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

Swiontek K[1], Planchon S[2], Ollert M[1,3], Eyer F[4], Fischer J[5], Hilger C[1].

[1]Department of Infection and Immunity, Luxembourg Institute of Health (LIH), Esch-sur-Alzette, Luxembourg

[2]Green Tech Platform, Luxembourg Institute of Science and Technology (LIST), Esch-sur-Alzette, Luxembourg

[3]Department of Dermatology and Allergy Center, Odense Research Center for Anaphylaxis, University of Southern Denmark, Odense, Denmark

[4]Department of Clinical Toxicology and Poison Control Center Munich, Technical University of Munich, Munich, Germany

[5]Department of Dermatology, Eberhard Karls University, Tuebingen, Germany

Corresponding author

Christiane Hilger, PhD

Department of Infection and Immunity

Luxembourg Institute of Health

29, rue Henri Koch

L-4354 Esch-sur-Alzette, Luxembourg

E-mail: christiane.hilger@lih.lu

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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, but might be underestimated and contribute to fatalities after snake bites. Allergic reactions to snake venom have been described following repeated snake bites [1,2], as well as by sensitization through inhalation [3,4].In particular, snake handlers are at risk of developing IgE-mediated symptoms to snake venom. Medeiros et al. reported an IgE prevalence to snake venom of 10.4% in professional snake handlers with manipulation of dried venom being a major risk factor identified in their cohort [5].

We describe a case of anaphylaxis to snake venom following skin sensitization without bite.An 18-year old male patient without known atopic dermatitis or allergy to insects, no history of anaphylaxis and lack of clinical evidence for mastocytosiswas admitted to the Toxicological Department presenting 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, local contact between the back of the left hand,which had small superficial skin lesions caused by outdoor activities, and the draining tap water occurred. 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 GCS-score of 13, systolic blood pressure was 60 mmHg, heart rate 170 beats/min (sinus rhythm) and oxygen saturation 60%. Due to laryngeal swelling with respiratory distress a rapid sequence intubation was performed, additionally 500 mg prednisolone, 4 mg dimetindene maleate and norepinephrine were administered intravenously and the patient was transported with a rescue helicopter to our center.

At admission he had a GCS of 3, blood pressure 110/80 mmHg, heart rate 95/min, saturation 100% at FiO2 of 0.6. No antivenin was administered due to minimal changes in clottingand clinically irrelevant swelling of

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the hand. With continuation of antiallergic therapy (250 mg prednisolone, 4 mg dimetindene)the laryngeal swelling improved rapidly so that the patient could be extubated on the day of admission.In the area of the back of the left hand an increasing necrosis was observed, which was debrided surgically. After wound debridement, a full-thickness skin graft with split skin from the forearm was performed seven days after admission. The reason for the generation of a necrosis with a putative minimal amount of venom is unclear, but may have been favoured by the superficial skin lesions present on the patient's hand.

Tryptase level, total IgE, specific IgE to CCD (cross-reactive carbohydrate determinants) and insect venoms were measured in patient's serum at hospital admission . The snake venom responsible for the anaphylaxis reportedly contained a mixture of venoms from *Naja nigricollis*(black-necked spitting cobra),*Bothropsatrox* and *Bothrops venezuelensis* (lanceheads). The venom mixture and single venoms were analyzed by SDS-PAGE, immunoblot and ELISA (Figure1; table 1 Online Repository) as described [6]. Venom proteins were resolved by 2D-gel electrophoresis and immunoreactive spots were identified by mass spectrometry.

Plasma tryptase concentration at admission was high with 49 μg/L (normal < 11.4 μg/L), and was normal with 3.02 μg/L two days after the event, corroborating the diagnosis of anaphylactic shock. Total IgE titerwas 1106 kU/L,specific IgE to CCD (MUXF3, 0.20 kU/L) and available venoms from insects, namely *Apis mellifera* and *Vespula vulgaris*(0.24 kU/L, resp. 0.51 kU/L), were very low, excluding a possible cross-reactivity to CCD or proteins from Hymenoptera venoms. Quantification by ELISA of specific IgE directed at the snake venom mixture showed a highly elevated titer (> 100 kU/L), which further confirms the diagnosis of allergic reaction to snake venom components (Table 1 Online Repository).

Following the resolution of snake venom by 2D-gel electrophoresis and immunoblot, several protein spots of approximately 16kDa were found to be immunoreactive with the patient's IgE antibodies (Fig. 1C). MALDI-TOF analysis identified these spots as phospholipase A2 (PLA2) from *Naja nigricollis*, *Naja mossambica* and *Bothrops asper*(Table 2 Online Repository).

The databases Uniprot and NCBI Protein contain only one sequence for *Naja nigricollis* phospholipase A2, but isoenzymes might exist in the venom of this species. In fact, several PLA2 isoenzymes have been

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described in the venom of a single species of *Naja* [7]. Sequences of phospholipases A2 from other species listed in the databases might therefore match better the results from the proteomics analysis, leading to the inconsistency between the listed and the actually handled species.

