M.G. Severino[1], B. Caruso[2], P. Bonadonna[3], D. Labardi[4], D. Macchia[1], P. Campi[1], G. Passalacqua[5]

Cross reactivity between European Hornet and Yellow Jacket venoms

[1] Allergy and Clinical Immunology Unit, Azienda Sanitaria di Firenze, Italy.
[2] Laboratory of Clinical Chemistry and Haematology, Verona General Hospital, Verona.
[3] Allergy Service, Verona General Hospital, Verona.
[4] Anallergo SpA, Florence.
[5] Allergy and Respiratory Diseases, DIMI, University of Genoa.

Key words

Hymenoptera venom allergy, Vespa crabro, Vespula germanica, Cross-reactivity, CAP-inhibition, immunoblotting

Summary

Background: *Cross-reactions between venoms may be responsible for multiple diagnostic positivities in hymenoptera allergy. There is limited data on the cross-reactivity between Vespula spp and Vespa crabro, which is an important cause of severe reactions in some parts of Europe. We studied by CAP-inhibition assays and immunoblotting the cross-reactivity between the two venoms.* ***Methods:*** *Sera from patients with non discriminative skin/CAP positivity to both Vespula and Vespa crabro were collected for the analyses. Inhibition assays were carried out with a CAP method, incubating the sera separately with both venoms and subsequently measuring the specific IgE to venoms themselves. Immunoblotting was performed on sera with ambiguous results at the CAP-inhibition.* ***Results****: Seventeen patients had a severe reaction after Vespa crabro sting and proved skin and CAP positive also to vespula. In 11/17 patients, Vespula venom completely inhibited IgE binding to VC venom, whereas VC venom inhibited binding to Vespula venom only partially (<75%). In 6 subjects the CAP-inhibition provided inconclusive results and their sera were analysed by immunoblotting. The SDS-PAGE identified hyaluronidase, phospholipase A1 and antigen 5 as the main proteins of the venoms. In 5 sera the levels of IgE against antigen 5 of Vespa crabro were higher than IgE against Vespula germanica, thus indicating a true sensitisation to crabro.* ***Conclusion:*** *In the case of multiple positivities to Vespa crabro and Vespula spp the CAP inhibition is helpful in detecting the cross-reactivities.*

Introduction

The choice of the vaccine for immunotherapy (IT) is crucial in hymenoptera venom allergy (HVA), since specific desensitization may confer an almost complete protection and avoid severe reactions (1). Therefore, it is important to know if the skin and CAP positivities to multiple venoms are due to independent sensitisations or, rather, if is due to cross-reacting epitopes. In this latter case, the vaccination with the primary sensitising venom is sufficient. Cross reactivities among venoms of different stinging insects, including *Polistinae* and *Vespinae* (2, 3) or bees and wasps (4), have been previously described, and in the case of Vespidae, the cross reactivity seems to be remarkably frequent (3). The CAP-inhibition technique maybe a helpful method to approach the problem.

European Hornet (*Vespa crabro*) is largely present in many European countries and is now recognized as an important cause of severe reactions in patients with HVA (5). There are, so far, few data available on the possible cross-reactivity between the venoms of *Vespa crabro* (VC) and *Vespula* spp (6-8).

We evaluated the presence and extent of cross-reactivity between the venoms of *VC* and yellow jacket in patients with severe reactions to VC stings. The cross reactivity was evaluated with CAP-inhibition techniques. In addition an immunoblotting was carried out on selected sera, for which the CAP-inhibition provided inconclusive results.

Methods

Sera from patients with severe reactions (grade III and IV according to Mueller), and who unequivocally recognized *VC* as the stinging insects responsible for the reaction were collected for the CAP-inhibition experiments. All patients underwent the standard diagnostic work-up (9), including clinical history, skin prick test, intradermal tests and specific IgE measurement by the commercial CAP-RAST (UniCap, Phadia, Uppsala, Sweden) assay. Prick tests were performed with standardized extracts at increasing concentrations from 0.01 to 100 μg/ml, whereas intradermal tests involved the injection of 0.02 mL extract at 0.001 to 1 μg/ml concentration. The tests were carried out with *Apis mellifera*, *Vespula* spp (Stallergènes, Milan, Italy), *Polistes dominulus* and *Vespa crabro* (Anallergo, Florence, Italy).

