

LEISHMANIA (VIANNIA) BRAZILIENSIS: CYSTEINE-PROTEINASE CAN BE LOCATED ONTO THE CELLULAR SURFACE OF PROMASTIGOTES.

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We have confirmed the presence of cysteine-proteinases (CPs) associated to membranes of *L. (V.) braziliensis*. Firstly, we have examined Concanavalin-A binding hydrophobic CPs from infective and non-infective promastigotes by DEAE chromatography. Both strains presented a peak of enzymatic activity coincident with the highest protein peak eluted from DEAE column. SDS-PAGE analysis of these peaks showed four main protein bands (63kDa, 43kDa, 30kDa and 27kDa). Enzymatic activity studies indicated that the 43kDa and 63kDa bands, from both strains, can hydrolyze gelatin and pEFLpNan at neutral pH and are sensitive to E-64; in addition, both enzymes were detected by a specific CPs antiserum in immunoblotting assay. Also, flow cytometry and immunocytochemistry assays with this antiserum revealed that these proteins may be located on the promastigote membrane surface. Moreover, the incubation of promastigotes with phospholipase C reduced the number of CPs-positive cells. Both anti-cross-reacting determinant (CRD) and anti-CPs antisera recognized the 63kDa and 43kDa bands in the supernatant from phospholipase C-treated cells, suggesting that isoforms of these proteins are glycosylphosphatidylinositol (GPI)-anchored to the plasma membrane. Finally, we determined that GPI-anchored CPs are in the detergent-resistant lipid pools. Financial Support: CAPES and PAPES IV (Fiocruz/CNPq-400148/2006-4).