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IMPLICATION OF CLASSICAL PHAGOCYTOSIS IN THE INITIAL EVENTS OF LEISHMANIA BRAZILIENSIS INFECTION IN MONOCYTE-DERIVED MACROPHAGES

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ABSTRACT

INTRODUCTION:*Leishmania braziliensis* (*Lb*), the primary causative agent of cutaneous leishmaniasis in Brazil, results in localized, mucosal, diffuse or disseminated forms. The initial parasite-macrophage (MΦ) interactions are crucial for determining infection outcomes, but remain poorly understood for *Lb*. **OBJECTIVE:**This study investigates these interactions, particularly focusing on phagocytosis and parasitophorous vacuole (PV) formation, and their roles in parasite survival within MΦ. **METHODOLOGY:**MΦ were treated with the AKT inhibitor GSK 690693 and the Akt inhibitor, and the PLC inhibitor U73122 to study their effects on parasite internalization and subsequent intracellular events. Infection rates, reactive oxygen species (ROS) production using the H2DCFDA probe, and endosomal markers Rab5 and Rab7 by fluorescent antibodies were assessed in PVs of infected cells and in cells incubated with zymosan as phagocytic control. Hydrolytic activity and vacuole acidification were evaluated using DQ-BSA or Lysotracker (Lys). Quantification was performed by fluorescence microscopy for ROS and confocal microscopy for quantifying markers. Additionally, the effect of chloroquine (Chl) on vacuole acidification was examined. **RESULTS**:Initial hours of infection showed a rise in amastigotes per MΦ, stabilizing from 24 to 72 h. U73122 reduced infection rates, indicating its role in parasite uptake. ROS production was elevated in the U73122-treated group, but not in the GSK 690693 group. Rab5 expression was higher at 2 h, and Rab7 at 24 h in PVs. Chl treatment reduced Lys positivity, confirming acidification of *Lb*-induced PVs. CONCLUSION: PLC inhibition diminishes *Lb* uptake, suggesting its involvement in phagocytosis via PIP_2 hydrolysis. In addition, the enzyme increases ROS levels by mechanisms should be elucidated. Moreover, the differential expression of Rab5 and Rab7 and the impact of Chl on vacuole acidification show that *Lb* follows a classical phagosome maturation pathway in MΦ.

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