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Categorization of venoms according to bonding properties: An immunological overview

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ABSTRACT

In this report, we present a study on the antigenic cross-reactivity of various venoms from the most dangerous Egyptian snakes and scorpions belonging to families Elapidae, Viperidae and Buthidae. The study was carried out with special reference to bonding properties between venoms and antivenoms and their involvement in the formation of specific and/or cross-reactive interactions. The homologous polyclonal antivenoms showed high reactivity to the respective venoms and cross-reacted with varying degrees to other non-homologous venoms. Assorting the antivenoms according to their susceptibility to dissociation by different concentrations of NH_4SCN revealed that most of the antibodies involved in homologous venom–antivenom interactions were highly avid; building up strong venom–antivenom bonding. Whereas cross-reactions due to heterologous interactions were mediated by less avid antibodies that ultimately led to the formation of venom–antivenom bonding of different power strengths depending on the antigenic similarity and hence on the phylogenetic relationship of the tested venom. A new parameter evaluating high and low avid interactions, designated as H/L value, for each antigen-antibody bonding was initiated and used as an indicator of bonding strength between different venom–antivenom partners. H/L values were many folds higher than 1 for homologous and closely related venoms, 1 or around 1 for cross-reactive venoms, whereas venoms from unrelated remote sources recorded H/L values far less than 1. Using well defined polyclonal antivenoms, H/L value was successfully used to assign eight unknown venoms to their animal families and the results were confirmed by species-specific ELISA and immunoblotting assays.

1. Introduction

Egypt has a collection of potentially dangerous venomous animals that inhabit rural areas in Eastern, Western and Sinai deserts as well as Nile Delta and Valley (Saleh, 1997; El-Hennawy, 2002). Both snake bites and scorpion stings are among the most important causes of envenoming and are responsible for significant morbidity and mortality (Fatani, 2014). Acute poisoning cases due to natural venomous creatures were estimated to be 733 admitted to one health facility (Poison Control Centre—Ain Shams University) during 12 months (Gamaluddin, 2004), with the snake and scorpions accidents were the most dangerous and life threatening. The burden of the problem is much heavier in deserts, country sides and other remote districts where the chances of morbidity and mortality being missed are perhaps even greater and medical care is minimal.

Unless obviously recognized, the bites and stings of the various venomous animals are sometimes undistinguishable especially when people are accidentally envenomed while being asleep at night. Signs and symptoms may not be clear enough to support particular antivenom. This happened in a clinical case in USA, where initial diagnosis suggested anaphylaxis due to a bee sting. Then after, serological tests using ELISA revealed that the animal involved was really a snake and not a bee (Banner, 1988). Moreover, the recognition of local tissue damage caused by bites of African spitting cobras, *Naja nigricollis* and *Naja mossambica,* was often delayed because many patients presenting with this sign were misdiagnosed as being bitten by *Bitis arientans* (Ho et al., 1986). The difficulty in discerning between elapid and viperid venoms in this case justified the preparation of polyspecific antivenom that could neutralize venoms from both families (Gutiérrez et al., 2005, 2011).

Classification of venoms within the respective family is an important issue for taxonomic analysis (Al Asmari et al., 2014)

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and also to provide insights of trends within venom components (Graham et al., 2008) for biomedical relevant investigation. Identification of the offending animal is critical in emergency treatment of animal envenomings (Selvanayagam and Gopalakrishnakone, 1999). False diagnosis could result in potentially fatal outcome (Banner, 1988; Fry et al., 2001).

Snake and scorpion venoms, described as mosaic of antigens, constitute a diverse cocktail of proteins and bioactive peptides as well as other small molecules of different proportions (Stocker, 1990; Abdel-Rahman et al., 2010). Many antigenic components are shared among closely related species and antivenoms cross-react along them (Berger and Bhatti, 1989). These similarities appear to be high at the species level (Wahby and Ibrahim, 2008; Ibrahim et al., 2013), little at the family level (Dong Ie et al., 2003; Ibrahim and Wahby, 2010) and become fewer and fewer among animals of diverse sources (Lipps and Khan, 2000). Antigenic cross-reactivity among venoms is mainly assessed by reaction of antivenoms with homologous and heterologous venoms in different immunoasssays (Higashi et al., 1995; Lipps amd Khan, 2000; Prieto da Silva et al., 2001; Ibrahim and Wahby, 2010). However, the nature of cross-reactive bonding, to what extent is it different from specific bonding regarding stability; affinity and ability to disrupt under different conditions are not well studied.

