Directing the killing of *Leishmania*: one biotechnological advance.

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**Abstract**
Molecular mechanisms involved in gene expression control in *Leishmania* are unusual and not fully comprehended. In the study, we characterized a new method to drive parasite death specifically to amastigote stage. The pFL-AMA plasmid including sequences encoding for toxic proteins were used to kill the parasite specifically to amastigote stage in *vitro* and in *vivo* conditions. We show in this work that this plasmid confers a restricted amastigote-stage gene expression in *Leishmania*, representing a genetic method capable to drive a programmed parasite death to pathogenic intracellular form.

**Key words:** *Leishmania*, plasmid, toxic gene

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**Introduction**

The parasitic protozoan *Leishmania* causes a wide spectrum of diseases known as leishmaniasis. Infection is initiated by inoculation of promastigotes forms from insect vector to host skin, where cells phagocyte the parasites and triggers the transformation to the amastigote stage. Amastigote differentiation process is driven by drastic switch in gene expression, activating and silencing life-stage specific genes. \(^1\)

**Results and Discussion**

Taking the gene expression control of pFL-AMA plasmid \(^2\) (which restrict expression specifically to amastigote stage), two putative toxic gene products were included in plasmid, obtained pFL-AMA-Toxic1 or –Toxic2. *Leishmania infantum* promastigote were transfected with both pFL-AMA-toxic plasmids and selected in presence of puromycin. As control, untransfected parasites or transfected with fluorescence version of the plasmid were used.

Firstly, we prove that promastigotes transfected with pFL-AMA-toxins are not affecting the viability using growth curves and propidium iodide. However, during axenic differentiation to amastigote (pH 5.5 and 37°C) parasites carrying toxins plasmids showed drastic decrease in viability, according to their capability to metabolize MTT and re-differentiation to promastigote (pH 7.2 and 26°C) compared with parasites controls.

Moreover, DH82 macrophage line was infected with promastigotes carrying toxins plasmids or controls and the infections capacity were evaluated using Giemsa staining along six days. No significant differences in its capacity to infect macrophages were observed between parasites transfected with toxins plasmids versions and controls.

Finally, to demonstrate parasite “suicide” *in vivo*, susceptible female Balb/c mice were infected with promastigotes carrying toxins plasmids or controls by intraperitoneal route. After ten weeks mice were euthanized to analyze parasite burden in spleen. Around fifty percent of mice inoculated with toxins plasmids did not present parasite, as demonstrated by limiting dilution and PCR. On the other hand, surviving parasites were cultivated in presence or absence of puromycin and no life-forms were observed in cultures with puromycin, except parasites extracted from mice infected with pFL-AMA-fluorescence plasmid. This manner, we demonstrated that amastigotes carrying pFL-AMA-Toxins plasmids also did not survive into the mammalian host.

**Conclusions**

We show in this work that pFL-AMA plasmid confers a restricted amastigote-stage gene expression in *Leishmania*. This system could permit new approaching to explore amastigote differentiation process and parasite-host interactions. In this manner, we describe for first time, a genetic method capable to drive a programmed parasite death to pathogenic intracellular form.

**Acknowledgement**

This work was supported by grant from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq): CHAMADA DE PROJETOS MEC/MCTI/CAPES/CNPQ/FAPS Nº 70/2013.

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