Gene expression and trafficking of the COOH-terminal region of cysteine proteinase B of *Leishmania (Leishmania) amazonensis* during *in vitro*differentiation

Santos-de-Souza, R.[1]; Souza-Silva, F.[1]; Pereira, B.A.S.[1]; Lopes-Ribeiro-Guimarães, M.[1]; Cysne-Filkenstein, L.[2]; Pereira, M.C.S[3],; Oliveira-Jr F.O.R[3]; Charret, K.S[1] ;Côrtes, L. M. C.[1] Alves, C.R.[1]

[1]Laboratório de Biologia Molecular e Doenças Endêmicas, [2]Laboratório de Imunoparasitologia and [3]Laboratório de Ultra-estrutura Celular, Instituto Oswaldo Cruz/Fiocruz-IOC, Rio de Janeiro, Brazil

Introduction: *Leishmania (Leishmania) amazonensis* presents adaptive mechanisms guided by their proteinases, as cysteine proteinase B (CPB), a well studied cathepsin L in these parasites. **Objectives**: The goal of this work is, to verify *(i)* whether gene expression *of cpb* (Pereira *et al*., 2012) varies along the parasite differentiation, and *(ii)* the destination of the COOH-terminal region of CPB after its final processing. **Material and Methods:** *In vitro* differentiation of promastigotes to amastigotes was performed using a combination of pH variation and temperature shock, followed up by optical and fluorescence microscopy, and flow cytometry. The *cpb* gene expression was evaluated at different time points by qRT-PCR. To assess cellular trafficking of the COOH-terminal region of CPB, specific polyclonal antibodies were produced by inoculation of rcyspep, obtained by a pET28-a expression system in *E. coli*, in mice. These specific Abs were then used to analyze subcellular fractions and culture supernatants of both promastigotes and amastigotes by surface plasmon resonance assays. **Results and Discussion:** Flow cytometry data, using cellular morphology and cytoplasmic granulation parameters, revealed high level of parasite differentiation throughout 96h in culture**.** We also observed that accompanying the morphological differentiation, there was an increase in the levels of *cpb* transcripts. In the course of this work, was opportune to assess pro-protein or processed CPB, in parasite's subcellular fractions and in culture supernatant. Comparatively, culture supernatants of amastigotes and promastigotes exhibited lower proportions of rcyspep. Additionally, we observed that parasite's proteins that were recognized by anti-IgG rcyspep and bound to trans-epoxysuccinyl-L-leucylamido(4-guanidino)butane were detected in higher quantities in promastigote culture supernatant. **Conclusions**: Collectively the results indicate that amastigotes of *L. (L.) amazonensis* exhibit higher expression of *cpb* gene than promastigote. Both parasite forms release the cyspep polypeptide to the extracellular environment, but only promastigotes releases that polypeptide still associated with CPB enzyme.

Key words: *Leishmania (L.) amazonensis,* cysteine proteinase, gene expression
Support : CNPq, FAPERJ and CAPES