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Are anaphylactic reactions to snake bites immunoglobulin E-mediated?

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Summary

Background Bites by poisonous European snakes of the genus Vipera lead to local tissue damage and systemic symptoms such as generalized oedema, hypotension, gastrointestinal symptoms, haemolysis and renal dysfunction. Not rarely anaphylactic symptoms like urticaria, localized angioedema and asthma are observed.
Objective To look for snake venom-specific immunoglobulin (Ig) E antibodies in patients with a history of bites by European vipers and for cross-reactions with Hymenoptera venoms, that have a similar composition.
Method Ten patients with a history of bites by *Vipera aspis* or *Vipera berus* were investigated. Three patients had been bitten only once, and two of these had developed only local reactions. Four reported previous allergic reactions to Hymenoptera stings. All patients, 10 Hymenoptera venom-allergic and five nonallergic individuals who served as controls underwent i.c. skin test endpoint titration with snake (*V. aspis*, *V. berus*) and Hymenoptera venoms (honey bee, *Vespula* spp.) and were investigated for specific serum IgE antibodies to the same venoms.
Results Seven of the eight patients with systemic snake bite reactions had both positive skin tests and serum IgE antibodies to snake venoms, while these tests were negative in the two patients with only local reactions to snake bites and all controls. Seven of the eight patients with systemic snake bite reaction also had positive skin tests and specific IgE with one or both Hymenoptera venoms. By RAST-inhibition with sera of four patients with high IgE to both Vipera and Hymenoptera venoms, partial cross-reactivity could be demonstrated in one.
Conclusions Anaphylactic reactions following snake bites may be IgE-mediated, especially in patients with repeated bites.

Keywords: anaphylaxis, Hymenoptera venom, skin tests, snake bites, snake venom, specific immunoglobulin E, *Vipera aspis*, *Vipera berus*

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Introduction

Free-living poisonous snakes in Europe belong almost exclusively to the family Viperidae, genus Vipera: *Vipera aspis* (Va) is found in France, Italy and in the Alpine region of Southern Germany, Austria and Switzerland, *Vipera ammodytes* in south-eastern Europe and *Vipera berus* (Vb) in most parts of Europe. The three European vipers are closely related. Consequently there are only minor differences in the composition of their venoms and symptoms of envenomation [1,2]. Occasional single bites by Va and Vb occur most commonly outdoors, whereas repeated bites are mainly observed in persons who keep snakes either professionally or as fanciers.

The venom of vipers contains a mixture of proteins with enzymatic and toxic activity, such as proteolytic enzymes, peptide hydrolases, hyaluronidase, phospholipase A2, phosphodieseterase and L-amino acid oxidase. Further constituents are amino acids, carbohydrates, toxic peptides and metalloproteins. Spreading of the venom in the tissue is

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facilitated by the action of hyaluronidase. Proteolytic enzymes and toxic peptides cause subcutaneous tissue damage including capillary endothelium with leakage of plasma and erythrocytes, and haemolysis. A spectrum of systemic effects follows the release of highly active endogenous substances such as histamine, bradykinin, prostaglandins and serotonin [1,3]. Local and systemic reactions may vary in their severity according to the venom amount injected. 'Dry bites' with no venom injected were observed at 13% in one study [2].

The most common symptoms of envenomation following bites by European vipers [4–6] are extensive local swelling with necrosis; abdominal pain, vomiting and diarrhoea; arterial hypotension or shock due to vasodilatation and vascular leakage with generalized oedema; haemolysis and renal failure. Anaphylactic symptoms like urticaria, localized angioedema and asthma are sometimes observed, which are thought by some to be due to the release of endogenous vasoactive agents such as bradykinin and histamine [1]. Considering the high frequency of immunoglobulin (Ig) E-mediated allergic reactions to other animal venoms, especially those of bees and wasps, and occasional reports suggesting allergic sensitization to snake venoms after repeated bites [7–9], we looked for IgE antibodies to Vipera venoms in 10 Swiss patients with a history of one or several bites by Va or Vb.

