Shift in expression of the transcription factor Cut-like homeobox 1 (CUX1) and miR-721 profile in macrophages infected with *Leishmania amazonensis*

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INTRODUCTION

Leishmania parasite lives inside phagocytic cells, such as macrophages, subverting inflammatory response to change infection outcome. The parasite can modulate host gene expression, altering transcriptional, mediated by transcription factors, and post-transcriptional mechanisms mediated by microRNAs to survive inside host cells. The murine BALB/c macrophage infection with *L. amazonensis* leads to an increase in miR-721 expression, an intronic miRNA into *Cux1* gene, and it negatively modulates *Nos2* expression and consequently lowers NO production, increasing infectivity. Cux1 codes for CUX1, a transcription factor that activate or repress the transcription of genes in many pathways. Therefore, we analyze the expression profile of *Cux*1 mRNA and miR-721 precursor in bone marrow derived macrophages from BALB/c and C57BL/6 mice infected with *L. amazonensis*, and stimulated by LPS. In the next steps, we will study the profile of CUX1 protein expression.

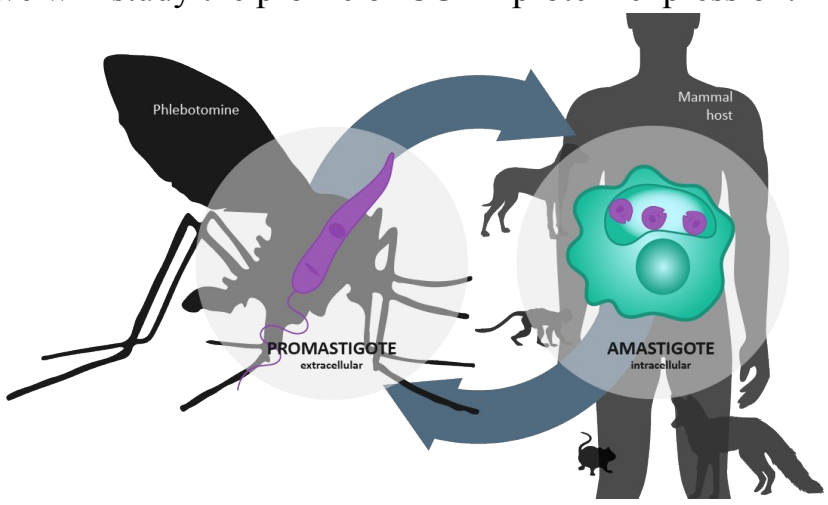

Figure 1 - *Leishmania* life cycle. The parasite in its promastigote form lives in the digestory tract of the Phlebotomine sand fly and is transmitted during the blood meal into skin of the mammals host. In the skin, phagocytic cells, as macrophages, phagocytosed the parasite that differentiates into amastigote form inside the phagolysosome.

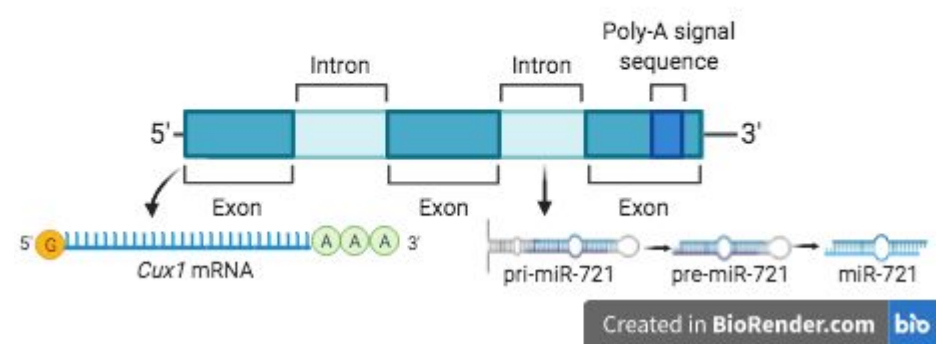

Figure 2 - Schematic representation of *Cux1* gene and miR-721 biogenesis. After splicing, the exons will form Cux1 mRNA and the intron containing unprocessed miR-721.

MATERIALS AND METHODS

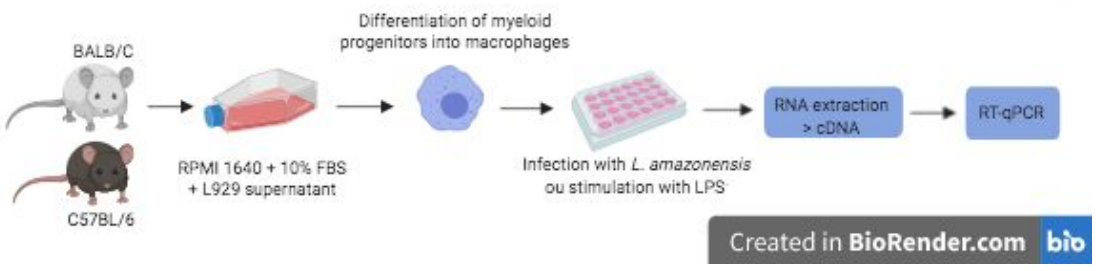

Figure 3 - Schematic representation of *in vitro* infection. Bone marrow is collected from BALB/c and C57BL/6 mice, the cells are kept in culture with RPMI 1640 medium supplemented with 10% SFB and supernatant of L929-conditioned media for a week to differentiate. The cells are distributed into 24-well plates and infected with *L. amazonensis* wild-type (MOI 5:1) or LPS. The RNA from these cultures is extracted and converted into cDNA, which we use for real time quantitative PCR. The GraphPad was used for T-Student test analysis.

