The serodiagnosis and protective efficacy of a *Leishmania* amastigote-specific hypothetical protein against visceral leishmaniasis

Gabriela O. Carvalho[1], Vívian T. Martins[2]; Daniela P. Lage[2]; Miguel A. Chávez-Fumagalli[2]; Lourena E. Costa[2]; Beatriz C.S. Salles[2]; Thaís T.O. Santos[2]; Fernanda F. Ramos[2]; Mariana P. Lima[2]; Danniele L. Vale[1]; Daniel S. Dias[2]; Patrícia A.F. Ribeiro[2]; Grasiele S.V. Tavares[2]; Débora V.C. Mendonça[2]; Isabela S. Gama[1]; Mariana C. Duarte[1,2]; Eduardo A.F. Coelho[1,2]
[1]Departamento de Patologia Clínica, COLTEC, UFMG, Brazil. [2]Programa de Pós-Graduação em Ciências da Saúde: Infectologia e Medicina Tropical, Faculdade de Medicina, UFMG, Brazil.

Life-long immunity to leishmaniasis in recovered patients has inspired the development of vaccines against disease. The present study aims to evaluate an amastigote-specific hypothetical *Leishmania* protein (LiHyp1) in an attempt to select a new candidate antigen for a serodiagnostic marker, as well as to compose a vaccine against visceral leishmaniasis (VL). For the serodiagnosis of canine VL (CVL), the rLiHyp1 (XP_001468941.1) protein was cloned, purified and used in the experiments. Sera samples from non-infected dogs living in an endemic area of leishmaniasis, from asymptomatic or symptomatic VL dogs, as well as sera from Leish-Tec® vaccinated dogs, and samples of animals experimentally infected by *Trypanosoma cruzi*, were used. The recombinant protein was recognizable by antibodies from sera of asymptomatic and symptomatic VL dogs, but it did not present any cross-reactivity to sera of dogs vaccinated with Leish-Tec, or with those experimentally infected with *Trypanossoma cruzi*. The immunogenicity and protective efficacy of rLiHyp1 plus saponin was evaluated in BALB/c mice challenged with *L. infantum*. Spleen cells of rLiHyp1 vaccinated mice showed a high production of IFN-, IL-12 and GM-CSF and, as compared to the control groups, the animals showed a significant reduction in the number of parasites in the liver, spleen, bone marrow and in the draining lymph nodes in the paws. Protection was associated with an IL-12-dependent IFN- production, produced mainly by CD4 T cells, which activate macrophages to eliminate the parasites. A decrease in the parasite-mediated IL-4 and IL-10 responses was also observed. The study showed that a new amastigote-specific *Leishmania* protein could be used for a more specific serodiagnosis of CVL and, when combined with a Th1-type adjuvant, has proven to be useful in the protection against VL.

Financial Support: Programa de Pós-Graduação em Ciências da Saúde: Infectologia e Medicina Tropical, Faculdade de Medicina, UFMG. FAPEMIG. CAPES. CNPq.