Evaluation of differentially expressed proteins during Leishmania major infection in murine macrophages lacking nitric oxide synthase

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Abstract

Leishmaniasis is a neglected tropical disease that can have 3 different presentations, cutaneous, mucocutaneous, or visceral leishmaniasis. It is caused by a diverse group of protozoan parasites, Leishmania, and is transmitted by certain types of sandflies. It is estimated that 1.5 million people are infected each year in more than 98 countries where the disease is endemic. Until now, vaccination and drug therapy have failed to control the disease. The main mechanisms responsible for controlling Leishmania replication involves the production of nitric oxide (NO), generated by inducible NO synthase (iNOS) following activation by IFN? and also reactive oxygen species (ROS), generated by the respiratory burst. Hence, studies evaluating changes in protein expression after Leishmania major infection in wild type macrophages, and macrophages lacking or superexpressing iNOS could help in the discovery of novel targets for the control of L. major. In order to identify proteins differentially expressed (DEPs) related to these functions, we analyzed protein extracts from C57BL/6J bone marrow derived macrophages (BMDMs) infected or not with Leishmania major and iNOS-/- BMDMs, using mass spectometry. Differential regulated proteins were selected based on several statistical analyses (t-test, LIMMA, ROTS and SAM), performed using RStudio and relevant packages, as to combine results from several sources and to choose the most suitable method to improve the confidence. DEPs were then submitted to biological network analyses using Enrichment Map and Gene Ontology related tools (such as g:Profiler and DAVID) to define enriched functionally related genes. In addition, we evaluated protein-protein physical and functional interactions with STRING database and also pathway abundances through Ingenuity Pathway Knowledge Base to improve these results. Thereby, we identified proteins differentially modulated during L. major infection course, which allowed us to define important altered biological processes, such as early endosome to late endosome transport. We expect our results to widen the understanding of the infection control and to unravel new information for further studies.

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