Phospholipase A2 Triggers Anaphylaxis to Snake Venom by Repeated Skin Sensitization: A Case Report

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Snake venoms are primarily known for their toxic properties. IgE-mediated systemic reactions to snake venom have been reported in the literature, although their frequency might be underestimated and contribute to deaths from snake bites. Allergic reactions to snake venom have been described following repeated bites [1,2], as well as by sensitization through inhalation [3,4]. Snake handlers are at particular risk of developing IgE-mediated symptoms to snake venom. Medeiros et al [5] reported the prevalence of specific IgE to snake venom to be 10.4% in professional snake handlers and identified contact with dried venom as a major risk factor in their cohort.

We describe a case of anaphylaxis to snake venom following skin sensitization without a bite. An 18-year-old man with no known atopic dermatitis or allergy to insects, no history of anaphylaxis, and no clinical evidence of mastocytosis was admitted to our Toxicology Department with anaphylactic shock following skin contact with snake venom. The patient kept venomous snakes in his apartment and had been milking their venom on a regular basis without wearing protective gloves. During the cleaning of objects used for milking the snakes, the back of the left hand, which had small superficial skin lesions caused by outdoor activities, came into contact with draining tap water. Immediately afterwards, the patient felt a burning sensation on the back of the left hand, followed by shortness of breath and sweating. Upon arrival of the emergency physician, the patient had a Glasgow Coma Scale (GCS) score of 13, systolic blood pressure of 60 mmHg, heart rate of 170 bpm (sinus rhythm), and oxygen saturation

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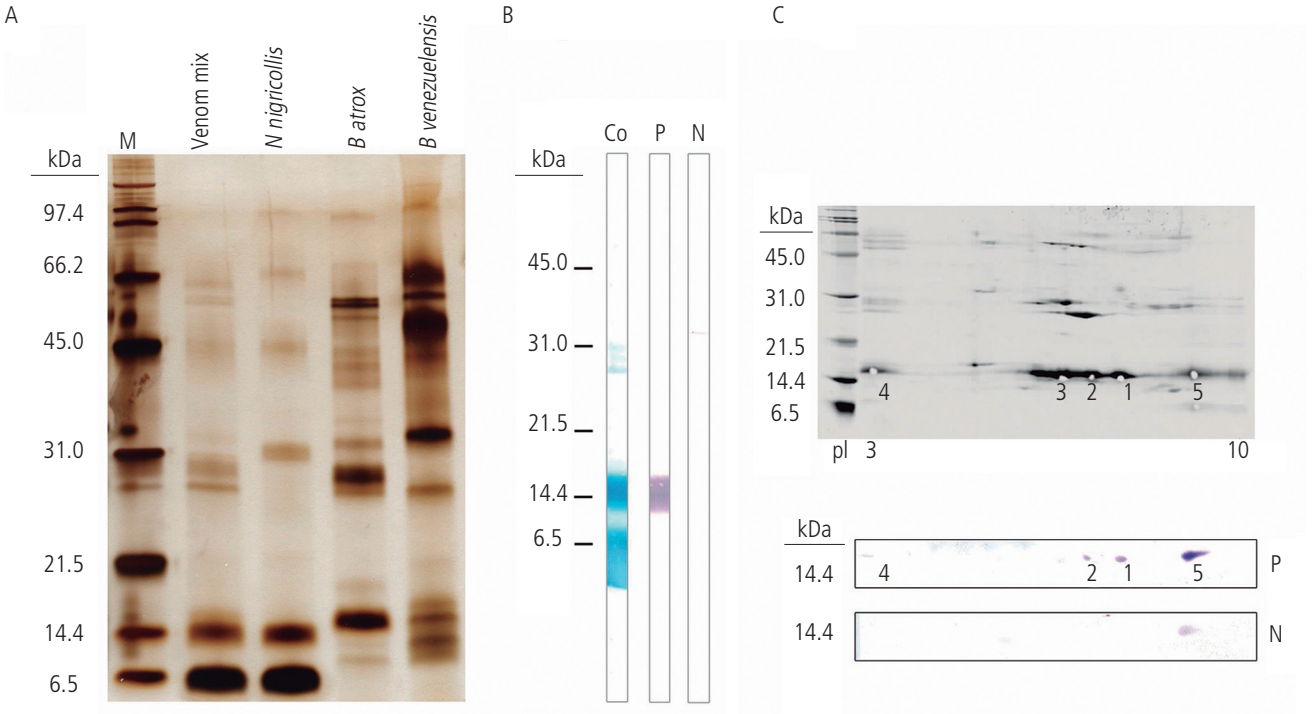

