**Proteases and phosphatases during Leishmania-macrophage interaction**

**Paving the road for pathogenesis**

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The outcome of Leishmania infection depends both on host and pathogen factors. Macrophages, the specialized host cells for uptake and intracellular development of Leishmania, play a central role in the control of infection. Leishmania has evolved strategies to downregulate host cell functions, largely mediated by the parasite-induced activation of macrophage protein tyrosine phosphatases (PTPs). We have recently identified PTP1B and TCPTP as two additional PTPs engaged upon Leishmania infection and have unraveled an intimate interaction between the Leishmania surface protease GP63 and host PTPs, which mediates a mechanism of cleavage-dependent PTP activation. Here we discuss new perspectives for GP63-mediated parasite virulence and propose putative mechanisms of GP63 internalization into host macrophages and access to intracellular substrates.

Infectious and parasitic diseases represent the second leading cause of deaths in the World after cardiovascular diseases.¹ Among these is leishmaniasis, a neglected tropical disease which currently affects more than 12 million people globally, with over two million new infections occurring per year.² Leishmaniasis is a sandfly-transmitted infectious disease caused by eukaryotic protozoan parasites of the genus Leishmania.³ It comprises a complex of diseases which, depending on the infecting Leishmania species, can range from self-healing cutaneous ulcers, disfiguring mucocutaneous lesions, to the fatal visceral form if left untreated. In the absence of an available vaccine, and the compromised usefulness of available chemotherapy due to toxicity and increased emergence of drug resistance, research on transmission, drug targets, vaccine development and host-pathogen interactions are priorities of high relevance to public health in the developing world.

The outcome of Leishmania infection depends both on host and pathogen factors. Macrophages, the specialized host cells for uptake and intracellular development of Leishmania, are central in the control of infection and parasite clearance. Despite this, most Leishmania species display strategies to overcome the innate immune response during the early stages of infection, rapidly triggering the downregulation of multiple host cell functions such as interleukin-12 (IL-12), nitric oxide (NO) and tumor necrosis factor-alpha (TNFα) production, phagolysosomal maturation and major histocompatibility complex class II (MHC II) antigen presentation (reviewed in ref. 4). Underlying macrophage effector and accessory functions is the activation of signaling pathways, largely controlled by events of protein phosphorylation. Consequently, the regulation of protein kinase and phosphatase activities is critical for signal transduction, and therefore, for the control of antimicrobial and inflammatory phagocyte functions. Although the complete mechanistic panorama has not been fully elucidated, and inter-species/subgenus variations need to be considered, the inactivation of macrophage protein

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kinases and activation of protein tyrosine phosphatases (PTPs) by Leishmania fundamentally contribute to the downregulation of host cell functions.5

By screening the PTP activity profile of Leishmania-infected and uninfected macrophages using an in-gel PTP activity assay, we have recently identified Protein-tyrosine phosphatase 1B (PTP1B) and T cell phosphatase (TCPTP) as two novel PTPs engaged upon Leishmania infection.5 Leishmania GP63 (glycoprotein of 63 KDa) is a glycosylphosphatidylinositol (GPI)-anchored zinc metalloproteinase6 and is the most abundant surface protein of Leishmania promastigotes.7 Although the important role of GP63 for Leishmania virulence and mammalian pathogenesis has been documented for more than two decades,8,9 the underlying events responsible for GP63-mediated regulation of mammalian signaling molecules that are proteolytically processed and/or modulated thus far remain elusive. We have identified a number of mammalian signaling molecules that are potentially for GP63-mediated regulation of host immune responses remained elusive.

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Figure 1. GP63 cleaves and modulates the activity of multiple intracellular signaling molecules. Once inside the host cell, GP63 directly interacts with and cleaves signaling molecules involved in actin cytoskeleton remodeling (p130Cas, PTP-PEST), cytokine and mitogen signaling (SHP-1, PTP1B, TCPTP, TAB-1, PTP-PEST) and gene transcription (transcription factor AP-1). Cleavage modulates the activities of these molecules, resulting in the alteration of cellular functions.

Conclusions

Our investigations have elucidated a novel regulatory mechanism of macrophage PTP activity by Leishmania based on the delivery and proteolytic activity within the host cell of the parasite virulence factor GP63. Early activation of
PTPs via GP63-mediated cleavage promotes an environment for the successful establishment of Leishmania parasites. Having identified TCPTP and PTP1B as additional host PTPs engaged upon \textit{L. major} infection, and having provided novel insights into the mechanism of Leishmania-induced host PTP regulation, many new questions arise: do TCPTP, PTP1B and SHP-1 have redundant functions in downregulation of immune cell functions, onset and progression of leishmaniasis? Do SHP-1, PTP1B and TCPTP physically co-exist in the same multi-protein complex upon Leishmania infection? Are these PTPs similarly important for the development of the various pathologies associated with Leishmania infection, including the devastating visceral form? Does GP63-mediated PTP cleavage alter PTP substrate specificity? Experiments in this direction will grant fruitful understanding of the delicate roles of these three macrophage PTPs in the context of leishmaniasis.

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