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CASPASE-8 ACTIVATION IS REQUIRED FOR CONTROL OF *LEISHMANIA AMAZONENSIS* REPLICATION IN MACROPHAGES

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Abstract

Leishmaniasis is a disease caused by the protozoan *Leishmania spp.*, considered a significant global health challenge. Macrophage-mediated innate immunity is essential for the parasite replication control. Central to this process is the NLRP3 inflammasome, which activates caspase-1, forming a protein complex that induces pyroptosis, an inflammatory form of cell death critical for parasite elimination. Caspase-1 activation drives the production of IL-1β, enhancing iNOS expression and nitric oxide synthesis, both essential for *Leishmania* elimination. Traditionally described as an initiator caspase involved in apoptotic cell death, caspase-8, in coordination with or via the activation of caspase-1, has also been shown to be important in controlling pathogen replication in macrophages infected by *Legionella* and *Yersinia,* and is associated with pyroptotic cell death. In this study, we explored the role of caspase-8 in regulating pyroptosis-related inflammatory processes during *Leishmania amazonensis* infection in macrophages. Using bone marrow-derived macrophages, we employed a combination of molecular and immunological approaches to uncover its function. Immunoblotting with a cleaved caspase-8-specific antibody, along with immunofluorescence microscopy, demonstrated that *L. amazonensis* infection induces caspase-8 activation in macrophages independently of pathways mediated by Ripk3 and caspase-1. Furthermore, flow cytometry analysis of macrophages from caspase-8/ripk3 double knockout mice revealed significantly increased susceptibility to *L. amazonensis*-RFP compared to wild-type controls after 24 hours of infection. These findings highlight the pivotal role of caspase-8 as a critical regulator of macrophage activation during *Leishmania* infection, enhancing their capacity to restrict parasite replication. By elucidating this pathway, our study provides valuable insights into the molecular mechanisms driving macrophage-mediated immunity against *Leishmania* infection.

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