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Activation of the complement system and leukocyte recruitment by *Tityus serrulatus* scorpion venom

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Abstract

The scorpion *Tityus serrulatus* is considered one of the most dangerous species in Brazil. Its venom evokes an inflammatory response, although the exact mechanism of this effect is still unknown. The aim of the present study was to investigate the effect of *Tityus serrulatus* venom (TsV) on the complement system (CS) and on leukocyte recruitment.

Complement consumption by TsV was evaluated using in vitro hemolytic assays, immunoelectrophoresis and two-dimensional immunoelectrophoresis of complement components (factor B and C3). In order to evaluate neutrophil migration induced in normal human serum (NHS) in the presence of TsV, in vitro chemotaxis assays were performed using the Boyden chamber model.

In vitro TsV induced a concentration- and time-dependent reduction in hemolytic activity of the classical/lectin and alternative complement pathways, with samples of 43.0 μg and 43.4 μg, respectively, inhibiting 50% of the lytic activity. Alterations in C3 and factor B electrophoretic mobility after incubation of NHS with TsV, were identical to those obtained with zymosan (positive control). Incubation of NHS with TsV induced neutrophil chemotaxis similar to that observed with zymosan-activated serum.

Abbreviations: AP, alternative pathway; CFD, complement fixation diluent; CP, classical pathway; CS, complement system; IC_{50}, venom concentration inhibiting 50% of the lytic activity; NHS, normal human serum; PBS, Phosphate-buffered saline; TEA, triethanolamine; TsV, *Tityus serrulatus* venom.

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Our results show that TsV activates the CS, leading to factor B and C3 cleavage, to reduction of serum lytic activity and generation of complement chemotactic factors. Therefore, CS may play an important role in the inflammatory response observed upon scorpion envenomation.

Keywords: *Tityus serrulatus*; Complement system; Neutrophil chemotaxis; Complement lytic activity; Scorpion venom; Immunoelectrophoresis

1. Introduction

Scorpion sting represents a significant and serious public health problem in certain regions of Brazil, as well as in other parts of the world, due to the frequency of their occurrence and to their potential for inducing severe—even fatal—clinical manifestations, especially among children [1]. In Brazil, most fatalities result from stings received from scorpions of the species *Tityus serrulatus*. The Brazilian Ministry of Health reports approximately 8000 scorpion stings/year, and the mortality rate among children is 1% [2].

Symptoms often displayed by victims of scorpion envenomation are, among others, fever, psychomotor agitation, excessive salivation, lachrymation, increased gastrointestinal tract motility, cardiac/respiratory arrhythmias and arterial hypertension—followed by hypotension, heart failure and shock. These manifestations can be explained by the ability of venom toxins to act on sodium and potassium channels in neuronal terminals, leading to depolarization of axonal membranes and consequent neuromediator release, which stimulates various organs, including the gut, heart and vascular tissue [1]. In the most severe cases, pulmonary edema is common and can be fatal [3]. Pulmonary edema induced by scorpion venom has both cardiogenic and noncardiogenic components. The latter appears to be related to the increased pulmonary vascular permeability that accompanies activation of the inflammatory cascade [4]. Recent results from our group show that leukocytosis is a common finding in scorpion envenomation [5].

Considering the role of the complement system (CS) in inflammation and the inflammatory reaction caused by scorpion stings [6,7], the aim of this study was to investigate the effects of *Tityus serrulatus* venom (TsV) on this system.

The complement system encompasses over 30 proteins, some of which circulate in the plasma as precursors. Depending on the stimulus, complement activation occurs along classical, alternative or lectin pathways (CP, AP and LP, respectively), leading to a cascade of component interactions and generation of products with biological activities such as anaphylaxis, chemotaxis, opsonization, immune complex solubilization, participation in the immune response, etc. After recognition, a series of proteases is activated, culminating in the construction of the "membrane attack complex" (MAC) within the membrane, leading to lysis or cell activation. Two important mediators of the inflammatory reaction, C3a and C5a, are produced as a consequence of CS activation [8].

Despite being a mechanism of protection, complement can cause self injury if not efficiently regulated and has been implicated in a great number of inflammatory and immunological diseases [9].

In a previous study, we showed that injection of TsV in rats affects CP/LP and AP lytic activity [10]. In the present study, we evaluated the capacity of TsV to activate the CS, investigating its in vitro effect on CP/LP and AP lytic activity and on C3 and factor B cleavage, as well as its ability to induce neutrophil chemotaxis. The results are discussed in the context of the role of the CS in the inflammatory reaction and the pathologic process resulting from scorpion stings.

2. Material and methods

2.1. Venom

Tityus serrulatus scorpion venom was purchased from Phoneutria Biotechnology and Services (Belo Horizonte, Brazil) and stored at −20 °C until use. The venom was dissolved in 0.9% (w/v) NaCl and centrifuged at 11270 ×*g* and at room temperature for 5 min. Protein concentration in the clear supernatant was determined using the microbiuret method