Specific IgE titer to Api m 1, the bee venom PLA2, was <0.1 kU/L, excluding any cross-reactivity with Api m 1 in our case. In addition, both phospholipases have a very low sequence identity of 11% (Fig.1 Online Repository). The calcium binding site of PLA2 (Y_{28}CGXXGXGXXXDDLDRCCQXHXXC_{51})is highly conserved among snake species [7], but only a few residues align on the calcium binding site of *Apis mellifera* PLA2 (Fig. 1 Online Repository). Phospholipases A2 and A1 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 allergen in a case of occupational respiratory allergy to rattlesnake venom [4]. Crotoxin is a heterodimeric protein formed by a non-toxic acidic subunit and a basic subunit, which has PLA2 activity. The identification of PLA2 as snake venom allergen in cobra suggests a 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 manipulating dried venom [3,4]. One case report described the occurrence of local urticaria at sites of skin contact with ringhals 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 snake venom skin exposure. IgE-reactive proteins in snake venom could be identified as PLA2.

We describe a case of anaphylaxis to snake venom following skin sensitization without bite. The culprit allergen was identified as PLA2.Phospholipases are major allergens of hymenoptera venoms, but due to low sequence identities between snake and hymenoptera PLA2s, a potential risk of cross-reactions is unlikely.Our report aims at drawing attention to allergic reactions to snake venom, which might be a critical and underestimated factor in patientmanagement.

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Informed consent

Informed consent was obtained from the patient for this study.

Poster

Data were presented in a poster session at the ISMA 2019 in Amsterdam.

Conflict of interest

None of the authors reports a conflict of interest related to this study.

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Figure 1. Snake venom extract contains IgE-reactive proteins.

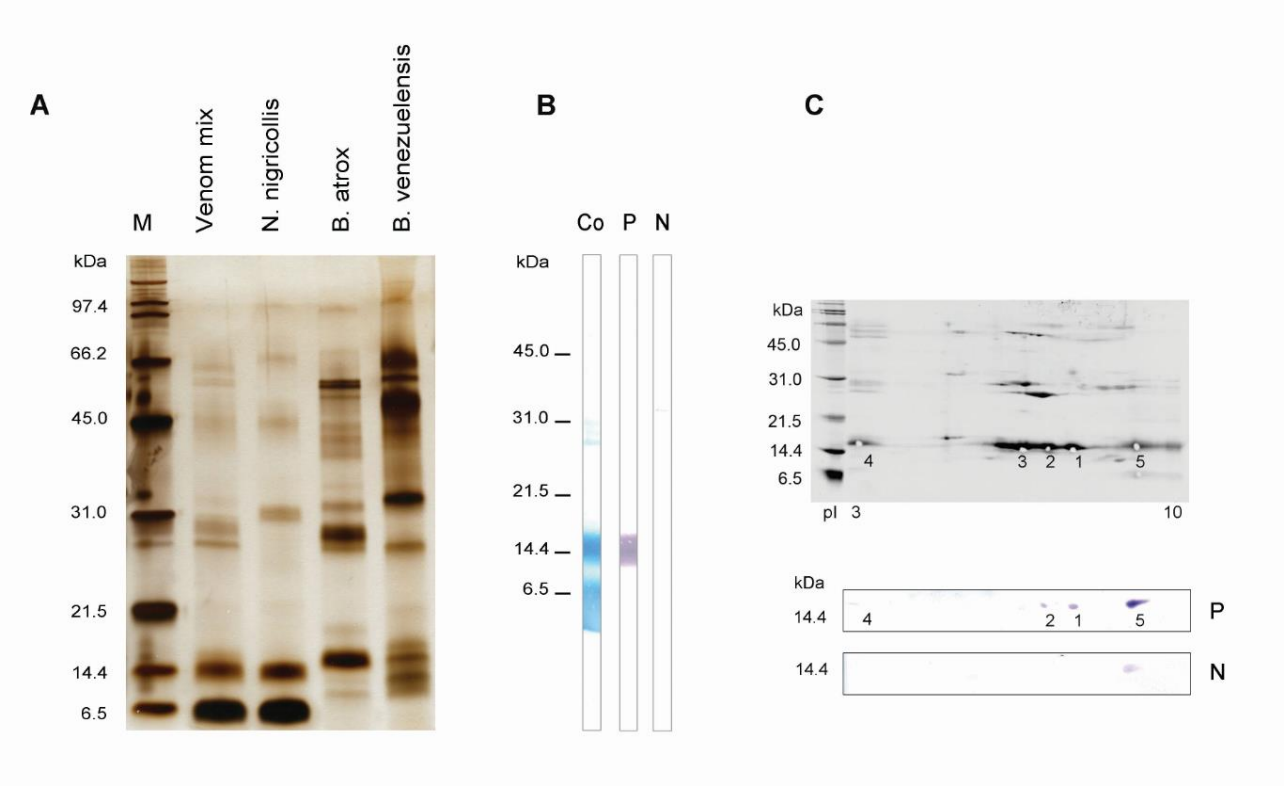

Figure legend: Venom extracts were separated by SDS-PAGE and immunoblotted with patient (P) and negative control serum (N). (A) silver stained gel, (B) IgE-immunoblot using venom mixture, (C) 2D SDS-PAGE of venom mixture stained with SyproRuby and immunoblot strips reacted with patient serum (P) and negative control serum (N). Co, Coomassie staining of immunoblotted venom mixture; numbers refer to spots isolated for MS analysis.