Bottling it all up: Using parasite population biology to identify susceptibility pathways in leishmaniasis

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Leishmania donovani causes the systemic multi-organ disease visceral leishmaniasis. However, little is understood about the mechanisms controlling parasite dissemination, survival and growth within and between different host tissues. Specifically, it is not known where or when parasite populations are reduced by immunological 'obstacles'; understanding this process could enable us to identify new methods to block dissemination and limit clinical disease.

We have combined CRISPR genome editing, high-throughput sequencing and Sequence Tag-based Analysis of Microbial Population Dynamics (STAMP) to determine dissemination patterns within a mouse model using an isogenic library of 102 *L. donovani* lines, each with a unique barcode. Using STAMP, we have assessed where bottlenecks occur in dissemination by calculating the 'Founder Population' (FP) in multiple tissues at different time-points post-infection. Additionally, we have measured the genetic distance between parasite populations in different tissues, to understand the potential mechanisms underlying dissemination patterns.

To investigate changes in dissemination over time, we infected male and female C57BL/6J mice with the promastigote barcoded library for 2 days, 14 days or 28 days, and assessed the parasite diversity in multiple tissues to calculate FP sizes. We have found that the liver is permissive to parasite colonisation and its diversity is relatively stable over time, whilst lymph nodes show a highly restrictive bottleneck, resulting in a small FP that increases significantly over a four-week infection. Spleen, lung, gut and bone marrow show intermediate FP sizes.

To understand the mechanisms for the earliest bottleneck events, we used a complement-deficient (B6.129S4-C3tm1Crr/J) mouse model; we found that complement knock-out increases the permissiveness of the bottleneck in multiple tissues at 2 days post-infection, with males exhibiting a more significant impact than females.

Using genetic distance measurements, we have additionally generated data supporting the hypothesis that the liver is the key source of parasites for the colonisation of other tissues. Overall, our findings provide an insight into the mechanisms regulating population bottlenecks and colonisation dynamics, which could have important implications for parasite and host evolution and in disease outcomes.