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Selected scorpion toxin exposures induce cytokine release in human peripheral blood mononuclear cells

Gerardo Corzo [b], Gerardo Pavel Espino-Solis [a, *]

[a] Baylor Institute For Immunology Research, 3410 Worth Street, Suite 600, Dallas, TX 75246, USA
[b] Departamento de Medicina Molecular y Bioprocesos, Instituto de Biotecnología, Universidad Nacional Autónoma de México, UNAM, Apartado Postal 510-3, Cuernavaca, Morelos 61500, Mexico

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ABSTRACT

A cytokine screening on human peripheral blood mononuclear cells (PBMCs) stimulated with selected scorpion toxins (ScTx's) was performed in order to evaluate their effect on human immune cells. The ScTx's chosen for this report were three typical buthid scorpion venom peptides, one with lethal effects on mammals *Centruroides suffussus suffusus* toxin II (CssII), another, with lethal effects on insects and crustaceans *Centruroides noxius* toxin 5 (Cn5), and one more without lethal effects *Tityus discrepans* toxin (Discrepin). A Luminex multiplex analysis was performed in order to determine the amounts chemokines and cytokines IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12-p40, IL-13, interferon alpha (IFN-α), interferon gamma (IFN-γ), tumor necrosis factor alpha TNF-α, and interferon-inducible protein-10 (IP-10) secreted from human PBMCs exposed to these toxins. Although, the ScTx Cn5 is not lethal for mammals, it was able to induce the secretion of cytokines IL-1β, IL-6, and TNF-α, IL-10 and IP-10 in comparison to the lethal CssII, which was able to induce only IP-10 secretion. Discrepin also was able to induce only IP-10. Interestingly, only low amounts of interferons α and β were induced in the presence of the ScTx's assayed. In a synergic experiment, the combination of Discrepin and Cn5 displayed considerable reverse effects on induction of IL-1β, IL-6, IL-10 and TNF-α, but they had a slight synergic effect on IP-10 cytokine production in comparison with the single effect obtained with the Cn5 alone. Thus, the results obtained suggest that the profile of secreted cytokines promoted by ScTx Cn5 is highly related with a cytokine storm event, and also it suggests that the mammalian lethal neurotoxins are not solely responsible of the scorpion envenomation symptomatology.

1. Introduction

Scorpions are venomous predatory arthropods which are members of the arachnidan class and order Scorpiones. They are living examples of 400 million years of successful evolution spread around the world, except in Antarctica (Ortiz et al., 2015). Scorpions are represented in 16 families with approximately 1700 different species. Scorpions can produce potent venoms to kill or paralyze their preys and to ward off possible competitors and predators and their venom could be the key for they successful predominance (Chippaux and Goyffon, 2008). The scorpion members of the buthidae family produce venom, which are highly toxic to mammals. In humans, the effects of a buthid scorpion sting can vary widely, from just local pain or inflammation to severe clinical complications, including death. Although, the severity of buthid scorpion envenomation is related to the presence of neurotoxins with pharmacological action on voltage-gated sodium channels (Nav) (Rodriguez de la Vega and Possani, 2005), the scorpion venoms and their toxins from different species and areas of the world caused different immunological and toxicological manifestations (Hadaddezfuli et al., 2015). Such different toxicological manifestations may be related to cytokines, which are increased after envenomation (Magalhaes et al., 1999). Increases of pro-inflammatory and anti-inflammatory cytokines in human serum, cellular lines and mice has also been reported after scorpion envenomation or venom/toxins incubation (Petricevich, 2010). Recent studies have been reported the effect of *Hemiscorpius lepturus* and *Androctonus crassicauda* scorpion venoms, which are able to induce the secretion of interleukin IL-12 in human monocytes (Hadaddezfuli et al., 2015; Saadi et al., 2015). Studies performed

* Corresponding author.
E-mail address: Gerardo.EspinoSolis@bswhealth.org (G.P. Espino-Solis).

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with the *Tityus serrulatus* venom and/or its major toxins, showed an inmunomodulatory activity in macrophages. High levels of tumor necrosis factor TNF-α, interleukin IL-1β, IL- 6, IL-8 and IL-10 were observed in supernatants of macrophages from mice exposed to *Tityus serrulatus* venom and its major toxins (Petricevich et al., 2007; Fialho et al., 2011). In the same way, increased levels of IL-1α, IL-1β, IL-6 and IL-10 were observed in sera from mice exposed to *Centruroides noxius* scorpion venom (Petricevich, 2006). Depending on the concentrations used, toxins isolated from the venom of the scorpion *Tityus serrulatus* Toxin 5 (TsV), Toxin 1 (Ts1) and Toxin 6 (Ts6) stimulated the production of nitric oxide (NO), IL-6 and TNF-α in J774.1 cells. Toxin 2 (Ts2) alone stimulated the production of IL-10, suggesting an anti-inflammatory activity of Ts2. These findings are important for understanding the mechanisms involved in macrophage activation following scorpion envenomation (Zoccal et al., 2011), and these data is summarized in Table 1.

Furthermore, there are clear examples of a cytokine cascade or cytokine storm when the presence of scorpion venom or toxins and the immune system goes out of the normal control and an inflammatory response also flares out of control. Although the general notion of an excessive or uncontrolled release of proinflammatory cytokines is well known, the concept of a cytokine storm and the biological consequences of cytokine overproduction are not clearly defined and the precise reason for this is not entirely understood, which may be caused by an exaggerated response when the immune system encounters a new and highly pathogenic invader (Tisoncik et al., 2012). It is known that the inflammation associated with a cytokine storm begins at a local site and spreads throughout the body via the systemic circulation. Redness, swelling or edema, heat, dolor, pain and loss of function are the hallmarks of acute inflammation. When localized in skin or other tissue, these responses increase blood flow, enable vascular leukocytes and plasma proteins to reach extravascular sites of injury, increase local temperatures (which is advantageous for host defense against bacterial infections), and generate pain, thereby warning the host of the local responses (Tisoncik et al., 2012). The pathology described above is highly related with a classic scorpion envenomation symptom.