The inhibition assays were performed following a slightly modified Straumann's procedure (4), thus a specific IgE level greater than 1 kU/L was required. Briefly, 200 μL of serum were incubated for 12 hours at 4°C with 100 μL of venom at increasing concentrations (0; 0.3; 3.0; 30, 300 μg/ml). Inhibitor venoms were the same used for IT and skin testing and the commercial reagent, containing American and European Vespula venoms (including germanica) was the substrate in the CAP inhibition. Subsequently, specific IgE against each of the venoms were determined in the samples prepared as above. The CAP inhibition test was carried out with a specific program in UniCap 250 (Phadia, Uppsala, Sweden). The extent of homologous (blockage of venom-specific IgE by the same venom) and heterologous (blockage of the venom-specific IgE by the other venom) inhibition at the maximum venom concentration was computed with the following formula: %inhibition= 100-[IgE inhibited

Table 1 - Characteristics of the patients and results of CAP-RAST and intradermal test. VC = *Vespa crabro*; Vspp = V*espula* Species

			Allergen-specific IgE (CAP)				Intradermal test wheal (concentration in μg/ml)			
N Pat	Age/ Sex	Total IgE kU/L	bee kU/L	Polistes dominulus kU/L	VSpp kU/L	VC KU/L	bee	Polistes dominulus	VSpp	VC
1	59/m	19	<0.35	1.00	2.30	1.79	8 mm (1)	6 mm (1)	8 mm (0.1)	9 mm (0.1)
2	51/m	118	<0.35	<0.35	2.08	1.11	7 mm (1)	NEG	9 mm (0.1)	10 mm (0.1)
3	63/f	91	<0.35	1.70	2.36	3.30	NEG	7 mm (1)	11 mm (0.1)	12 mm (0.1)
4	43/m	127	0.85	<0.35	4.95	1.36	8 mm (1)	8 mm (0.1)	10 mm (0.01)	9 mm (0.1)
5	50/f	80	<0.35	1.0	10.5	5.25	NEG	8 mm (0.01)	8 mm (0.001)	12 mm (0.001)
6	39/f	346	0.88	<0.35	11.0	2.28	NEG	NEG	11mm (0.01)	10 mm (0.1)
7	51/m	260	0.70	0.60	4.50	3.50	6 mm (1)	6 mm (1)	9 mm (0.1)	10 mm (0.1)
8	47/f	173	0.35	0.49	4.29	2.81	NEG	8 mm (0.01)	9 mm (0.01)	8 mm (0.1)
9	17/m	85	0.75	0.90	2.90	1.60	7 mm (1)	8 mm (1)	9mm (0.1)	10mm (0.1)
10	52/m	209	0.92	0.94	84.9	5.50	NEG	8 mm (0.01)	9 mm (0.0001)	11mm (0.0001)
11	30/m	69	0.35	1.40	1.60	3.49	8 mm (1)	9 mm (0.1)	10 mm (0.1)	11 mm (0.1)
12	46/m	191	0.80	0.35	4.27	5.26	NEG	11 mm (0.01)	10 mm (0.01)	12 mm (0.01)
13	33/m	168	7.90	2.40	6.50	10.8	9 mm (1)	NEG	10 mm (0.1)	9mm (0.1)
14	75/f	280	4.12	12.0	15.2	6.57	10 mm (1)	11.5 mm (1)	11 mm (0.1)	13 mm (1)
15	70/m	175	9.27	0.68	6.78	7.37	10 mm (1)	11 mm (1)	13 mm (0.1)	12 mm (0.1)
16	60/m	116	0.77	1.72	6.06	2.92	NEG	11 mm (1)	12 mm (0.1)	11.5 mm (0.1)
17	30/m	151	3.00	1.93	2.66	2.75	6.5 mm (1)	10 mm (0.1)	10.5 mm (0.1)	10 mm (0.1)

sample (kU/L)X100/IgE antivenom (kU/L) at zero concentration]. An inhibition ≥75% was considered indicative of full cross-reactivity.