RESULTS

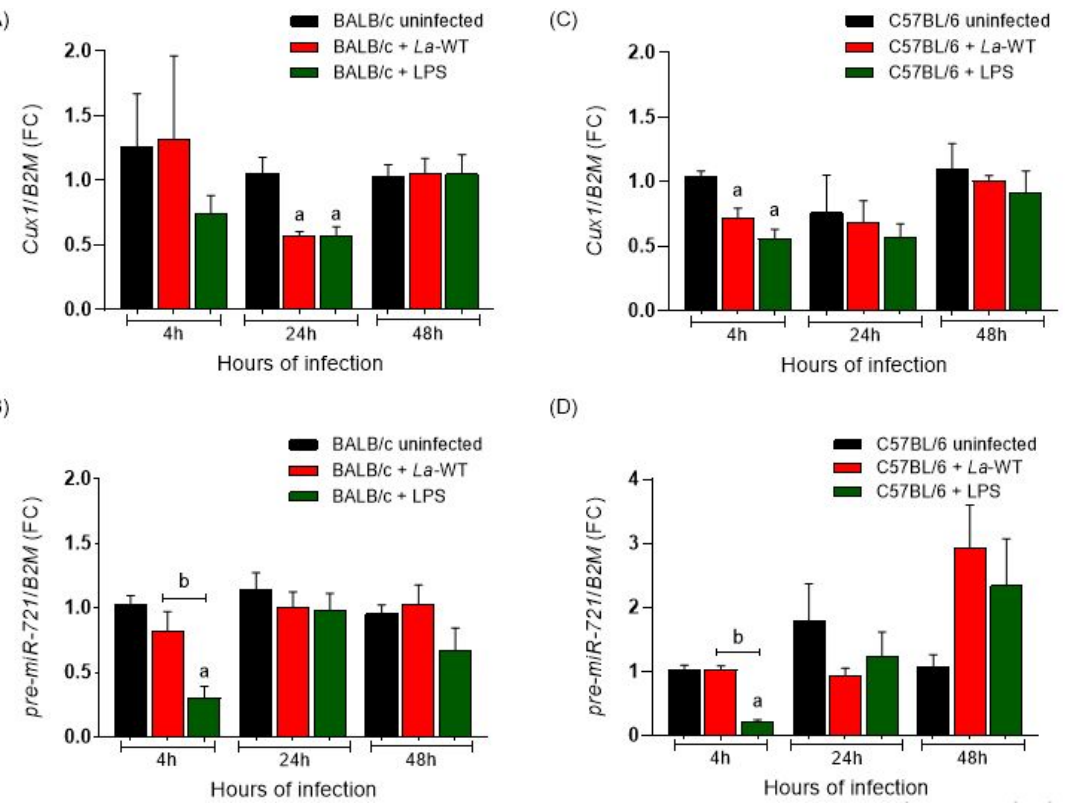

Figure 4 - Comparative analysis of genetic expression of Cux1 and miR-721 precursor in murine macrophages infected with *Leishmania amazonensis* and stimulated with LPS. Analysis of relative expression (ΔΔCt), gathered from qRT-PCR reaction, that used cDNA samples from BALB/c or C57BL/6 mice BMDMs infected by *L. amazonensis* or stimulated with LPS. The bars are representative of Fold Change (FC) values ± SEM, using uninfected macrophages as reference to each condition (FC=1) The graphics are representative of 2 independent experiments (n=6-8). a = $p \leq 0.05$, comparison between infected or stimulated samples and non-infected macrophages, b = $p \leq 0.05$, comparison between stimulated with LPS and infected macrophages.

CONCLUSIONS

The levels of *Cux1* was decreased in infected BALB/c mice after 24 h of infection, a mice strain susceptible to infection; while in C57BL/6 macrophages, *Cux1* levels are maintained. LPS stimulation regulated Cux-1 levels in the same way of infection.

The levels of miR-721 precursor was maintained in infected macrophages, but was reduced in LPS stimulation.

The miR-721 expression pattern worked in a distinctly way, its levels in control samples are small to none. It indicates that the genetic background of the macrophages impact the regulation of *Cux1* levels during infection and that the biogenesis of miR-721 is independent.

Also, in silico analysis shows that both the TF CUX1 and mature miR-721 are able to target genes related to to the immune response, such as *Tnf* and *Nos2*.

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