Exploiting susceptible and resistant rodent systems to uncover immune signatures underlying *L. donovani* symptomatic infection

P. Jenkins, P. Pescher, T. Cokelaer, I. Vitrenko and G. F. Späth

Institut Pasteur , Université Paris Cité, INSERM 1201, Unité de Parasitologie Moléculaire et Signalisation, Paris, France

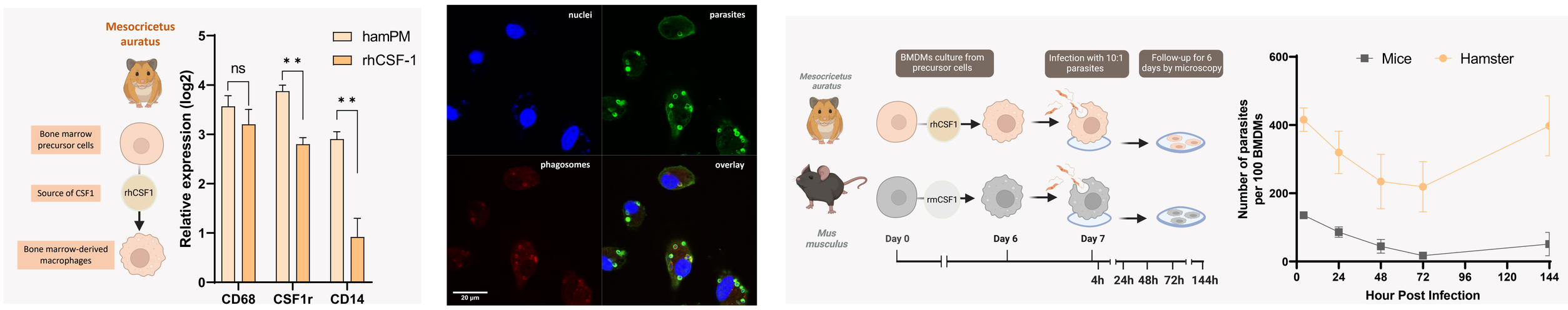
ID 123

Macrophages are important innate immune cells that instruct the immune system for an appropriate adaptive response against infection. *Leishmania* resists macrophage cytolytic activities and exploits these cells as hosts for intracellular proliferation. Chronic infection in humans can be either asymptomatic or causing devastating immuno-pathologies. The host determinants and immune mechanisms underlying this dichotomy are largely unknown.

We propose to address this open question using *Leishmania donovani* resistant (mouse) and susceptible (hamster) rodents

The central role of hamster bone marrow-derived macrophage (hBMDM) in the establishment of *L. donovani* infection

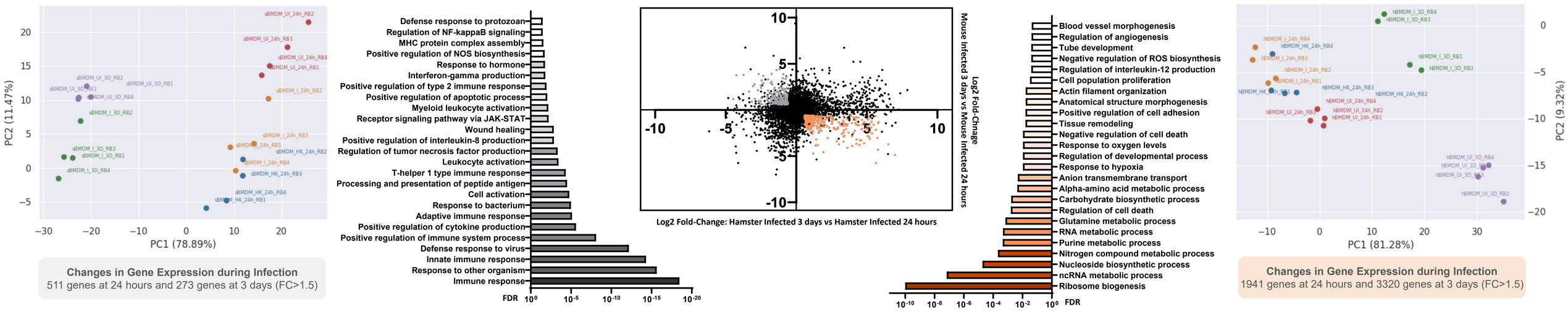
(A) hBMDMs express macrophage markers — (B) hBMDMs phagocytose *L. donovani* — (C) hBMDMs are highly permissive to *L. donovani* infection