Polyclonal antibodies induced in response to invasion by complex antigens are heterogeneous with respect to both specificity and affinity. In addition to antibodies raised towards the different epitopes on the antigen, each epitope could induce the production of a collection of antibodies with a range of affinities (Mak and Saunders, 2006). Specific interactions between epitopes and paratopes are a result of attractive non-covalent forces; Van der Waal, hydrogen-bonding, hydrophobic and electrostatic, all of which are weak forces but combine to forge a very tight bond. Overall strength of binding depends on the goodness of fit between the two partners and their total contact area. The more complementary the shapes of the antigenic epitope and paratope, the more contact sites will simultaneously be brought into close proximity, increasing the number of non-covalent bonds of all types and resulting in a stronger overall binding (Reverberi and Reverberi, 2007; Mak and Saunders, 2006). However, binding of antigens and antibodies is fully reversible and liable to dissociate by the application of high salt concentration, non-physiological pH, detergents and chaotropic agents, at a rate that depends on the resistance and number of the binding forces being disrupted. Dimitrov et al. (2011) demonstrated that different chaotropic agents having different abilities to dissociate immune complexes and those capable of disturbing both polar and non-polar interactions are more suitable for elution of immune complexes and determination of avidity.

In a previous report we studied the immunological cross-reactivity among the venoms of snakes and scorpions belonging to families Elapidae, Viperidae and Buthidae in different immunassays (Ibrahim and Wahby, 2010). The common antigenic components responsible for cross-reactivity were also detected. In the present study, the cross-reactivity extents were evaluated in the scope of apparent avidities of three polyclonal antivenoms; anti-elapid, anti-viperid and anti-scorpion, against nine different snake and scorpion venoms. The diversity of bonding between antigens and antibodies and the subsequent contribution to the formation of specific and/or cross-reactive interactions were discussed. Moreover, a novel parameter evaluating the ratio of the high and low avid interactions, designated as H/L value, for each venom–antivenom couple was created and used as an indicator of bonding strength of a venom to a certain antivenom and hence to categorize a venom under specified animal family.

2. Materials and methods

2.1. Snake venoms

The venoms were obtained from adult elapid snakes; *Naja haje*, *N. nigricollis and Walterinnessia aegyptia* ($n = 1–4$) and viperid snakes; *Cerastes vipera*, *Echis carinatus* and *Pseudoserastes fieldi* ($n = 30–36$ except for *P. fieldi* $n = 2$). The animals were collected by experts from their natural habitat and kept in captivity. The venoms of each species were milked by trained individuals by manual compression of the venom glands, pooled, centrifuged and the supernatants were lyophilized and stored at −20 °C until used.

2.2. Scorpion venoms

Scorpions of *Leiurus quinquestriatus*, *Androctonus australis*, and *Androctonus amoreuxi* ($n = 100–200$) were collected and identified by experts before extraction of the venom. The venom of each species was extracted by well-trained individuals using electrical stimulation, dissolved in distilled water, centrifuged and the supernatants were lyophilized and kept at −20 °C until used.

2.3. Anti-venom antisera

Monovalent antivenoms to snake venoms were raised in rabbits according to the following method: New Zealand rabbits (2–3 kg) were injected individually with graded doses of venoms (from 25 to 50 μg/0.5 ml saline) at two week intervals. The first dose was emulsified in Freund's complete adjuvant while subsequent booster doses were delivered in Freund's incomplete adjuvant. Animals were bled 7 days after each booster injection from the ear vein. The blood was incubated at 37 °C for 2 h then placed overnight at 4 °C in the refrigerator. The serum was separated from blood cells by low speed centrifugation (2000 × *g*) for 10 min at room temp. Equal volumes of monovalent antivenoms against *N. haje, N. nigricollis and W. aegyptia* were pooled and considered as polyvalent anti-elapid antivenom; while a pool of monovalent antivenoms against *C. vipera, E. carinatus* and *P. fieldi* were considered as a polyvalent anti-viperid antivenom. Polyvalent scorpion antivenom was purchased from the Egyptian organization for biological products and vaccines. It was prepared from purified plasma of healthy horses that have been immunized against the most dangerous scorpions.

Previously prepared species specific antivenoms against one elapid venom, *N. haje*, and two viperid venoms, *C. cerastes* and *E. coloratus*, were used for confirmatory tests. They were prepared by affinity chromatography according to Wahby et al. (2005).

2.4. Indirect ELISA

The indirect ELISA was performed according to Theakston et al. (1977) with minor modifications. Microtiter plates (Dynatech) were coated overnight at 4° C with 100 μl of a constant concentration (5 μg/ml) of each venom in the coating buffer (5 mM carbonate buffer pH 9.6). The plates were washed 3 times with the PBS-T buffer (100 mM PBS pH 7.5 containing 0.05% Tween 20) and incubated for 1 h at 37° C with 150 μl/well of the blocking buffer (PBS containing 2% gelatin). After wash, a defined concentration of the antivenoms under test (100 μl, dilution 1:5000 for the polyvalent antivenoms or 2 μg/ml for the species-specific antivenoms) was dispensed into different venom-coated wells and binding to the venoms was allowed for 1 h at 37° C. After another washing cycle, the appropriate horseradish peroxidase (HRPO) conjugate (either rabbit anti-horse HRPO conjugate for the anti-scorpion antisera or goat anti-rabbit HRPO conjugate for the anti-snake antisera, dilution 1:3000) was added (100 μl) and incubation was allowed at 37 °C for 1 h. The plates were washed for 3–5 times with PBS-T