CELLULAR LOCALIZATION AND IDENTIFICATION OF GP63 HOMOLOGOUS METALLOPROTEASES IN Leishmania (Viannia) braziliensis STRAINS

<u>Patricia Cuervo</u>^{1,2}; André L.S. Santos³; Carlos R. Alves⁴; Gustavo C. Menezes⁵; Bianca A. Silva³; Constança Britto⁴; Octavio Fernandes²; Elisa Cupolillo¹; Jose Batista De Jesus⁴

Departamentos de ¹Imunologia, ²Medicina Tropical, e ⁴Bioquímica e Biologia Molecular, IOC-FIOCRUZ, RJ, Brasil ³Instituto de Microbiologia Prof. Paulo de Góes, UFRJ, RJ, Brasil ⁵Instituto de Biofísica Carlos Chagas Filho, UFRJ, RJ, Brasil.

Leishmania (Viannia) braziliensis is the major causative agent of American cutaneous leishmaniasis, a disease that encompasses a broad spectrum of clinical manifestations. In previous study, we showed that *L. braziliensis* strains, isolated from patients with distinct clinical manifestations, display different pattern of metalloprotease activities. Here we investigated the cellular localization of these molecules and their relation to the major surface protease (gp63) of Leishmania. A comparative analysis of metalloprotease expression among clinical isolates was also performed. Western blot analysis, using an anti-gp63 antibody, revealed polypeptides with a similar profile to that of the zymographic analysis, and additional bands without proteolytic activity. Flow cytometry and confocal fluorescence microscopy analyses indicated the presence of gp63 homologous metalloproteases on the parasites cell surface and revealed differences in the expression level of such molecules among the strains. Cellular distribution of metalloproteases, assessed by confocal analysis, showed the existence of intracellular gp63 homologous metalloproteases, predominantly located near to the flagellar pocket. Finally, it was observed that differential zymographic profiles of metalloproteases exhibited by L. braziliensis strains remain unaltered still after continuous and prolonged in vitro culture.