Figure. Snake venom extract containing IgE-reactive proteins. Venom extracts were separated by SDS-PAGE and immunoblotted with patient (P) and negative control serum (N). A, Silver-stained gel. B, IgE-immunoblot using venom mixture. C, 2D SDS-PAGE of venom mixture stained with SyproRuby and immunoblot strips reacting with patient serum (P) and negative control serum (N). The numbers within the panel refer to the spots isolated for mass spectrometry analysis. Co indicates Coomassie staining of the immunoblotted venom mixture.

of 60%. He was quickly intubated owing to laryngeal edema with respiratory distress, and 500 mg of prednisolone, 4 mg of dimetindene maleate, and norepinephrine were administered intravenously. The patient was transported to our center in a rescue helicopter.

At admission, he had a GCS score of 3, and his blood pressure was 110/80 mmHg, heart rate 95 bpm, and oxygen saturation 100% at an FiO_2 of 0.6. No antivenin was administered owing to minimal changes in clotting and clinically irrelevant swelling of the hand. The laryngeal edema improved rapidly with continuation of antiallergic therapy (250 mg prednisolone, 4 mg dimetindene), and the patient was extubated on the day of admission. Increasing necrosis observed on the back of the hand was surgically debrided. Seven days later, a full-thickness skin graft was performed with split skin from the forearm. The reason for the generation of necrosis with a putative minimal amount of venom is unclear, although it may have been favored by the superficial skin lesions present on the patient's hand.

Serum tryptase level, total IgE, and specific IgE to cross-reactive carbohydrate determinant (CCD) and insect venom were measured at admission. The snake venom responsible for the anaphylaxis reportedly contained a mixture of venoms from *Naja nigricollis* (black-necked spitting cobra), *Bothrops atrox*, and *Bothrops venezuelensis* (lanceheads). The venom mixture and single venoms were analyzed using SDS-PAGE, immunoblot, and ELISA (Figure; Table 1, Online Repository) as described elsewhere [6]. Venom proteins were analyzed using 2D-gel electrophoresis, and immunoreactive spots were identified by mass spectrometry.

The plasma tryptase concentration at admission was high (49 µg/L, normal value, <11.4 µg/L) and returned to normal 2 days after the event, thus corroborating the diagnosis of anaphylactic shock. The total IgE titer was 1106 kU/L, specific IgE to CCD (MUXF3) was 0.20 kU/L, and titers for available insect venoms (*Apis mellifera* and *Vespula vulgaris*) were very low (0.24 kU/L, resp. 0.51 kU/L), thus excluding possible cross-reactivity to CCD or proteins from hymenoptera venoms. ELISA of specific IgE directed at the snake venom mixture revealed a highly elevated titer (>100 kU/L), thus further confirming the diagnosis of allergic reaction to snake venom components (Table 1, Online Repository).

Following the analysis of snake venom by 2D gel electrophoresis and immunoblot, several protein spots of approximately 16 kDa were found to be immunoreactive with the patient's IgE antibodies (Figure, C). MALDI-TOF analysis identified these spots as phospholipase A2 (PLA2) from *Naja nigricollis, Naja mossambica*, and *Bothrops asper* (Table 2, Online Repository).

The Uniprot and NCBI Protein databases contain only 1 sequence for *Naja nigricollis* phospholipase A2, although isoenzymes might be present in the venom of this species. In fact, several PLA2 isoenzymes have been reported in the venom of a single species of *Naja* [7]. Sequences of PLA2 from other species listed in the databases might therefore better match the results from the proteomics analysis, leading

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to the inconsistency between the listed species and those actually handled.

The specific IgE titer for the major bee venom allergen PLA2 (Api m 1) was <0.1 kU/L, thus excluding cross-reactivity with Api m 1 in the present case. In addition, both phospholipases have a very low sequence identity of 11% (Figure 1, Online Repository). The calcium-binding site of PLA2 (Y_{28}CGXXGXGXXXDDLDRCCQXHXXC51) is highly conserved in snake species [7], although only a few residues align on the calcium-binding site of *Apis mellifera* PLA2 (Fig. 1, Online Repository). PLA2 and PLA1 are known allergens of bees, bumble bees, hornets, wasps, yellow jackets, and fire ants [8,9]. Recently, crotoxin, a rattle snake neurotoxin, was identified as an allergen in a case of occupational respiratory allergy to rattlesnake venom [4]. Crotoxin is a heterodimeric protein formed by a nontoxic acidic subunit and a basic subunit, which has PLA2 activity. The identification of PLA2 as a snake venom allergen in cobra points to the high allergenic potential of this protein family.