For immunoblotting, the proteins of venoms were separated through an SDS-PAGE (Bio-Rad, gel Criterion XT 12% and Bio-Rad, XT Reducing Agent 20x) in MES buffer (Bio-Rad, MES Running buffer) under reducing and denaturing conditions. Eight mcg of venom, 5 mcg of molecular weight standard and 8 mcg of Parietaria extract (as control) were run for 1 hour at 200V. Parietaria was chosen since it is an uncommon allergen in North-east Italy, where all patients proved skin negative for it. Protein bands were revealed by Coomassie blue staining and quantified by densitometry. In parallel, another gel was run for immunoblotting onto nitrocellulose membranes. Membranes were incubated with patients' sera, then with peroxydase-conjugate anti-IgE (Sigma, St.Louis, Anti-Human IgE peroxidase Conjugate). Bound IgE were detected by a chemiluminescent reaction (GE Healthcare, ECL Plus, catalog RPN2132). Final results were expressed as the ratio between the staining intensity obtained in the immunoblotting and that of the Coomassie blue, in order to avoid the bias due to the different content of proteins in the separation bands.

Results

Patients. Seventeen patients (12 male, mean age 45.3 years) had a severe reaction (10 grade IV and 7 grade III) unequivocally provoked by VC. Thirteen of them had previous stings by yellow jacket, two could not recognize the insect at previous stings and two (patients 1 and 2) reported one VC sting in the past. In all cases, the previous stings had provoked only local reactions. All subjects had skin and CAP-RAST positivity to both VC and *Vespula* spp. with specific IgE of 3.98 ± 2.55 and 10.2 ± 19.6, respectively (p= NS). Some patients had also a positive skin test and/or CAP-RAST for honeybee and four for Polistes dominulus, but they have had never been stung by these insects. The results of the diagnostic workup are summarized in Table 1.

Table 2 - Results of the CAP-inhibition assays. The inconclusive results are highlighted in light grey. VC= *Vespa crabro*; VSp= *Vespula* Species

	Heterologous		Homologous	
	% inhibition of VC-specific IgE by VSp venom	% inhibition of VSp-specific IgE by VC venom	% inhibition of VSp-specific IgE by VSp venom	% inhibition of VC-specific IgE by by VC venom
1	75	48	85	80
2	90	39	89	70
3	67	73	94	79
4	82	62	94	83
5	96	67	99	98
6	87	42	95	70
7	95	36	92	90
8	75	88	100	95
9	75	52	98	75
10	90	87	98	93
11	83	52	85	79
12	98	32	94	99
13	77	61	89	77
14	95	68	94	98
15	97	83	90	98
16	92	92	93	95
17	86	78	94	83

CAP inhibition. At the CAP-inhibition assays, pre-incubation with each venom efficiently blocked the specific IgE for that venom (homologous inhibition >90%) as expected. Concerning the heterologous inhibition, in 11/17 patients, *Vespula* venom completely inhibited IgE binding to VC venom, whereas VC venom inhibited binding to *Vespula* venom only partially (<75%) (Table 2). This means that pre-incubation with VC venom did not bind the Vespula-specific IgE. In 6 subjects (n. 3, 8, 10, 15-17) the CAP-inhibition test provided inconclusive results, therefore the sera of these patients were analysed by immunoblotting. Examples of different inhibition curves (patients 14 and 16) are shown in Figure 1.

SDS-PAGE. The SDS-PAGE procedure separated three major bands, corresponding to hyaluronidase (45 kD), phospholipase A1 (35 kD) and antigen 5 (23 kD) (11) (Fig. 2). The 45 kD band had a too low intensity and was not analysed. At the immunoblotting, the serum from patient 15 proved positive also for the negative control (Parietaria) and was not included in the evaluation. In the 5 sera evaluated, the levels of IgE (optical density) against phospholipase of VC and V germanica were similar. On the other hand, IgE against Vespula antigen 5 were significantly lower than IgE against VC antigen 5 (Fig. 3), thus indicating at least a greater affinity of the IgE for the VC antigen 5 epitopes.

