CRISPR-Cas9-based deletion of glutathione synthetase in Leishmania donovani demonstrates its essential role in growth and virulence

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Redox homeostasis is critical for the survival of *Leishmania* parasites, particularly under oxidative stress generated within the host's macrophages. Unlike most eukaryotes, trypanosomatids, including *Leishmania*, rely on a unique thiol-based redox system centered around trypanothione. Glutathione synthetase is a key enzyme in this pathway, catalyzing the conjugation of glycine to γ-glutamyl-cysteine to form glutathione, which subsequently serves as a precursor for trypanothione biosynthesis. In this study, we employed a CRISPR-Cas9-based gene knockout strategy to generate *Leishmania donovani* null mutants lacking glutathione synthetase. Phenotypic analysis revealed that loss of this enzyme severely compromised parasite growth and amastigote infectivity, underscoring its essential role in *Leishmania* pathogenesis. Our findings establish glutathione synthetase as a crucial enzyme for parasite survival and virulence, positioning it as a promising target for novel antileishmanial therapeutics.