LEISHPORIN, A PORE-FORMING-PROTEIN OF *LEISHMANIA AMAZONENSIS*: BINDING TO LIPID BILAYERS, STRATEGY OF PURIFICATION AND NEW INSIGHTS ON ITS IDENTITY AND MECHANISM OF LYSIS.

Castro-Gomes, T.[1]; Giulianetti-Almeida, M.P.[1]; Silva, R.A.[1]; Andrade, H.M. [1]; Chapeaurouge, A.[4]; Perales, J.[4]; Noronha, F.S.M.[2]; Frézard, F.[3]; Horta, M.F.[1]

Departamentos de [1]Bioquímica e Imunologia, [2]Microbiologia, [3]Fisiologia e Biofísica, ICB-UFMG; [4]FIOCRUZ-RJ.

Leishporin is a pore forming protein of *L.amazonensis*, optimally active at pH5.5 and 37ºC. It may act in the mammalian host and be involved in the rupture of fagolisosome and macrophages, an important step of the infection. To lyse cells, leishporin must be activated by proteolysis which seems to render the molecule able to bind to lipids. We used these features in an attempt to purify the cytolysin and further study its lytic mechanism. We found that liposomes w/o cholesterol remove all the hemolytic activity, while binding 5 major proteins from promastigote extracts. Calcein-containing liposomes are lysed after binding these proteins, which indicate that leishporin is among them. MALDI-ToF-ToF analysis of 4 of these proteins, identified 3 of them: gp63, GAPDH and ß-tubulin. The fourth (48kDa), an unknown protein of *L.amazonensis*, may be leishporin. Previous results indicated that leishporin is quite resistant to proteolysis. Taking advantage of this resistance, we have treated extracts with high concentrations of proteinase-K, which destroyed all proteins bigger than 20 kDa but did not reduce the lytic activity. This result demonstrates that the smaller lytic unit of leishporin is smaller than 20kDa (probably derived from a larger precursor).