Discussion

The cross-reactivity among different allergens is quite common and occurs, in fact, with vegetables, pollens (10) and drugs. In the case of HVA, cross-reactions among venoms may produce multiple diagnostic positivities, with the consequent prescription of multiple vaccines, also when one single IT would be sufficient. This frequently occurs with Vespidae, whose venoms are quite similar in the allergenic composition. In our experience, the positivity to both yellow jacket and VC (European hornet), often makes difficult the choice of the vaccine, although it has been previously suggested that one wasp venom can protect also against VC (8). Thus, we attempted to define if a patient truly had IgE against unique epitopes in both venoms or if the reactivity with one of the venoms was entirely due to cross-reactivity. The CAP-inhibition assay, indeed evidenced that the two venoms extensively cross-react in 67% of patients, and that vespula venom efficiently binds the VC-specific IgE. In those patients, yellow jacket vaccination is reasonably expected to be adequate. Similar findings were reported some years ago in a case series of 24 patients

Figure 1 - Examples of two inhibition experiments. On X axis the concentration of the inhibitor, and on Y axis the % of inhibition. Homologous and heterologous inhibitions with VC and *Vespula* spp venoms are shown.

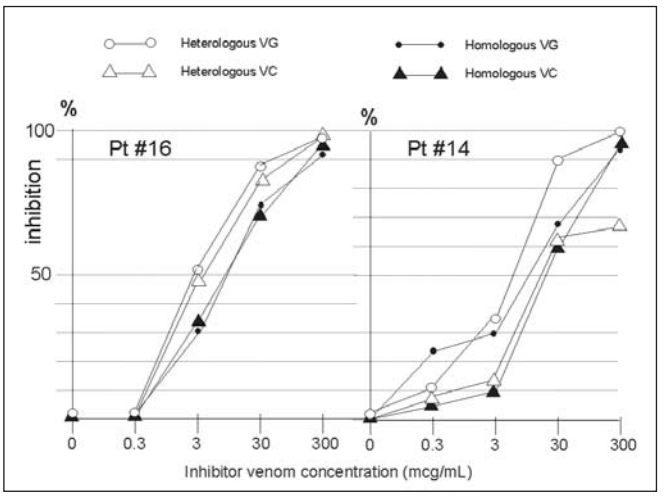


Figure 2 - Immunoblotting assays of the 6 sera with inconclusive results at the CAP inhibition assay. From left to right lanes: molecular standard, *Vespula*, VC, parietaria. The serum #15 (right) proved positive also for the negative control and was excluded

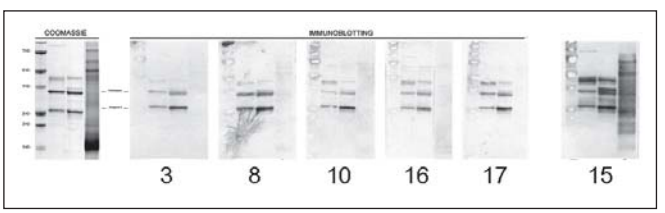
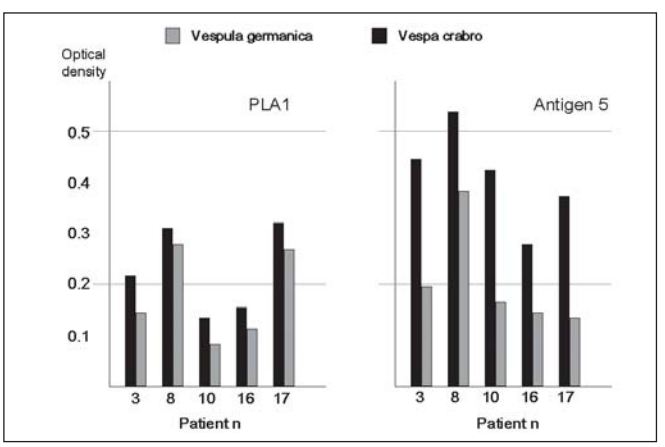


Figure 3 - Specific IgE (optical density) against phospholipase (left) and antigen 5 (right) of VC and *Vespula* in the five sera shown in figure 1



(8). In such cases, it can be hypothesized that patients are primarily sensitised to yellow-jacket, and the cross-reactivity of venoms is responsible for the severe reactions to European hornet. Our results partly differ from those reported in a Spanish study (7), but this may be attributed, at least in part, to the different presence and distribution of the insect in different geographical regions. As a partial limitation, in this study we could not identify the exact nature of the cross-reactive epitope, although it is conceivable that part of the cross-reactivity is due to carbohydrate determinants, as previously described for honeybee and yellow jacket (12, 13). Another possible limitation is that the extract used for skin tests and as inhibitor is a mix of different Vespula species, including *Vespula germanica*. This is due to the fact that a purified Vespula germanica venom for in vitro and in vivo diagnosis is not available.

