Species-specific differences in macrophage activation and cholesterol metabolism in hamster and mouse macrophages correlate with host susceptibility to *L. donovani* infection

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Leishmania (*L.*) infection ranges from asymptomatic to devastating immunopathology. Even though these parasites thrive inside macrophages, the role of these important immune cells on defining disease outcome remains to be established. To address this important question, we took advantage of two experimental rodent systems described as resistant (C57/BL6 mice) or susceptible (Syrian hamsters) to *L. donovani* infection. We first established a protocol to produce bone marrow-derived macrophages from hamsters (hamBMDMs) and demonstrated their increased susceptibility to intracellular *L. donovani* growth compared to mouse (m)BMDMs, supporting a possible crucial role of the initial parasite-macrophage interaction in disease outcome.

Comparative RNA-seq analyses of uninfected and *L. donovani*-infected hamBMDMs and mBMDMs revealed marked differences in both intrinsic and infection-induced transcriptional programs. Notably, hamBMDMs displayed a bias toward an anti-inflammatory, M2-like polarization state. We further identified candidate genes associated with hamster susceptibility linked to cholesterol homeostasis and metabolic regulation, including the transcription factors *Srebf2* and *Cebp/β* as well as the enzymes *Arg1* and *Tgm2*.

To functionally validate these candidates, we employed pharmacological approaches targeting cholesterol levels and the activities of ARG1, TGM2 and CEBP/β. Mouse and hamster macrophages treated with specific inhibitors were subsequently challenged with *L. donovani* to assess changes in the resistance or susceptibility phenotypes. Using an optimized semi-automated microscopy-based quantification method, we revealed that only cholesterol depletion with methyl-β-cyclodextrin (MβC) but none of the other treatments significantly impaired intracellular parasite replication, highlighting cholesterol availability as a critical determinant of infection outcome. Importantly, MβC had no direct effect on free parasites, indicating that the observed reduction in parasite burden is host cell–dependent. Moreover, we observed a more pronounced reduction of parasite replication in mBMDMs compared to hamBMDMs, suggesting species-specific differences in cholesterol dependency during infection.

We are currently assessing such species-specific differences in cholesterol biosynthesis by (i) using specific chemical inhibitors to target SREBF2/LXR, cholesterol efflux, HMG-CoA processing and squalene or cholesterol synthesis steps, and (ii) comparative metabolomic profiling of resting and parasite-exposed hamster and mouse BMDMs.

Overall, our data suggest that divergent susceptibility to *L. donovani* infection in hamster and mouse macrophages may rely on distinct macrophage activation states and metabolic profiles.

Our integrative strategy provides a useful blueprint to investigate host factors associated with resistance to microbial infection that can uncover novel targets for host-directed therapeutic avenues.