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Short communication

Tityus bahiensis scorpion venom injected to dams during pregnancy affects some cytokines of fetuses

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ABSTRACT

Due to the high incidence of scorpion stings in Brazil, pregnant women are among the possible victims. Cytokines are important during the pregnancy, and scorpion venoms can change their release. We evaluated the levels of some cytokines in the fetuses after the treatment of pregnant rats with the *Tityus bahiensis* scorpion venom. The concentration of some of them is altered and can be responsible for the effects previously observed on innate reflexes, and the physical and behavioral development of the offspring.

Scorpion stings are considered a serious public health problem in Brazil (Cologna et al., 2009). The most important scorpion species found in the country are *Tityus serrulatus*, *Tityus bahiensis*, *Tityus stigmurus* and *Tityus obscurus*, with the first two being the main ones responsible for accidents in humans (Brazilian Ministry of Health 2009; Pardal et al., 2014).

Scorpion stings result in a massive release of neurotransmitters, mainly from the autonomic nervous system (Alves et al., 2005; Teixeira et al., 2001); however, the central nervous system is also affected (Lourenço et al., 2002; Nencioni et al., 2009; Ossanai et al., 2012). The injection of scorpion venom also causes damages to the tissue, which induces a systemic release of mediators that affect inflammatory processes, including kinins, eicosanoids, platelet-activating factor, nitric oxide, and cytokines (Fukuhara et al., 2003; Petricevich, 2010).

Due to the high incidence of scorpion stings, pregnant women are among the possible victims. During pregnancy, the immune system plays an important role ensuring the normal development of the pregnancy, also preventing the development of complications (Kwak-Kim et al., 2005). Cytokines also play a fundamental role in the development of neurons, including the proliferation, survival, differentiation and axo-dendritic growth, and regulation of neuronal synapses (Gilmore et al., 2003).

Patients and experimental animals, when exposed to some scorpion venoms, have an increase on the serum levels of some cytokines (Fukuhara et al., 2003; Petricevich, 2010). On the other hand, it has already been demonstrated that the scorpion venom alters the reproductive performance and physical and behavioral development of rats experimentally treated with scorpion venom during pregnancy (Barão et al., 2008a,b; Dorce et al., 2009, 2010, 2014). Therefore, the purpose of this study was to determine whether there are changes on the cytokine levels in the fetus/placenta unit of dams treated with the venom during pregnancy, and whether these changes may be correlated with the alterations observed in the offspring.

Male (n = 25) and female (n = 75) Wistar rats, weighing 250–270 g, provided by the Central Animal Facility of the Butantan Institute and maintained under a controlled temperature (20 ± 2 °C), with a 12:12 h dark:light schedule and free access to food and water, were used. All the experimental procedures were conducted with prior permission of the Ethics Committee for Experiments on Animals of the institution (Protocol No. 513/08).

