Proteomic approach for the evaluation of oxide nitric dependent pathways during Leishmania major infection

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Abstract

Leishmaniasis is a tropical and subtropical endemic disease caused by parasites of the genus Leishmania. The disease clinical manifestations depend on the infecting Leishmania species and the host immune response, and can be classified into two types: tegumentary and visceral. Macrophages represent the primary line of defense against infection, and nitric oxide (NO) production are one of the major mechanisms involved in eliminating parasites. In order to identify proteins involved in the parasite control through the nitric oxide pathway, we performed a proteomic analysis. Bone marrow derived macrophages (BMDMs) from wild type and NOS2-deficient C57BL/6J mice were infected with Leishmania major, and protein levels were quantified by mass spectrometry. The analysis was based on statistical calculations available in packages and functions of the R language, such as t.test function, and limma and ROTS packages. The differentially expressed proteins (DEPs) were defined as those that obtained p-value less than 0.05. Gsr, Arg1, F13a1, Pcna, Plin3 and Cd36 proteins were more differentially expressed in the context of Leishmania infection. Through pathway enrichment analysis using packages such as clusterProfiler, ReactomePA, WebGestalt and EGSEA, we identified activated pathways related to the regulation of adaptive immune response, immune effector process and leukocyte mediated immunity. Cd14, Cd36, Arg1, Ctsl, Dctn2, Rab7 e Arf1 were selected as acting in the regulation of the identified pathways by protein interaction analysis. The reliability of the detection of differentially expressed proteins was increased when using different approaches to statistical analysis, mainly when compared to traditional methods. The evaluation of the protein interaction network allowed to identify important proteins of modulated pathways during the infection, improving the understanding of infection control in the absence of nitric oxide.

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