Patients and methods

Patients and controls

We investigated 10 patients with a history of bites by Va or Vb. Two of these patients (patient 3, patient 10) were referred because of systemic symptoms suggestive of an allergic reaction, the remaining eight were recruited from a Swiss snake-fanciers society. Two of these individuals reported only local reactions, the other six systemic symptoms (Table 1). Ten patients with Hymenoptera venom allergy (five to honey bee venom, five to Vespula venom) and five individuals with no Hymenoptera venom allergy and without any exposure to snakes served as controls. All patients and controls were adults and a written informed consent was obtained from each. The study protocol was accepted by the Ethical Committee of the Medical Faculty of the University of Bern, Switzerland.

Allergens

The venoms of *Vipera aspis* and *Vipera berus* were obtained from Biomedical ICN Pharmaceuticals (Costa Mesa, CA, USA) and Sigma (St Louis, MO, USA), honey bee and Vespula venom from ALK, Hørsholm, Denmark and Sigma (St Louis, MO, USA).

Skin tests

For skin testing, snake venom was diluted in commercial skin test diluent (ALK) and sterilized by filtration. First, prick tests with 10^{-1}g/L of Va and Vb venom were done. Then i.c. skin tests with increasing concentrations of snake venom (10^{-8}g/L to 10^{-4}g/L) were performed at intervals of 20 min until the test was positive. A weal of ≥ 5 mm in diameter with erythema was considered as a positive reaction. i.c. skin tests with honey bee and *Vespula* spp. venom were done as described previously [10]. Prick tests to 12 common inhalants were also performed. The inhalants included were: dog, cat, horse, *D. farinae*, *D. pteronyssinus*, mixed grass pollen, tree pollen, ash tree, mugwort, latex, *Cladosporium herbarum* and *Alternaria alternata* (ALK).

Specific serum IgE

Specific serum IgE antibodies to Hymenoptera venoms were estimated by the ImmunoCAP, system (Pharmacia-Upjohn, Uppsala, Sweden), the results are given in kU/L or CAP classes from 0 to 6. Snake venom-specific IgE were estimated by an enzyme immunoassay (EAST, IBL, Hamburg, Germany). Experimental snake venom disks were prepared by coupling 10 μg of snake venom protein per CNBr-activated disk as described previously [11]. The results are given in U/mL or EAST classes from 0 to 4. Inhibition assays were performed as described previously [11]. Briefly 50 μL of patient serum in appropriate dilution were incubated with 100 μL of serial tenfold dilutions of either allergen from 1000 to 0.1 μg/mL for 3 h at room temperature. This was followed by estimation of specific IgE by EAST as above. Percent inhibition was calculated as follows:

$$\%\text{inhibition} = (1 - {}^{ES}/_{EO})100$$

where EO is the extinction with the unabsorbed serum and ES the extinction with the absorbed serum.

SDS-electrophoresis and Western blot [10]

Snake, honey bee and Vespula venom at a concentration of 5 mg/mL was separated in a 17.5% polyacrylamide gel at 200 V for 45 min using a BioRad, mini gel electrophoresis system (BioRad, Hercules, CA, USA) and stained with Coomassie Brilliant Blue, 0.1% in 40% methanol, 10% acetic acid. For western blot the separated unstained bands were blotted onto nitrocellulose in transfer buffer for 1 h at 100 V/250 mA and then blocked by incubation with TBS (TRIS-buffered saline)/casein. Thereafter, 4 mm strips were cut and incubated overnight in a 1 : 4 solution of patient serum in TBS. Strips were then washed and incubated for 18 h in a monoclonal mouse antihuman IgE serum coupled to alkaline phosphatase (Alablot, DPC Bierman,

Table 1. Clinical data of 10 patients with snake bites

Patient	Age (years)	Atopy?	Snake (bite)	Exposure to snakes?	Symptoms*	Hymenoptera allergy?	Sensitization to snake venom (skin test/EAST)
1	26	Yes	*V. aspis*	Yes	Local reaction only	No	No
2	28	No	*V. aspis*	Yes	Local reaction only	No	No
3	39	Yes	*V. aspis*	No	AE, D, GID	Yes	No
4	33	No	*V. aspis*	Yes	GID, HT	No	Yes
			V. aspis		U		
5	40	No	*V. aspis*	Yes	AE, GID	Yes	Yes
			V. aspis		U, AE		
6	39	Yes	*V. aspis*	Yes	U, D	Yes	Yes
7	37	No	4–5× *V. aspis*	Yes	LE	No	Yes
			V. aspis		Conjunctivitis, D, LE, GID, HT		
			V. aspis		Chest tightness		
			V. berus		Chest tightness		
8	35	Yes	*V. aspis*	Yes	GID	Yes	Yes
			V. aspis		AE, GID, HT, LC		
9	55	Yes	*V. aspis*	Yes	U, AE, D, HT	No	Yes
10	51	Yes	*V. aspis*	Yes	U, GID, HT, LC	No	Yes

