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REACTIVITY OF THE A2 AMASTIGOTE-STAGE-SPECIFIC RECOMBINANT PROTEIN OF LEISHMANIA DONOVAN WITH SERA FROM BRAZILIAN PATIENTS AND DOGS WITH VISCERAL LEISHMANIASIS. *CARVALHO, F. A. A., GUIMARÃES, D. A. M., vCHAREST, H., *GAZZINELLI, R. T., *TAVARES, C. A. P., i MATLASHEWSKI, G., wGENARO, O., JBARRAL, A., *PAGANINI, E. V. AND FERNANDES, A.P.* Dep. Bioquímica e Imunologia - w Dep. Parasitologia - ICB - UFMG - Dep. Análises Clínicas e Toxicológicas - Faculdade de Farmácia, UFMG - JFundação Oswaldo Cruz, Salvador - BA - *Dep. Bioquímica e Farmacologia, UFPI - Teresina, PI. i MacGill University, Quebec, Canada - vNational Institutes of Health, Bethesda, USA.

A2 proteins are composed predominantly by a repetitive element, which makes it an attractive antigen for diagnosis. These proteins are preferentially expressed in the amastigote stage of *Leishmania donovani*. The genes coding for A2 are also present in *L. mexicana* strains, but not in *L. major* or *L. braziliensis*. The fusion protein A2-GST has been previously evaluated in ELISA assays with sera of Indian and Sudanese patients with kala-azar and being reactive with 60 and 82% of the tested sera, respectively (GHEDIN et al., 1997). In this study the reactivity of A2, fused to GST or to a tag of histidins, was evaluated with a large panel of canine kala-azar sera (previously tested by IFA) and also of patients with visceral or tegumentar leishmaniasis, tuberculosis and hanseniasis. The recombinant proteins were expressed in *Escherichia coli* and purified with glutathione beads or by means of a nickel affinity chromatography. ELISA was performed with either A2-GST, GST, A2-His or total extracts of promastigotes of *L. chagasi* as antigens. Due to the high reactivity of kala-azar negative canine sera with GST alone, we found that A2-GST is not adequate to kala-azar serologic tests. However, using the A2-His as antigen, anti-A2 antibodies were detected by ELISA in 88% of the parasitological or IFA positive canine sera tested, in 40% of the sera of patients with visceral leishmaniasis and only in 15% of patients with the tegumentar disease. Using the total extract as antigen we found 95%, 60%, 55% of reactivity, respectively. The reactivity of A2 with sera of patients with *Mycobacterium* infection was 10%. Similar results were obtained by Western blotting analysis of selected sera. Our findings suggest that A2 may be an useful antigen to improve the serodiagnosis of visceral leishmaniasis.

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