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Inflammatory effect of a PLA2 isolated from *Bothrops diporus* venom

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In northeastern Argentina the vast majority of snakebite envenomings are caused by *Bothrops diporus*. Proteomic studies have shown that about 24.1% of its venom consists of phospholipases A2 (PLA2s) that induce inflammatory events. However, there are no previous reports on the specific inflammatory mediators released by immune and endothelial cells in response to these toxins. Thus, in this work we quantified a panel of cytokines on peripheral blood mononuclear cells (PBMC) previously incubated with a PLA2 isoform from *B. diporus* venom.

Briefly, PLA2 was isolated by reverse phase chromatography (RP-HPLC) on a C18 column. *B. diporus* venom (2 mg) was dissolved in 200 µL of 0.1% trifluoroacetic acid (TFA) and elution was performed at 1 mL/min in acetonitrile gradient with 0.1% TFA. Specific phospholipase activity, concentration at 280nm and molecular mass by MALDI-TOF MS (Shimadzu MALDI-8030) were determined.

In order to analyze the inflammatory response induced by this toxin on human PBMC, Luminex multiplex technology was used. After 10h incubation of $1x10^6$ PBMCs, from three different donors in technical duplicate, with PLA2 (25 µg/mL) or positive control (PMA/Ionomycin) the Human Panel Th17 for GM-CSF, IFNγ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12p70, IL-13, IL-15, IL-17A, IL-17E/IL-25, IL-17F, IL-21, IL-22, IL-23, IL-27, IL-28A, IL-31, IL-33, MIP-3α, TNFα and TNFβ was used. The statistical analysis was performed using the Two-Way ANOVA and FDR Benjamini-Hochberg method.

Results clearly showed that the PLA2 (14,048Da) isoform isolated from *B. diporus* venom, induced a significant increase in the release of the pro-inflammatory cytokines Il6 and TNFα and the macrophage inflammatory chemokine MIP3a, after 10h incubation. Even the IL-10, an anti-inflammatory cytokine, was also over expressed; the predominantly inflammatory effect induced by PLA2 was confirmed.

Further studies, eg. on oxidative stress, will complete this information that could be useful to develop new strategies in anti-venom therapy.