*U = urticaria, AE = angioedema, LE = laryngeal edema, D = dyspnea, GID = gastrointestinal disturbances, HT = hypotension, LC = loss of conciousness

Bad Nauheim, Germany). After extensive washing, IgE-binding bands were visualized by incubation with brom-chlor-indolyl-phosphate toluidine salt/nitroblue tetrazolium chloride substrate solution.

Statistics

Mean values of snake venom-specific IgE between snake-exposed patients and controls were compared by Mann–Whitney *U*-test, correlations of specific IgE to Vb and Vb by Spearman's rank correlation coefficient.

Results

Clinical data

All the patients with snake bites were adult (age 26–57 years) and male. One bite was due to Vb, all other to Va. Two of the patients had been bitten only once (patients 1 and 3), the other eight had experienced up to 15 snake bites. Nine of the patients dealt with snakes professionally or were snake fanciers. Patient 3, a vintager, was bitten only once by a snake in his vineyard. The clinical data are summarized in Table 1. Eight patients had local and systemic symptoms, two (patients 1 and 2) developed only a local reaction. Systemic symptoms were usually typical for an IgE-mediated allergic reaction (urticaria, angioedema, laryngeal oedema and asthma) or at least compatible with such a pathogenesis (arterial hypotension, abdominal cramps, vomiting, diarrhoea). Gastrointestinal symptoms and/or hypotension often occurred already after first bites by European vipers (patients 4, 8) or exotic snakes (patients 5, 6, 9, 10), symptoms typical of IgE-mediated reactions mostly only after repeated bites (patients 4, 5, 6, 9, 10). Exceptions are patients 3 and 7 who developed generalized angioedema or laryngeal oedema, respectively, already after the first snake bite.

Skin tests and specific serum IgE antibodies (Table 2)

In seven of eight patients with a systemic reaction a positive i.c. skin test to Vipera venom at 10^{-8} g/L was found, while the test was negative up to 10^{-4} g/L in the two patients with only local reactions. The patient with a systemic reaction but a negative skin test (patient 3) had no previous exposure to snakes. In patients with positive skin tests, specific IgE levels to snake venoms were 2.4 to > 17.5 U/mL to Vb and 2.9 to > 17.5 U/mL to Va venom. None of the controls had specific IgE above 1.0 nor did the three snake-bitten patients with a negative skin test. As a consequence only a specific IgE titre over 1.0 U/mL was considered as a positive result. Mean values of Va- and Vb-specific IgE were significantly higher in the 10 snake-bitten patients than in Hymenoptera sting-allergic (Va $P = 0.02$; Vb $P = 0.007$)

