactive antigen is added, the inhibition is only partial.

Patients and Methods

Patients

Twenty-four consecutive patients (mean age, 40±12 years), who experienced anaphylactic reaction after European hornet sting, recognized the insect, and had detectable specific IgE antibodies against the European hornet venom, were included in the study. None of the patients had been treated with immunotherapy before the study.

Specific IgE

Specific IgE antibodies against venoms of *Vespula germanica, Dolichovespula arenaria,* and *Vespa crabro* were determined by a commercial test, Uni-CAP fluorescence enzyme immunoassay (FEIA, Pharmacia, Uppsala, Sweden). Values >0.35 kUA/L were regarded as positive.

Inhibition Tests

Uni-CAP FEIA inhibition tests (3) were performed with homologous and heterologous venoms of German yellow jacket, European hornet, and Yellow hornet. Patient's serum (50 μL) was preincubated with equal amount of venom (100 mg protein/L) on a shaker for 1 h at room temperature. Venoms used for the inhibition were produced by ALK-Abelló (Hørsholm, Denmark), except for the *Vespa crabro* venom (Lofarma, Milano, Italy). Venoms were used as purchased and not tested for total protein content or presence of known allergenic proteins. The Uni-CAP assay (Pharmacia) was performed on a mixture of serum and venom. The inhibition was expressed as a percentage of saline diluted serum (1:1). Inhibition over 80% was regarded complete (3). Not all inhibition tests were performed in all patients.

Results

All patients had measurable levels of specific IgE antibodies against wasp and hornet venoms (Table 1).

Preincubation of sera with the wasp venom resulted in complete inhibition of IgE against the venom in all patients (Table 2). On the other hand, European hornet venom inhibited only 6 of 13 European hornet IgE positive sera.

Seventeen out of 24 patients were sensitized to wasp venom (complete inhibition of wasp and European hornet IgE antibodies with wasp venom, but not with European hornet venom) and two were sensitized to completely cross-reactive epitopes (complete inhibition of both wasp and European hornet IgE antibodies with wasp and European hornet venoms). One patient was sensitized only to a European hornet venom (complete inhibition of wasp and European hornet IgE with European hornet venom, but not with wasp venom) and 4 were sensitized to separate epitopes of both venoms (no complete inhibition with either venom). The sensitization to epitopes completely covered with commercially available Yellow hornet venom was found in only 3 patients (Table 1).

Table 1. Specific IgE against *Vespula germanica* and *Vespa crabro* in 24 patients who experienced allergic reaction after Vespa crabro sting[a]

Allergen	Median	Max.	Min.
Vespula germanica (kUA)/L)	4.9	39.2	0.79
Vespa crabro (kUA/L)	2.0	23.1	0.51

[a]Antibodies were measured by Pharmacia Uni-CAP. Values >0.35 kUA/L are positive.

Discussion

We found that at least 70% of patients who experienced systemic allergic reaction after European hornet sting were actually sensitized to wasp venom.

In Slovenia, like in other European countries with temperate climate, wasp stings are the most common cause of allergic reactions to vespid venom. Reactions to *Vespa crabro* venom occur only occasionally. This is the reason why the group of patients included in our study was relatively small.

Unfortunately, the patients who have frequent allergic reactions did not see or do not recognize the insect that stung them. Selection of the correct venom for immunotherapy is then based either on the skin test results or on the finding of specific IgE antibodies. As a rule, multiply positive allergy diagnostic tests to *Vespula germanica, Vespa crabro,* and paper wasp are regularly found in cases of allergy to vespid venoms. The reason is cross-reactivity to the constituents of the venoms, namely phospholipases, hyaluronidases, and antigen 5 (2,4-7). Despite pronounced cross-reactivity, some determinants found in venoms of different *Vespidae* species are quite unique (8). Phospholipases of wasps and hornets show significant differences in their aminoacid composition (9).

Selection of a proper venom for immunotherapy is important for two reasons. First, immunotherapy with a venom to which patient is not primarily sensitized can lead to incomplete protection and treatment failure. Second, treatment with cross-reactive venom only or with a mixture of venoms can lead to the formation of specific IgE antibodies against epitopes to which the patient was not sensitized prior to immunotherapy (10).

The European Academy of Allergy and Clincial Immunology (EAACI) (11) advises the treatment with *Vespula germanica* venom in patients who are positive for multiple *Vespula* venoms. In the USA, the mixture of all venoms to which the patient tests positive is used (12). In Europe, Blanca (10) proposes the treatment with vespid venom that caused the allergic reaction, to provide optimal concentration of relevant proteins for desensitization and to avoid the appearance of IgE antibodies to allergens which the patient was not initially sensitized.

Since we found that the majority of patients allergic to *Vespa crabro* venom is primarily sensitized to the *Vespula germanica* venom, this venom seems the most appropriate for the treatment of such patients. Epitopes in commercially available Yellow hornet venom are significantly different from that inducing sensitization in wasp and European hornet allergies. Surprisingly, *Vespa crabro* extract we used for the inhibition did not bind well to most epitopes of the patients' sera, either. We suspect that some important epitops detected with the Uni-CAP system were lost during purification of commercially available *Vespa crabro* venom. Alternative explanation is that the concentration of certain epitopes in commercially avail-

Table 2. Results of inhibition experiments performed on 24 sera of patients who experienced severe systemic anaphylactic reaction to a *Vespa crabro* sting

		Inhibition (%)			
Specific IgE antibodies to	with	median	max	min	Patients with inhibition ≥80%
Vespula germanica	*Vespula germanica*	99	100	81	19/19
Vespa crabro	*Vespa crabro*	68	100	21	6/13
Vespa crabro	*Vespula germanica*	91	99	36	19/24
Vespula germanica	*Vespa crabro*	53	96	17	3/13
Vespula germanica	*Dolichovespula arenaria*	47	96	2	3/24
Vespa crabro	*Dolichovespula arenaria*	48	98	6	4/24

able *Vespa crabro* venom extract is too low to sufficiently absorb the specific antibodies in patients' sera. Therefore, we think that commercially available *Vespa crabro* venom is not appropriate for immunotherapy because it does not contain all allergenic epitopes, as shown by autologous inhibition test. However, we did not analyze the concentration of different allergenic proteins in commercially available venoms.

We also found that in the majority of patients with anaphylactic reaction to *Vespa crabro* sting the sensitization was induced by *Vespula germanica* venom. The rational conclusion from this observation would be that *Vespula germanica* venom remains the most appropriate immunotherapeutic agent for most patients.

FEIA Uni-CAP inhibition should be considered convenient and cost-effective tool for the identification of primary sensitization in patients allergic to the vespid venoms. *Vespa crabro* venom alone or together with *Vespula germanica* venom should be used only in the immunotherapy of patients with proven sensitization to specific *Vespa crabro* venom epitopes.

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