In order to evaluate what kind of scorpion peptide toxins are involved in cytokine induction on human immune cells, three typical buthid scorpion venom peptides were selected, one with lethal effects on mammals (CssII), another with lethal effects on insects and crustaceans (Cn5) and one more without lethal effects (Discrepin). Both CssII and Cn5 are 66 amino acid residue long peptides with four disulfide bridges isolated from the Mexican scorpions *Centruroides suffussus suffusus* and *Centruroides noxius,* respectively (Corzo et al., 2009; Saucedo et al., 2012), and Discrepin is a 38 residues short peptide with three disulfide bridges originated from the Venezuelan scorpion *Tityus discrepans*. CssII and Cn5 modifies the voltage-gated sodium channel currents, and Discrepin blocks preferentially the Ia currents of the voltage-gated potassium channels of rat cerebellum granular cells; however, it is no toxic to neither mammalians nor crustaceans (Prochnicka-Chalufour et al., 2006). Tridimensional structure and multiple alignment of scorpion toxins assayed are displayed on Fig. 1. Therefore, we performed a cytokine screening on human PBMCs stimulated with such scorpion toxins in order to evaluate their effect on human immune cells.

2. Material and methods

2.1. Peptide toxins

CssII and Cn5 were isolated from *C. suffusus suffusus* lyophilized crude venom (10 mg). Discrepin was chemically synthesized according to Prochnicka-Chalufour et al. (2006). Briefly, the crude venom was dissolved in 500 μL of 0.1% aqueous trifluoroacetic acid (TFA), and the insoluble material was removed by centrifugation at 14,000 *g* for 5 min. The soluble venom was used directly for high performance liquid chromatography (HPLC) fractionation using a reverse-phase semi-preparative C_{18} column ($5C_{18}$MS, 10 × 250 mm Nacalai-Tesque, Japan) equilibrated in 0.1% trifluoroacetic acid (TFA), and eluted with a linear gradient of acetonitrile from 0 to 60% in 0.1%TFA, run for 60 min at a flow rate of 2 mL/min. Effluent absorbance was monitored at 280 nm. Fractions were collected in 1.5 mL tubes and dried out under vacuum. The HPLC fractions of interest were further purified by cation-exchange chromatography on TSK-gel sulfopropyl column (SP-5PW, 4.6 × 75 mm, Tosoh, Japan). The fractions were diluted to 200 μL with 20 mM ammonium acetate in 1 M acetic acid pH 2.9, and they were further fractionated using a linear gradient of 2 M ammonium acetate in 1 M acetic acid pH 5.9 from 0 to 50%, in 50 min (1 mL/min). Eluted proteins were monitored by conductivity. If required, a final step purification, essentially for extra desalting, was performed in a C_{18} reverse-phase column (4.6 × 250 mm, Nacalai Tesque, Japan) equilibrated in 0.1% TFA, and eluted with a linear gradient of acetonitrile from 20 to 60% in 0.1% TFA, run for 60 min at a flow rate of 1 mL/min. Effluent absorbance was monitored at 230 nm. The identity of the scorpion peptides was confirmed by mass spectrometry as described (Zamudio et al., 1992).

2.2. Peripheral blood mononuclear cells (PBMCs) isolation

Human healthy PBMCs were isolated from whole blood by using the Ficoll-Paque method reported by the reagent provider (GE-Healthcare Bio-Sciences AB, Uppsala, Sweden). Fresh PBMCs were maintained in complete RPMI -1640 media (cRPMI), supplemented

Table 1
Pro and anti-inflammatory cytokines are induced by the effect of scorpion venoms and toxins.

Scorpion/toxin	Experimental model	Cytokines produced	References
Androctonus australis hector	Rats	IL-1β, IL-4, IL-6, IL-10 and TNF-α	(Adi-Bessalem et al., 2008)
Buthus martensi Karch	Rats	NO and paw edema	(Liu et al., 2008)
Centruroides noxius	Mice	IL-1β, IL-1a, IFN-γ, IL-6, IL-10 and TNF-α	(Petricevich, 2006)
Leiurus quinquestriatus	Rabbits	IL-6, IL-8, NO, and TNF-α	(Abdoon and Fatani, 2009)
Tityus serrulatus	Human and Rabbits	IL-1β, IL-6, IL-8, IL-10, NO. TNF-α, IL-1α, IFN-γ and GM-CSF	(Magalhaes et al., 1999; Fukuhara et al., 2003)
Tityus discrepans	Human	IL-1β, IL-6 and TNF-α	(D'Suze et al., 2003)
Tityus discrepans	Rams	IL-6 and TNF-α	(D'Suze et al., 2004)
Tityus discrepans inflammatory toxins	Mice macrophages	NO and TNF-α	(Ramirez-Bello et al., 2014)
Ts1, Ts2 and Ts6 toxins	Mice, cell lines	NO, IL-6, IL-10 and TNF-α	(Zoccal et al., 2011, Zoccal et al. 2013)
Hemiscorpius lepturus	Human monocytes	IL-12	(Hadaddezfuli et al., 2015)
Androctonus crassicauda	Human monocytes	IL-12	(Saadi et al., 2015)
Tityus serrulatus	Mice	IL-1β, Inflammasome	(Zoccal et al., 2016)