Table 2. Skin tests and specific IgE to Hymenoptera and snake venoms

Patient*	Honey bee		Vespula		*Vipera aspis*		*Vipera berus*	
	Skin test (g/L)	Specific IgE CAP (class [kU/L])	Skin test (g/L)	Specific IgE CAP (class [kU/L])	Skin test (g/L)	Specific IgE EAST (class [U/mL])	Skin test (g/L)	Specific IgE EAST (class [U/mL])
1	10^{-3}	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	1 (0.65)
2	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	2 (0.7)
3	10^{-4}	Neg	10^{-4}	2 (2.10)	Neg.	1 (0.6)	Neg.	1 (0.65)
4	10^{-3}	3 (10.7)	10^{-3}	1 (0.53)	10^{-8}	4 (>17.5)	10^{-8}	4 (>17.5)
5	Neg.	Neg.	10^{-4}	2 (0.98)	10^{-8}	3 (3.4)	10^{-8}	3 (4.6)
6	10^{-4}	3 (11.7)	10^{-4}	3 (4.56)	10^{-8}	2 (3.3)	10^{-8}	3 (4.7)
7	10^{-4}	2 (2.05)	Neg.	Neg.	10^{-8}	2 (2.9)	10^{-8}	2 (2.4)
8	10^{-4}	3 (4.9)	10^{-4}	2 (1.44)	10^{-8}	3 (7.0)	10^{-8}	3 (13.5)
9	Neg.	Neg.	Neg.	Neg.	10^{-8}	2 (3.4)	10^{-8}	3 (4.8)
10	10^{-4}	2 (1.42)	10^{-3}	1 (0.56)	10^{-4}	3 (8.0)	10^{-8}	3 (8.5)
11	10^{-4}	3 (6.55)	10^{-3}	1 (0.69)	Neg.	1 (0.57)	Neg.	1 (0.41)
12	10^{-6}	3 (9.97)	10^{-3}	Neg.	Neg.	1 (0.43)	Neg.	2 (0.95)
13	10^{-6}	4 (19.7)	Neg.	2 (3.17)	Neg.	1 (0.68)	Neg.	2 (0.9)
14	10^{-4}	1 (0.64)	Neg.	Neg.	Neg.	1 (0.63)	Neg.	2 (1.0)
15	10^{-6}	3 (7.27)	Neg.	Neg.	Neg.	Neg.	Neg.	1 (0.5)
16	Neg.	Neg.	10^{-6}	3 (4.66)	Neg.	1 (0.44)	Neg.	1 (0.68)
17	Neg.	Neg.	10^{-6}	2 (2.52)	Neg.	1 (0.58)	Neg.	1 (0.6)
18	Neg.	1 (0.52)	10^{-6}	2 (2.51)	Neg.	1 (0.45)	Neg.	1 (0.57)
19	Neg.	Neg.	10^{-4}	1 (0.48)	Neg.	1 (0.36)	Neg.	1 (0.53)
20	Neg.	Neg.	10^{-4}	2 (1.26)	Neg.	Neg.	Neg.	2 (0.93)
21	Neg.	Neg.	Neg.	Neg.	Neg.	1 (0.5)	Neg.	2 (0.75)
22	10^{-4}	1 (0.62)	Neg.	Neg.	Neg.	1 (0.63)	Neg.	1 (0.65)
23	Neg.	1 (0.54)	10^{-4}	Neg.	Neg.	Neg.	Neg.	1 (0.6)
24	Neg.	Neg.	Neg.	Neg.	Neg.	1 (0.53)	Neg.	2 (0.88)
25	Neg.	Neg.	Neg.	Neg.	Neg.	1 (0.41)	Neg.	1 (0.6)

*1–10: patients with snake bites, 11–20 patients with Hymenoptera venom allergy (11–15 honey bee, 16–20 Vespula), 21–25 nonallergic controls.

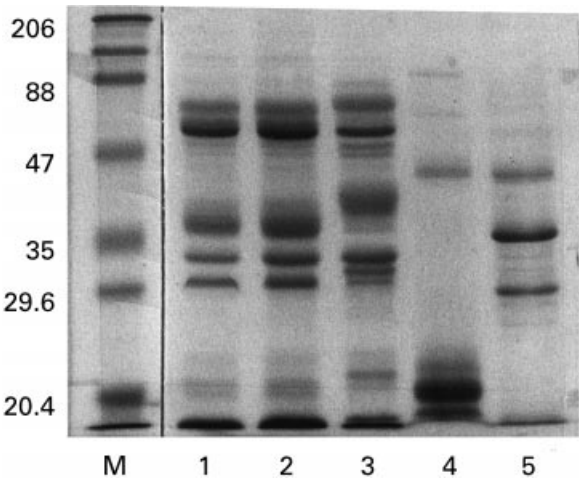

Fig. 1. SDS-electrophoresis of venoms from: (1) and (2) *Vipera berus*, (3) *Vipera aspis*, (4) honey bee (*Apis mellifera*) and (5) *Vespula vulgaris*. M = markers

and in nonallergic controls (Va $P = 0.07$; Vb $P = 0.03$). The correlation between IgE to Va and Vb was very close (Spearman's rank: rho = 0.924, $P = 0.006$).

Seven of eight patients with systemic reactions to snake bites also had a positive skin test and specific serum IgE to Hymenoptera venoms. Out of these seven, four gave a history of systemic allergic reactions to Hymenoptera stings. Four of the seven patients with positive skin tests and specific serum IgE antibodies to snake venom were atopic according to skin tests to common inhalants.

