PROTEOMIC INVESTIGATION OF THP-1 MACROPHAGES POLARIZED TO M1, M2 AND UNDER THE EFFECT OF BOTHROPS JARARACA VENOM

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Snakebite envenomation is responsible for thousands of deaths per year worldwide. Most reported cases of snakebite accidents in Brazil are caused by snakes of the genus Bothrops, whose complex venom toxins play different roles in several local and systemic effects. Among the motivating findings of this project, studies by our group have shown that B. jararaca envenomation causes an effect on spleen proteome noted by the differential abundance of proteins, involved in inflammatory, apoptotic, and immunological pathways. Considering that monocytes differentiate to phagocytic cells essential for the immune response and that their polarization to M1 or M2 depends on the antigen presented and on adjuvant stimulant in the development of characteristic phenotypes, it is interesting to investigate the effect of a snake venom on phagocytic cells which, among other functions, play a fundamental role in the presentation of antigens to adaptive immune cells in primary and secondary lymphoid organs, such as the spleen. Human monocytic leukemia-derived THP-1 cells have the ability to differentiate into macrophage-like cells when treated with phorbol 12-myristate 13-acetate (PMA), mimicking the differentiation of peripheral monocytes to macrophages. After differentiation, THP-1-derived macrophages can be polarized into M1 or M2 by specific stimulus, reflecting respectively pro-inflammatory or anti-inflammatory phenotypes of tissue repair. In this project, we aimed to investigate the proteome of differentiated THP-1 macrophages polarized to M1, M2, and under the effect of B. jararaca venom to assess the effects of a complex mixture of toxins on human myeloid cells in vitro. THP-1 cells cultured in RPMI 1640 medium were plated with 40 ng/mL PMA for development of adherent macrophage phenotype. After 48 h, the cells received medium containing the stimulus for polarization to M1 with lipopolysaccharide and M2 with transforming growth factor beta. After 24 h, cells were lysed and the protein content was estimated by the BCA assay.Proteins were subjected to reduction and alkylation, followed by digestion with trypsin using SP3 protocol. Samples were analyzed by LC-MS/MS on a Vanquish Neo UHPLC system coupled to an Orbitrap Exploris 480 mass spectrometer, operating in DIA mode, collecting full MS from 350-950 m/z with 10 m/z isolation windows. Peptide identifications and protein inference were performed using DIA-NN 1.9 search engine, and statistical analyses were carried in R using the limma and mixOmics packages with a Quarto markdown document specific to the project. RESULTS: We identified in total 4,227 unique proteins and, on average, 3,690 proteins per sample. Data sparsity revealed, on average, 13.3% of missing proteins and 86.7% of proteins present in all samples, and the sparsity was tested under the null hypothesis of Missingness Completely At Random (MCAR). Although the coefficients of variation indicated homogeneity among replicates (CV < 20%), supervised statistical analyses were not able to discriminate all treatment groups, except cells stimulated with TGF-ß and venom, as detected by the proteins identified with significant differential abundance in each treatment group. Preliminary results suggest responses regarding cellular signaling as a consequence of the recognition of foreign epitopes and contribute to the understand the effects of a complex mixture such as *B. jararaca* venom on cultured human monocytes THP-1.

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