Systematic identification of genes encoding cell surface and secreted proteins that are essential for in vitro growth and infection in *Leishmania donovani*.

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Abstract

Leishmaniasis is an infectious disease caused by protozoan parasites belonging to the genus Leishmania for which there are no approved human vaccines. Infections localise to different tissues in a species-specific manner with the visceral form of the disease caused by *Leishmania donovani* and *L. infantum* being the most deadly in humans. Although *Leishmania spp*. parasites are predominantly intracellular, the visceral disease can be prevented in dogs by vaccinating with a complex mixture of secreted products from cultures of *L. infantum* promastigotes. With the logic that extracellular parasite proteins make good subunit vaccine candidates because they are directly accessible to vaccine-elicited host antibodies, here we attempt to discover proteins that are essential for in vitro growth and host infection with the goal of identifying subunit vaccine candidates. Using an in silico analysis of the Leishmania donovani genome, we identified 92 genes encoding proteins that are predicted to be secreted or externally anchored to the parasite membrane by a single transmembrane region or a GPI anchor. By selecting a transgenic *L. donovani* parasite that expresses both luciferase and the Cas9 nuclease, we systematically attempted to target all 92 genes by CRISPR genome editing and identified four that were required for in vitro growth. For fifty-five genes, we infected cohorts of mice with each mutant parasite and by longitudinally quantifying parasitaemia with bioluminescent imaging, showed that nine genes had evidence of an attenuated infection although all ultimately established an infection. Finally, we expressed two genes as full-length soluble recombinant proteins and tested them as subunit vaccine candidates in a murine preclinical infection model. Both proteins elicited significant levels of protection against the uncontrolled development of a splenic infection warranting further investigation as subunit vaccine candidates against this deadly infectious tropical disease.