The dried venom was dissolved in 1.46% NaCl before use (the

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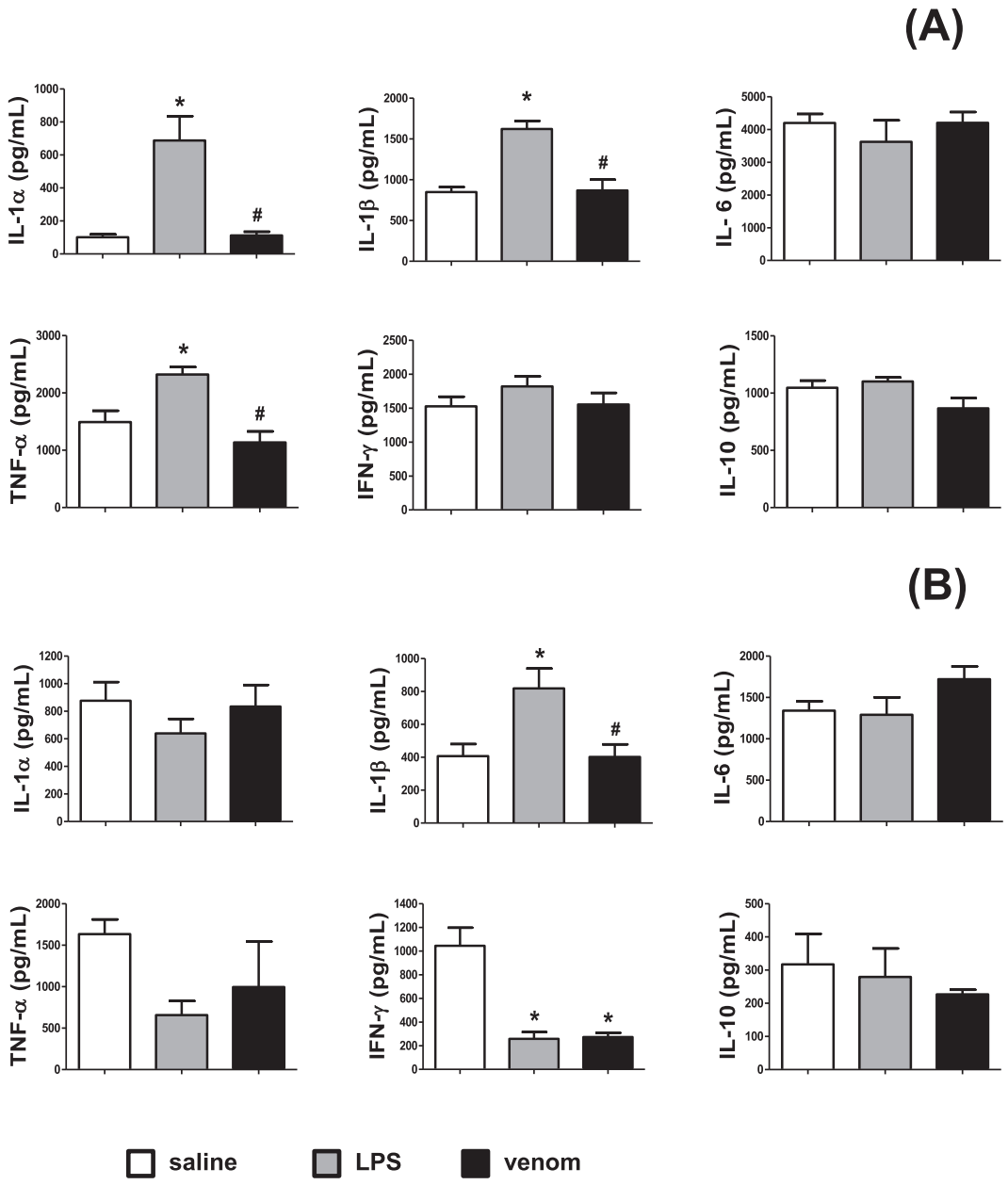

Fig. 1. Cytokine levels in the fetal-placental unit 6 h (A) and 24 h (B) after the *T. bahiensis* scorpion venom was administered to dams on GD10. Values represent the mean ± SEM. * Significantly different from control; # significantly different from LPS; $p < 0.05$, ANOVA followed by Tukey–Kramer test.

venom is soluble only at this concentration). A solution of lipopolysaccharide (LPS) of *Escherichia coli*, serotype 005:B5 (Sigma–Aldrich, reference L2880) was used to verify whether the venom would act as an inflammatory agent.

Groups of pregnant rats on the 10th (GD10) or 16th (GD16) gestational day received a single subcutaneous injection (on the back) of 2.5 mg/kg of the *T. bahiensis* venom or 1 ml of saline 1.46%, or a single intraperitoneal injection of 100 μg/ml LPS. Five animals in each group were used. The subcutaneous route was chosen to simulate the venom kinetics and dynamics after an envenomation. GD10 corresponds to the middle of the organogenesis period, which consists of a series of processes culminating in the formation of organs, and GD16 corresponds to the neonatal development, in which the cerebral maturation and the brain development happen.

6 or 24 h after the treatments, the females were deeply anesthetized with carbon dioxide (CO_2) and subjected to laparotomy to section the uterus. The fetuses and their placenta were removed and weighed. After that, the anesthesia was deepened until the death of the animal.

The tissue samples (fetus/placenta) were immediately placed in a protease inhibitor solution (protease inhibitor cocktail Sigma P8340). The samples were macerated in a Polytron® tissue homogenizer and centrifuged (6707 G, 4 °C, 10 min) and the supernatant was collected and stored at −80 °C until analysis. Macerates of two fetus/placenta units were used to assay each cytokine. The cytokines IL-1α, IL-1β, TNF-α, IL-6, IL-10, and IFN-γ were assayed by a sandwich enzyme-like immunosorbent assay (ELISA), according to the manufacturer of the kit (IBL International GMBH, Hamburg, Germany).

A statistical analysis was performed using ANOVA followed by the Tukey–Kramer post-hoc test. P-values lower than 0.05 were considered statistically significant. All graphs were generated using