SDS-Electrophoresis and Western blot (Figs 1 and 2)

Figure 1 shows SDS electrophoresis of Va and Vb venom in comparison with honey bee and *Vespula vulgaris* venom. Several major protein bands are found in Vipera venoms between about 10 and 70 kDa, in agreement with previous reports [12]. They are situated in similar positions in Va and Vb venoms. The 16–20 kDa band in honey bee venom corresponds with phospholipase A2, the 43 kDa band to hyaluronidase. The three bands in Vespula venom are antigen 5 (25 kDa), phospholipase A_1B (35 kDa) and hyaluronidase (43 kDa) [13]. In Western blot (Fig. 2), IgE binding was observed in three or more patients to protein bands of both venoms at about 15, 24 and 30–35 kDa. The patterns with Va and Vb are similar, but not identical.

Inhibition studies

In four patients (patients 4, 6, 8 and 10) with high IgE to both bee and snake venom, inhibition studies were performed. As shown in Figs 3 and 4, results in patient 6 with up to 33% inhibition of IgE binding to Vb by honey bee venom and vice versa, suggests a partial cross-reactivity between the two venoms. No indication of cross-reactivity was found in patients 4, 8 and 10.

Discussion

All eight patients with a generalized reaction had symptoms typical of an IgE-mediated allergic or of an anaphylactoid reaction, such as urticaria, angioedema or bronchial obstruction. Other less specific symptoms, like arterial hypotension, loss of consciousness, vomiting, abdominal cramps, etc., were also observed in most patients. In seven of eight patients with systemic reactions to snake bites we could clearly demonstrate IgE antibodies to Vipera venom both in skin tests and RAST. All these seven patients had intensive and protracted contact with snakes either professionally or as fanciers, while patient 3, the only one with negative skin tests and specific IgE, was a vintager with no professional snake contacts who had only been bitten once by Va. The detection of snake venom-specific IgE in both serum and skin tests together with a history of symptoms typical for an IgE-mediated reaction in seven of eight patients strongly suggests an IgE-mediated pathogenesis of the reaction in these seven patients. The observation of patient 3 with angioedema and dyspnea after a Vipera bite, who had no

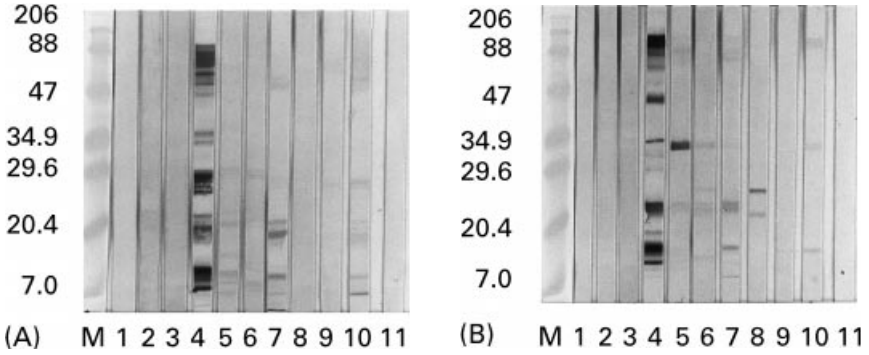

Fig. 2. Western blot with venoms of (a) *Vipera aspis* and (b) *Vipera berus* on the right. M = markers, 1–10 snake-bitten patients, 11 pool of sera from individuals without snake exposure

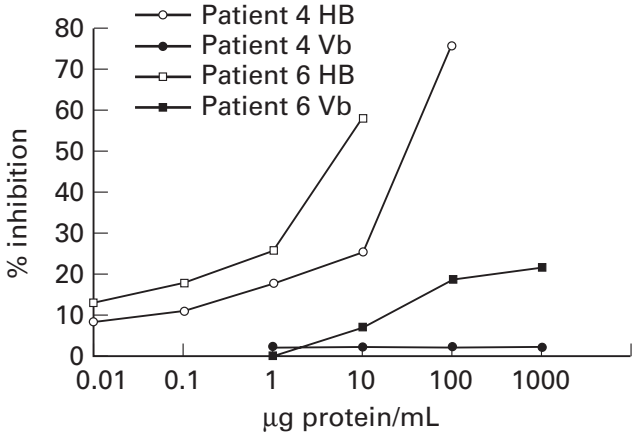

Fig. 3. Inhibition of IgE binding to honey bee venom discs by absorption of sera from patients 4 and 6 with venoms of the honey bee (HB) or *Vipera berus* (Vb)

detectable venom-specific IgE indicates that such symptoms may be non-IgE-mediated or anaphylactoid, e.g. involving complement activation or the release of bradykinin due to snake venom toxins [1].

