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# MANIPULATION OF THE COMPLEMENT SYSTEM BY ANIMAL VENOMS

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*Interaction of Snake Venoms With Complement (C) Components.* — Poisonous snakes belong to four families: ELAPIDAE, CROTALIDAE, VIPERIDAE, and HYDROPHYDAE, and their venoms contain a vast number of substances with different biochemical and pharmacological activities. The venom from some members of the ELAPIDAE family contains a 144 KDa glycoprotein, designated cobra venom factor (COF) (H.J. Müller-Eberhard and K.E. Fajëllstrom, J. Immunol. 107: 1666-1672, 1971). This protein interacts with factor B in the presence of $Mg^{++}$, the resulting COF-B being transformed into the enzymatically active complex COF-Bb upon cleavage of bound B by factor D (N.R. Cooper, J. Exp. Med. 137: 451-460, 1973). COF-Bb cleaves C3 in C3a and C3b fragments. C3a is released into the fluid phase (W. Dias da Silva and I.H. Lepow, J. Exp. Med., 125: 921-946, 1967), while C3b binds to the complex, the resulting COF-BbC3b enzyme being able to form the C5b-C9 cytolytic complex. (Pickering et al., Proc. Nat. Acad. Sci. USA 62: 521, 1969; Miyama et al., Biken's J. 18: 193, 1975). Related COF protein has not been found in venoms from Brazilian crotalids but their sera contain a serum protein immunochemically related with human C3 (W. Dias da Silva et al., Acta Pathol. Microbiol. Scand, Sect. C: Supll. 284: 97, 1984).

In this communication we are showing that normal human serum (NHS) loses, its hemolytic activity upon incubation with *Micrurus (M. ibiboboca* and *M. spixii), Bothrops (B. jararaca, B. moojeni* and *B. cotiara*) and *Naja (N. melanoleuca* and *N. nigricollis*) but not with *Crotalus (C. durissus terrificus*) venoms, as evaluated by the capacity of the serum samples pretreated with the venoms to lyse sheep red blood cells (Es) optimally sensitized with rabbit serum (A) anti-Es (EsA). Venom from *N. naja*, cleaves C3 in NHS in presence of 5$\mu$M $Mg^{++}$ even in absence of $Ca^{++}$ ions by adding 10$\mu$M EGTA. In contrast, venoms from *M. ibiboboca, N. nigricollis*, and *M. spixii*, only cleave C3 in NHS in the presence of $Ca^{++}$ ions. C consumption and C3 cleavage induced by venom from *N. melanoleuca* apparently was due to a mechanism similar to that described for *N. naja*, since a COF-like molecule was detected in that venom by Western-blot analyses, using as probe monospecific antisera against COF or human C3. Equivalent molecules were not detected in the venoms from the other snakes analysed. These observations indicate that, as venom from *N. naja*, venom from *N. melanoleuca* also activates C and cleaves C3 through activation of the alternative C pathway (AP).

C3 cleavage in NHS by the venoms from *N. nigricollis, M. ibiboboca and Bothrops* could be due to the presence of proteases, since it was observed that these venoms induce cleavage of purified human C3. Proteolytic enzymes, able to cleave casein, were detected in *Bothrops* and, with lesser intensity, in venom from *C. durissus terrificus*. When gelatin was included in a 10% polyacrylamide gel, several very large and 3-4 faint bands with proteolytic activity were detected in *Bothrops* and in *C. durissus terrificus* venoms, respectively, after the electrophoretic separation. Venoms from genera *Micrurus* and *Naja* were unable to

cleave both, casein and gelatin. Venoms from *M. spixii* and *N. nigricollis* had some thrombin-like activity.

In summary: C consumption induced by snake venoms in NHS are due to; a) COF, a glycoprotein detected earlier in venom from *N. naja* and in the present studies in the venom from *N. melanoleuca*, that interacts with factor B in the presence of $Mg^{++}$ ions to generate the complex COF-B potentialy able to be transformed into the C3 convertase COF-Bb, upon cleavage of B in Bb and Ba by factor D, the enzyme responsible for the C3 conversion into C3a and C3b; b) proteolytic enzymes capable of cleaving directly C3 and maybe other C components are present in the venoms from *Bothrops, M. ibiboboca* and *N. nigricollis; c) thrombin-like factors described in the venoms from M. spixii* and *M. nigricollis* may interact with C3 leading the formation of the C5b-C9 complex, as early studies have suggested to occur with human thrombin (M.J. Polley, J. Exp. Med., 150: 633-645 (1979).

*Interaction of Spider Venom with C Components*: — spiders of the genera *Loxosceles, Phoneutria, Lactrodectus and Grammostola* are largely distributed in South, Central, and North America as well as in the Mediterranean area and Europe. These genera include many species throughout the world, some of them being medically relevant due to the frequency with which the casualty may occur and to the complications which may follow. *Phoneutria's* bite is very painful; loxoscelic poison has proteolytic and hemolytic activities causing large tissues necrosis and hemolysis; *Lactrodectus* causes muscular contractures, violent abdominal colic pain and also intense generalized pain (J.H. Correa Rodrigues et al., Revista HCPA, 6: 91-96, 1986). *Loxosceles* venom appears to be composed of at least eight different proteins, mostly enzymes (B. Elpert et al., Fed. Proc. 223, 1973, Abstract; J. Finke et al., Annual Meeting of The Am. Soc. Mic. p. 97, 1073). The direct lytic action of this venom on red blood cells has been attributed to the sphingomielinase D (L.J. Forrester et al., Arch. Biochem. Biophys. 187: 355-365 (1978) and its dermonecrotic activity to a 35-33 KDa protein (K. Barbaro et al., Toxicon, 30: 331-338, (1992). ''In vitro'' venom acts directly on cell wall of human strain cells to disrupt its organization causing immediate cell death; ''in vivo'', venom causes a rapid intravascular coagulation and occlusin of small cappilaries followed by necrosis of skin. As the coagulation cascade proceedes, other inflammation mediators are formed, the vascular permeability is increased and platelets are trapped over the endothelial cells (P.C. Anderson et al., Mo. Med. 74: 549-552 (1977). Animals injected in the skin with *L. reclusa* venom showed an immediate and local edema, hemorrhage and necrosis. Based on the analogy of these events with those observed in the classical Arthus phenomenon, it was suggested that the C system could play a role in the induction of the lesions in spiders bite (C.W. Smith et al, Lab. Invest., 22: 90-93, 1970).

In this report we present evidence that venoms from *L. gaucho and Phoneutria* consume, also in a dose — related fashion, human C by activating both the classical (CP) and AP pathways. Evidences for that were the pronounced loss of the capacity of the human serum samples, pretreated with various amounts of venom for 1h at 37º C, to lyse EsA or rabitt blood cells (Er). The biochemical and functional characterization of the molecule(s) involved in this events are in process.

The data presented in this communication together with those described in early studies, should be taken into consideration in the interpretation of the mechanisms involved in the pathogenesis of tissue lesions produced by animal venoms.