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Neutralizing antibodies obtained in a persistent immune response are effective against deleterious effects induced by the *Thalassophryne nattereri* fish venom

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Abstract

Thalassophryne nattereri envenoming represents a great cost to North and Northeast Brazilian communities in terms of public healths, leisure and tourism. Victims rapidally develop symptoms as pain, local swelling, erythema followed by intense necrosis that persist for long days. The aim of this work was tested the immune competence of neutralizing antibodies in pre-immunized mice against principal toxic activities induced by venom. During the primary antibody response in mice, an elevation of IgG antibody levels was only observed on day 28. After boosting, high antibody levels were detected between days 49 and 70, with a 12-fold increase in IgG level over control values at day 49. We confirmed the *in vitro* neutralizing capacity of *T. nattereri* anti-venom against toxic effects and thereafter we show that neutralizing antibodies obtained in a persistent immune response are more effective, inclusive against edematous reaction. After boosting during the secondary response mice with high antibody levels do not present any alterations in venule or arteriole after topical application of venom on cremaster muscle. In addition, CK activity diminished in these mice with high neutralizing antibody levels corroborating the attenuation of the myonecrotic effect by venom. In addition, we determined the presence of high IgG antibodies levels in patients 6 months after injury by *T. nattereri*. In conclusion, the presence of neutralizing antibodies against to *T. nattereri* venom in the serum of pre-immunized mice could change the outcome of lesion at site of posterior envenoming. Antigen-specific antibodies of high affinity in consequence to specific immune response, dependent of *T* lymphocyte activation, could minimize the symptoms of intense and immediate inflammatory reaction caused by *T. nattereri* venom. These finding prompt us to the possibility of development of immune therapeutic strategies using specific anti-venom as an efficient intervention for protecting human victims.

Keywords: Fish venom; *Thalassophryne nattereri*; Local effects; Neutralization; Antigen-specific antibodies of high affinity; Experimental poisoning

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1. Introduction

Thalassophryne nattereri envenoming represents a great cost to North and Northeast Brazilian communities in terms of public healths, leisure and tourism (Fonseca and Lopes-Ferreira, 2000; Haddad Jr. et al., 2003; Faco et al., 2005). *T. nattereri* venomous fish are member of the Batrachoididae family, and in Brazil they are known by severity of accidents that provoke in fishermen and bathers. Victims rapidly develop symptoms as pain, local swelling, erythema followed by intense necrosis that persist for long days (Fonseca and Lopes-Ferreira, 2000). Venom is delivered when the spine pierces the tissue of the victim, the integumentary sheath enclosing the spine and venom disrupted, and the venom injected into the victim (Fróes, 1933).

Experimental studies performed with the *T. nattereri* venom showed that low doses of the venom (0.3 μg/animal) induced local effects as the nociception and edema, similar to that described in humans, independently of the presence of hemorrhagic, phospholipasic A_2 or coagulant activities (Lopes-Ferreira et al., 1998). The histological analysis of the lesion provoked by the venom in the gastrocnemius muscle evidenced acute mionecrosis, presence of thrombi, a scarce infiltrate of polymorphonuclear leukocytes and macrophages, and the skeletical muscle regeneration was partially impaired (Lopes-Ferreira et al., 2001). Recently, local acute inflammatory response induced by the venom was characterized (Lima et al., 2003). Cytokines as TNF-α, IL-1β and IL-6 and a weak leukocyte influx were detected on footpad of mice. Additionally, a cytotoxic effect of the venom on mononuclear cell was also observed.

Most accidents with *T. nattereri* venom occur in the fishing communities and, due to the lack of efficient therapy, victims may take weeks, or even months before returning to work. The immediate treatment for the fish vemon accidents has been to place the wound region in hot water, or administration of local anaesthetics or analgesics, resulting in slight decrease of the symptoms of the envenomation (Sutherland, 1983). The anti-inflammatory drugs used (dexamethasone and indomethacin) are not efficient in reducing the clinical symptoms. In the absence of an appropriate treatment, it is common the occurrence of concomitant bacterial infection and in many cases the local lesion evolve to permanent sequela (Fonseca and Lopes-Ferreira, 2000). In adittion, our loboratory showed recently in a experimental model that nociception and edema induced by the venom were not reduced either by treatment with inhibitors of serotonin and histamine or by non-steroidal and steroidal anti-inflammatory drugs, but only by the administration of kallikrein-specific inhibitor (Lopes-Ferreira et al., 2004).

The capacity of venoms or toxins from venomous marine animals to induce humoral immune responses with neutralizing antibodies has been described previously (White, 1998; Currie, 2003). Evidence supporting the *in vitro* efficacy of *T. nattereri* antivenom was initially demonstrated by Lopes-Ferreira et al. (2000) using the antiserum produced in rabbits. In a recent study, we also reported that lower doses of *T. nattereri* venom mixed to alum for a optimal conditions of T cell activation confer a strong and sustained active humoral immune response in mice (Grund et al., 2006). It is interesting to register that unlike other poisons that can provoke sensitization and shock (bee venoms) the fishermen habitual victims of the *T. nattereri* develop progressive resistance after successive attacks, with gradual decrease of the effect of the venom with minimization of the pain and of the inflammatory effects in the repeated lesions (Auto, 1992).

Since systemic antibody levels could be induced by *T. nattereri* venom, we tested the immune competence of these neutralizing antibodies in pre-immunized mice. Our results showed that the immunization with *T. nattereri* venom induced not only a strong long-lasting B-cell memory against venom antigens but also neutralizing antibodies against the principal toxic effects.

2. Materials and methods

2.1. Patients

Seven patients injured by *T. nattereri* in Maceió (Alagoas State) were observed during 6 months, which allowed the collection of the blood for determination of antibody levels 6 months after accidents.

2.2. Animals and venom

Outbreed Swiss male, weighing 18–22 g were provided by the Butantan Institute and used in accordance with the guidelines provided by the Brazilian College of Animal Experimentation, and