The CAP-inhibition assay, which is a sensitive technique, largely used in allergy since decades, is helpful in identifying those patients. Where the CAP-inhibition provided ambiguous results, the immunoblotting assay clearly showed that the patients had higher levels of IgE against one allergen of VC, thus they should be vaccinated with a VC extract, which is of note available only in few European countries. Certainly, the clinical evaluation remains the basic criteria, but the CAP-inhibition, which is relatively simple, can be regarded as an useful tool to better detail the diagnosis and the consequent therapeutic approach. In this regard, the identification of the correct venom to use for vaccination may counterbalance the cost of the technique itself.

References

1. Bonifazi F, Jutel M, Biló BM, Birnbaum J, Mueller U; EAACI Interest Group on Insect Venom Hypersensitivity. Prevention and treatment of hymenoptera venom allergy: guidelines for clinical practice Allergy 2005; 60: 1459-70.
2. Hamilton RG, Wiesenauer JA, Golden DB, Valentine MD, Adkinson NF Jr. Selection of Hymenoptera venoms for immunotherapy on the basis of patient's IgE antibody cross-reactivity. J Allergy Clin Immunol 1993; 92(5):651-9.
3. Caruso B, Bonadonna P, Severino MG, et al. Evaluation of the IgE cross-reactions among vespid venoms. A possible approach for the choice of immunotherapy. Allergy 2007; 62 (5): 561-4.
4. Straumann F, Bucher C, Wüthrich B. Double sensitization to honeybee and wasp venom: immunotherapy with one or with both venoms? Int Arch Allergy Immunol 2000; 123:268-74
5. Antonicelli L, Bilo MB, Napoli G, Farabollini B, Bonifazi F. European hornet (*Vespa crabro*) sting: a new risk factor for life-threatening reaction in hymenoptera allergic patients? Allerg Immunol (Paris) 2003; 35 (6): 199-203.
6. Panzani R, Blanca M, Sánchez F, Juarez C. Sensitivity to European wasps in a group of allergic patients in Marseille: preliminary results. J Investig Allergol Clin Immunol 1994; 4 (1): 42-4.
7. Blanca M, Garcia F, Miranda A, et al. Determination of IgE antibodies to *Polistes dominulus, Vespula germanica* and *Vespa crabro* in sera of patients allergic to vespids. Allergy 1991; 46: 109-14.
8. Kosnik M, Korosec P, Silar M, Music E, Erzen R. Wasp venom is appropriate for immunotherapy of patients with allergic reaction to the European hornet sting. Croat Med J 2002; 43 (1): 25-7.
9. Bilò BM, Rueff F, Mosbech H, Bonifazi F, Oude-Elberink JNG & the EAACI Interest Group on Insect Venom Hypersensitivity. Diagnosis of Hymenoptera venom allergy. Allergy 2005; 60: 1339-49.
10. Mothes N, Horak F, Valenta R. Transition from a botanical to a molecular classification in tree pollen allergy: implications for diagnosis and therapy. Int Arch Allergy Immunol 2004; 135: 357-73.
11. Hoffman DR, Jacobson RS, Zerboni R. Allergens in hymenoptera venom. XIX. Allergy to *Vespa crabro*, the European hornet. Int Arch Allergy Appl Immunol 1987;84(1):25-31.
12. Hemmer W, Focke M, Kolarich D, et al. Antibody binding to venom carbohydrates is a frequent cause for double positivity to honeybee and yellow jacket venom in patients with stinging-insect allergy. J Allergy Clin Immunol 2001;108: 1045–52.
13. Jappe U, Raulf-Heimsoth M, Hoffmann M, Burow G, Hubsch-Muller C, Enk A. In vitro hymenoptera venom allergy diagnosis: improved by screening for cross-reactive carbohydrate determinants and reciprocal inhibition. Allergy 2006; 61: 1220-9