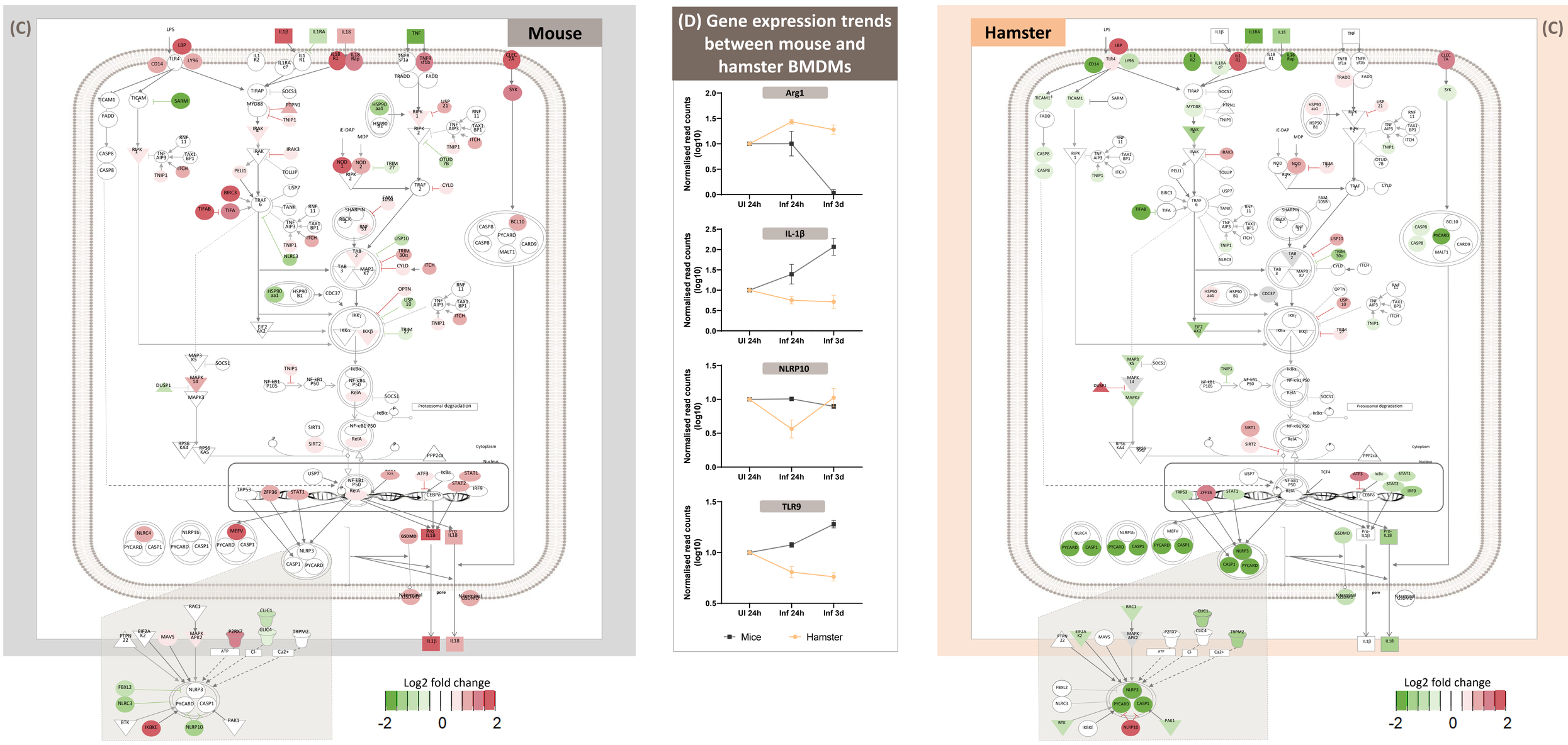
(A) Schematic workflow for the production of bone marrow-derived macrophages (rhCSF1= recombinant human CSF1) and relative transcript expressions of macrophage markers against bone marrow precursor cells (hamPM= hamster peritoneal macrophage, ns = non-significant, *p < 0.05, **p<0.005). (B) Microscopic images of hamster BMDMs 24 hours after infection with metacyclic enriched *L. donovani* promastigotes. (C) Workflow for the infection experiment and number of parasites per 100 hamster or mice BMDMs. Cells and parasites nuclei were stained after 4, 24, 48, 72 and 144h of infection with metacyclic enriched parasites (mean n=3, SD).

RNA-seq analysis of hamster and mouse BMDMs informs on pathways underlying *L. donovani* permissivity

(A) Clusterisation of mouse samples — 3 days vs 24 hours — (B) Inversely enriched biological processes between rodents during infection — 3 days vs 24 hours — Clusterisation of hamster samples (A)



(A) Principal component analysis (PCA) of mouse (sBMDM) and hamster (hBMDM) samples (UI= Uninfected, HK= Metacyclic-enriched Heat-killed parasites, I= Infected with metacyclic enriched promastigotes, 24h= 24-hours post infection, 3D= 3 days post-infection). (B) Double ratio plot representing the log2 fold change of transcript abundance between hamster BMDMs 3 days versus 24 hours after infection (x-axis) and mouse BMDMs 3 days versus 24 hours after infection (y-axis). All transcripts with at least 5 base mean reads are represented (black dots). Transcripts showing inversed fold-change over time between mouse and hamster (FC > 1.5, adjusted p-value < 0.05) are highlighted in grey and orange respectively. Gene ontology analysis for the biological process after functional network enrichment using String (FDR= False Discovery Rate). Twenty five biological processes are represented for mouse (grey) and hamster (orange).



(C) Changes in gene expression between 3 days and 24 hours after infection with *L. donovani* metacyclic-enriched promastigotes for the NF-κB pathway in mouse (left) and hamster (right) BMDMs. Log2 fold-changes (p-adjusted<0.05) are reported according to the legend in the graphs. White boxes = p-adjusted > 0.05 or FC<1.25. (D) Expression trends of differentially regulated genes among conditions. Read counts were normalised between mouse and hamster BMDMs at 1 and plotted as a log10 value (y-axis). UI= Uninfected, Inf= Infected, 24h= 24 hours and 3d= 3 days.

Conclusion

Our comparative experimental system allowed us to firmly correlate the differences in parasite survival to differences in the rodent macrophage responses, suggesting that the initial parasite-host cell interaction can define the trajectory towards either acute symptomatic or silent chronic *L. donovani* infection.