All patients reacted similarly to the venoms of Va and Vb both in skin test and RAST, and the titres of specific IgE to the two venoms were closely correlated, suggesting extensive cross-reactivity. In Western blot IgE binding was observed in six of the seven patients with positive skin tests. A considerable number of IgE binding fractions between 7 and 100 kDa was observed in both Va and Vb venoms. Fractions which bound IgE from three or more, up to six patients, indicating a major allergen were observed in both venoms, although the patterns were not identical as could be expected in two different species.

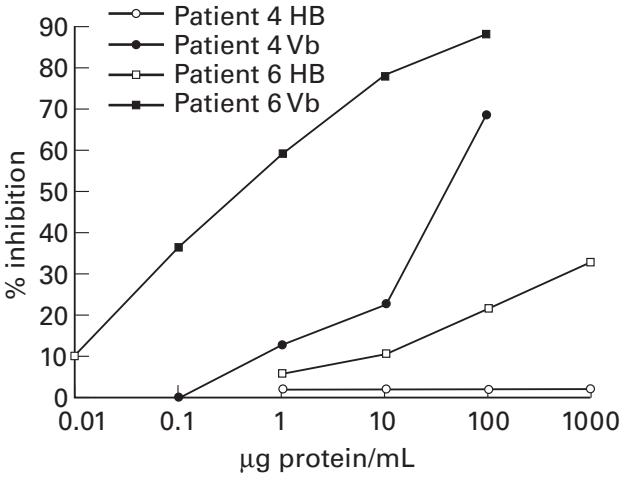

Fig. 4. Inhibition of IgE binding to *Vipera berus* venom discs by absorption of sera from patients 4 and 6 with venoms of the honey bee (HB) or Vipera berus (Vb)

In the literature there are occasional reports suggesting IgE-mediated allergy in patients with repeated snake bites [7,8,14,15]. Parish [15] found a positive scratch test in four of 13 patients with repeated snake bites and was able to passively transfer this sensitivity in Prausnitz-Küstner test. Schmutz and Stahel [8] observed seven life-threatening anaphylactoid reactions among 74 snake-bitten individuals which all occurred only after the second or later bites. Diagnostic tests were, however, not done in this study. Kopp *et al*. [7] report on a snake biologist who developed a severe local haemorrhagic oedema, dyspnoea and abdominal pain after his first, angioedema of the face and wheezing after the second bite by Va. The patient had a positive skin prick test with Va venom and Va venom-specific IgE was demonstrated in his serum.

Sensitization to snake venoms may also occur by inhalation or through skin contact. Zozoya [16] was the first to describe a patient who developed symptoms of allergic rhinitis when handling dried snake venom. Sensitivity to the venom was confirmed in a scratch test and could be transferred in Prausnitz Küstner test. Similar case reports have been published by Mendes *et al*. [17] and Wadee and Rabson [9]. Some of these professionally-exposed patients also developed anaphylactic symptoms when bitten by snakes. Our observation that four of the seven snake handlers with IgE-mediated reactions to snake bites were atopic indicates the possibility of sensitization by inhalation. However, none of these four reported symptoms of respiratory allergy when handling vipers.

Four of our eight patients with systemic reactions to snake bites gave a history of an allergic reaction to Hymenoptera stings and positive diagnostic tests were observed in seven of the eight patients. This suggested the possibility of cross-reactions between individual venom proteins of Hymenoptera and snake venoms, since a history of generalized allergic reactions to Hymenoptera stings is only given by 1–5% of the European population and positive diagnostic tests are observed in 10–30% at most [13]. Cross-reactivity could be due to proteins hyaluronidase and phospholipase, which are contained in both Hymenoptera and snake venoms [18,19]. During RAST inhibition studies in four patients with both high specific IgE to Vipera and honey bee venom partial cross-reactivity was detected in one individual only. Together with the observation that none of the 10 Hymenoptera venom-allergic patients had detectable specific IgE to Vipera venoms this indicates, that such cross-reactivity is probably rare. In an occasional patient it could, however, be of clinical relevance.

In conclusion, our results suggest that generalized anaphylactic reactions in patients with repeated snake bites may often be IgE-mediated.

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