The *Leishmania donovani* ortholog of the GPI-anchor biosynthesis co-factor PBN1 is essential for host infection

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Abstract

Leishmania donovani, a kinetoplastid parasite for which no licenced vaccine is available. To identify potential vaccine candidates, we systematically identified genes encoding putative cell surface and secreted proteins essential for parasite viability and host infection. We identified a protein encoded by *LdBPK_061160* which, when ablated, resulted in a remarkable increase in parasite adhesion to tissue culture flasks. Here, we show that this phenotype is caused by the loss of glycosylphosphatidylinositol (GPI)-anchored surface molecules, and that *LdBPK_061160* encodes a non-catalytic component of the *L. donovani* GPI-mannosyltransferase I (GPI-MT I) complex. GPI-anchored surface molecules were rescued in the *LdBPK_061160* mutant by the ectopic expression of both human genes PIG-X and PIG-M, but neither gene could complement the phenotype alone. From further sequence comparisons, we conclude that *LdBPK_061160* is the functional orthologue of yeast PBN1 and mammalian PIG-X, which encode the non-catalytic subunits of their respective GPI-MT I complexes and we assign LdBPK_061160 as LdPBN1. The LdPBN1 mutants could not establish a visceral infection in mice, a phenotype that was rescued by constitutive expression of LdPBN1. Although mice infected with the null mutant did not develop an infection, exposure to these parasites provided significant protection against subsequent infection with a virulent strain. In summary, we have identified the orthologue of the PBN1/PIG-X non-catalytic subunit of GPI-MT I in trypanosomatids, shown that it is essential for infection in a murine model of visceral leishmaniasis, and demonstrated that the LdPBN1 mutant shows promise for the development of an attenuated live vaccine.