Phenotypic and functional genetic analysis of virulence attenuation in *Leishmania donovani*

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Abstract:

Leishmania donovani cycles between insect and mammalian hosts and has evolved strategies to survive inside phagocytic cells by subverting host antimicrobial activities through expression of virulence factors. Mechanisms that govern intracellular *Leishmania* survival remain widely unknown and only few parasite virulence factors have been identified to date.

Our previous results revealed a progressive loss of *L. donovani* infectivity during culture adaptation. A phenotypic characterization of virulent promastigotes derived from hamster splenic amastigotes (culture passage P2) and attenuated long-term cultured parasites (culture passage P20) showed differences *in vitro* in terms of growth and stress resistance.

In order to assess mechanisms underlying loss of infectivity and identify novel virulence factors, we use our experimental *Leishmania* system for a functional genetic screen (CosSeq). Genomic DNA from promastigotes freshly derived from hamster splenic amastigotes was used to generate a cosmid library. Using HTseq analysis, we validated nearly complete coverage of the *Leishmania* genome in our library, with only few genes missing. P20 parasites were transfected with the homologous cosmid library and injected into hamsters for *in vivo* selection of parasites carrying a cosmid with genes that enhance or restore infectivity. The hamster weight is monitored weekly and parasites will be extracted 10 weeks after inoculation. After mapping of virulence genes encoded in the cosmid inserts by transposon mutagenesis, genes will be individually validated *in vivo* and *in vitro* for their capacity to increase parasite infectivity and resistance to various cytotoxic host activities.