Several cases of allergic symptoms such as allergic rhinitis, conjunctivitis, wheezing, and asthma have been described in professional snake handlers who come into contact with dried venom [3,4]. One case report described the occurrence of local urticaria at sites of skin contact with rinkhals cobra venom (*Hemachatus haemachatus*), followed by increasing systemic symptoms, shortness of breath, and dizziness on later occasions [10]. To the best of our knowledge, this is the first report on severe anaphylaxis caused by exposure of the skin to snake venom. IgE-reactive proteins in snake venom could be identified as PLA2.

We describe a case of anaphylaxis to snake venom following skin sensitization without a bite. The culprit allergen was identified as PLA2. Phospholipases are major allergens of hymenoptera venoms, although given the low sequence identities between snake and hymenoptera PLA2, a potential risk of cross-reaction is unlikely. Our report aims to draw attention to allergic reactions to snake venom, which might be a critical yet underestimated factor in patient management.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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SUPPLEMENTARY MATERIAL

Table 1. Tryptase level, total IgE and specific IgE titers of the patient's serum from the day of anaphylaxis.

Tryptase level	49 µg/L
Total IgE	1106 kU/L
sIgE *Apis mellifera* (native) Api m 1 Api m 2 Api m 3 Api m 5 Api m 10	0.24 kU/L <0.1 kU/L <0.1 kU/L 0.11 kU/L <0.1 kU/L <0.1 kU/L
sIgE*Vespula vulgaris* (native) Ves v 1 Ves v 5	0.51 kU/L 0.13 kU/L 0.6 kU/L
CCD (HRP) CCD (MUXF3)	0.24 kU/L 0.20 kU/L
sIgE snake venom mix (ELISA)	> 100 kU/L

Normal tryptase levels are < 11.4 µg/L, normal total IgE titers are < 150 kU/L, normal sIgE titer are < 0.1 kU/L. sIgE titer to snake venom was measured by using a standard curve of serial dilutions of a patient serum with a known sIgE titer to cat serum albumin.

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Table 2. Identification of immuno-reactive spots from 2D analysis by mass spectrometry (MALDI-TOF-TOF; database SwissProt 2017_04.

Spot	ID	Accession number	Number of peptides identified
1	Basic phospholipase A2 CM-II OS=*Naja mossambica*	P00603	4
2	Basic phospholipase A2 CM-II OS=*Naja mossambica*	P00603	4
3	Acidic phospholipase A2 CM-I OS=*Naja mossambica*	P00602	5
4	Phospholipase A2 "basic" OS=*Najanigricollis* Basic phospholipase A2 homolog 2 OS=*Bothrops asper* Acidic phospholipase A2 CM-I OS=*Naja mossambica*	P00605 P24605 P00602	4 1 2
5	Phospholipase A2 "basic" OS=*Najanigricollis*	P00605	1

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Figure 1.

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P00605_NAJNG PLA2  1   NLYQFKNMIHCTVPSRPWWHFADYGCYCGRGGKGTPVDDLDRCCQVHDNCYEKAGKMGCWPYLT------LYKYKCSQGK 74
P00630_APIME PLA2  1   ----------IIYPGTLWCGHGNKSSGPNELGR---FKHTDACCRTHDMCPDVMSAGESKHGLTNTASHTRLSCDCDDKF 67

P00605_NAJNG PLA2  75  LTCSGGNSKCGAAVCN----CDLVAANCFAGARYIDAN----------YNIN---------FKKRCQ 118
P00630_APIME PLA2  68  YDCLKNSADTISSYFVGKMYFNLIDTKCYKLEHPVTGCGERTEGRCLHYTVDKSKPKVYQWFDLRKY 134
```

Alignment of the protein sequence of PLA2 from snake *Naja nigricollis*(Uniprot accession number P00605) and honey bee *Apis mellifera*, Api m 1(Uniprot accesion number P00630). Identical residues are highlighted in dark grey, similar residues are highlighted in light grey (Clustal W program). The box highlights the calcium binding site of snake PLA2 as described[1].

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