Protective efficacy of different vaccine candidates using *Leishmania* proteins to protect against visceral leishmaniasis

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Visceral leishmaniasis (VL) is a major health problem in the world. Due to difficulties in implementing effective prophylactic methods and in the treatment of the disease, the development of alternative measures, such as vaccines, is attractive. Attempting to select different candidate antigens to compose a vaccine against VL, immunoproteomic and bioinformatics tools have been widely used. Several proteins have been identified and tested; however, the search for improving a vaccine keeps going. The aim of this study was to compare the immunogenicity and the protective efficacy of three recombinant proteins isolated (LiHyp1: LinJ.35.1290, LiHyp6: LinJ.36.0580, and HRF: LinJ.24.1560), using in a mix, as well as a chimeric protein composed by T cell epitopes derived from these proteins, all of them added with saponin, against VL. The DNA sequences were cloned, and the recombinant proteins were expressed and purified. The chimera was synthesized with immunodominant regions composed by CD4+ and CD8+ T cell epitopes specific for mice and human haplotypes, from the three proteins. The single or mixed proteins, as well as the chimera, were added with saponin and used to immunize BALB/c mice. Animals were infected with *L. infantum* and the immunogenicity and protective efficacy were evaluated. All the vaccinated mice showed an increase in specific production of IFN-γ, IL-12, and GM-CSF after *in vitro* stimulation, which were maintained after infection; however, the level of these cytokines was higher in the mix and chimera groups, when compared with the isolated proteins and control groups (saline and saponin). Immunized animals also presented significant reductions of the parasite burden in the evaluated organs (spleen, lymph node, liver and bone marrow) but the mix and chimera groups showed a higher protection in relation to the other groups. The vaccine efficacy was associated with a raised production of IFN-γ and nitric oxide, besides of anti-leishmanial IgG2a isotype antibodies. In addition, a decrease in the secretion of IL-4 and IL-10 was also observed in protected animals. In conclusion, recombinant proteins isolated, when added with saponin, can induce protection in BALB/c mice against VL; however, the mix of proteins, as well as the chimeric protein, showed a better efficacy. Due to the efficiency and to the possibility of cost reduction, a recombinant chimeric protein plus saponin could be considered as a prophylactic alternative to prevent VL.

Keywords: Polyproteins vaccine; visceral leishmaniasis; Th1 imune response.

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