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Dynamic and Correlative Imaging of host cell infection by Leishmania donovani reveals intracellular parasite motility, Recruitment and Exocytosis of Lysosomes and Host Cell Wounding

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L. donovani is the responsible agent of visceral leishmaniasis, one of the major neglected diseases. Despite the important impact of this disease in endemic areas, and its fatal outcome if left untreated, our understanding on how flagellated infectious promastigote parasites enter and colonize vertebrate host cells is limited. According to the current view, the parasite is engulfed by the host cell through a classical phagocytic event and then transiently inhibits parasitophorous vacuole maturation by preventing fusion with lysosomes.

Here we used a robust experimental system that combines primary macrophages infected with virulent metacyclic promastigotes and high spatio-temporal resolutive microscopy technologies to dissect in real time the initial encounter of promastigotes with phagocytic host cells and provide a complete view of the early infectious process. On the parasite side, we revealed that highly polarized and motile promastigote parasites enter host cells in a polarized manner through the flagellar tip while dead parasites were not phagocytosed. Once internalized the parasites re-oriented quickly such that their flagellum faced toward the cell periphery. At this point persistent flagellar activity resulted in a long lasting oscillating movements of the intracellular parasite toward and outward the host cell center. On the host side, we demonstrated that *L. donovani* infection was associated with recruitment, docking and exocytosis of lysosomes at the parasite oscillating site. Finally, we correlated focal lysosomal exocytosis at the parasite location with local wounding of the host cell plasma membrane.

Our study provides new insights into the mechanism used by *L. donovani* to gain access to the host cell that involve parasite polarity and motility, parasite-mediated host cell injury and exocytosis of lysosomes consistent with membrane repair.

Keywords: Leishmania donovani, Host cell infection, Motility, lysosomes exocytosis