Genomic and transcriptomic profiling of tissue-derived *Leishmania donovani* amastigotes identifies δ-amastins as key fitness factors in splenic infection

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Leishmania donovani survival and pathogenicity during mammalian infection depends on the parasite's capacity to adapt to different environments, including distinct host tissues such as liver and spleen. The parasite fate strictly depends on its capacity to subvert tissue-specific immune responses in these organs. While *L. donovani* adaptation to external cues and selection for fitness largely depends on gene dosage-driven expression changes, how the parasite responds to different immune pressures and adapts to tissue-specific constraints during infection is unknown. To address this important open question, we conducted independent in vivo evolutionary experiments (EEs) in immunocompetent and immunocompromised hamsters and mice infected with *L. donovani* Ld1S parasites. Genomic analysis of tissue amastigotes purified from liver and spleen revealed only minor changes in karyotype and gene copy number in individual biological replicates between parasites from the different rodent systems (hamster vs mouse), the different host immune status (immunocompetent vs immunocompromised) and the different tissues (liver vs spleen). However, the absence of converging genomic changes between replicates indicates that the observed signals are rather due to bottle neck effects than natural selection, suggesting that these parasites do not undergo genomic adaptation *in vivo*, at least under our experimental conditions. In contrast, additional infection experiments assessing the transcriptomes of liver and spleen-derived amastigotes revealed significant changes in mRNA abundance of a cluster of 7 δ-amastins located on the chromosome 31 that was observed across all four spleen-derived amastigote replicates. This spleen-specific signature aligns with harsher phagolysosomal conditions imposed by splenic macrophages (M1-skewed, hypoxic) vs permissive liver Kupffer cells. Ongoing normalization to genomic content will establish if these changes are independent of gene dosage, thus potentially revealing post-transcriptional regulation as an important driver of parasite phenotypic adaptation during infection. In conclusion, our data reveal tissue-specific transcriptomic adaptation in *L. donovani* and define chr 31 δ-amastins as key amastigote fitness factors in hamster infection.