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Leishmania tarentolae as a vehicle for the delivery of RNA molecules to Antigen Presenting Cells (APCs)

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Background

Due to their ability to target phagocytic cells of the myeloid line, the protozoans of the genus *Leishmania* could be exploited as a vehicle for the delivery of RNA and other molecules to macrophages and dendritic cells (DCs). *Leishmania tarentolae* is a paramammals andiles, is not pathogenic to humans and other mammals, and is classified as a biosafety level class I organism. This parasite has already been developed as a vaccine platform, as a tool to produce antigens from different viruses, and for the delivery of the antigens to dendritic cells and lymph nodes. However, the potential of *L. tarentolae* to deliver RNA molecules to APCs, activating RNAi-mediated gene knockdown, is still unexplored.

Materials and methods

In our study, we carried out a proof-of-principle experiment, loading promastigotes of *L. tarentolae* with heterologous RNAs and testing whether these determined an interference in gene expression in the targeted APCs. Firstly, we set a protocol to load small interfering RNAs into *L. tarentolae* cells using electroporation. Secondly, we carried out an infection experiment to assess the ability of siRNA-loaded promastigotes of *L. tarentolae* to enter macrophages and deliver the RNA molecules. For this purpose, we used fluorescent siRNA, visible under confocal microscopy. Upon confirming the successful delivery of fluorescent siRNA to APCs, we proceeded to transfect *L. tarentolae* with different siRNAs targeting the mouse Mannose receptor C type 1 (Mrc1) mRNA. Subsequently, we determined the expression of this gene.

Results

The transfection protocol of *L. tarentolae* using electroporation was successful for the loading of siRNA in *L. tarentolae* cells (Figure 1). Moreover, our experiments show that siRNA-loaded *L. tarentolae* maintain their capability to target macrophages, delivering the fluorescent siRNA to the cells at different time points. The fluorescence was visible either 4h or 24h after the infection of macrophages with siRNA-loaded *L. tarentoale* (Figures 2 and 3). Finally, the gene expression experiment showed that there was no downregulation both after 4h and 24h from the infection while we appreciated a weak downregulation of the Mrc1 gene 48h after the infection of macrophages with siRNA-loaded *L. tarentolae*.

Conclusions

In conclusion, we set up a successful protocol for the loading of siRNA in *L. tarentolae* using electroporation. Moreover, the infection experiment confirmed that the siRNA-loaded promastigotes of *L. tarentolae* were able to enter the macrophages and deliver the RNA molecules into them. Even with the successful loading and delivery of RNA molecules to macrophages, we only appreciated a weak downregulation of the Mrc1 gene in the gene expression experiment. The weak downregulation could be either caused by the formation of aggregates of siRNAs during the electroporation or by the choice of the Mrc1 target which is a recognition receptor and not a cytokine expressed during the inflammation. Future steps will include assays on different loading protocols and on different genes to be downregulated.

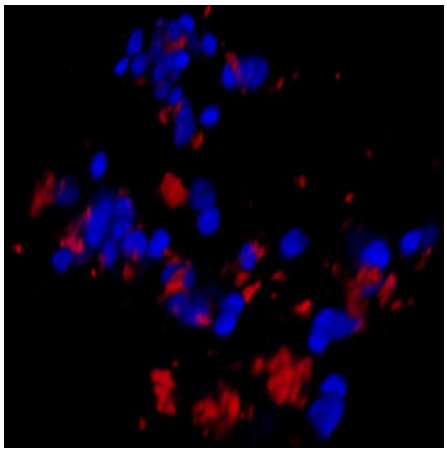

Figure 1 - Transfection of *L. tarentolae* with Cy3 marked siRNA. Red: Cy3 marked siRNA; Blue*: L. tarentolae* nuclear DNA (DAPI)

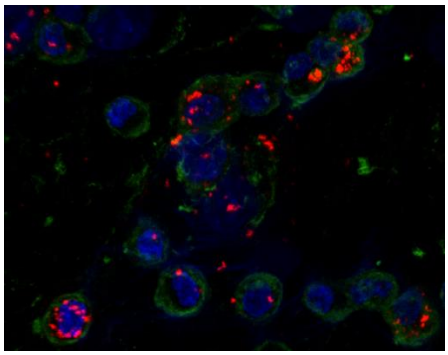

Figure 2 - Macrophages infected with *L. tarentolae* previously loaded with Cy3 marked siRNA 4h after the infection. Red: *L. tarentolae* cells loaded with the marked siRNA; Blue: macrophage nuclear DNA (DAPI); Green: microtubules of macrophages (phalloidin)

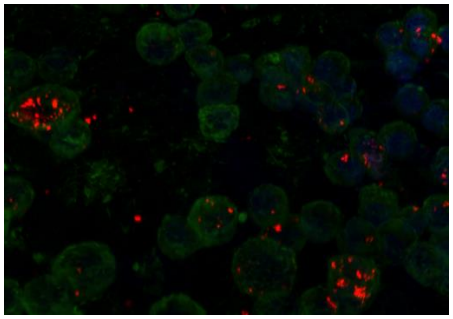

Figure 3 - Macrophages infected with *L. tarentolae* previously loaded with Cy3 marked siRNA 24h after the infection. Red*: L. tarentolae* cells loaded with the marked siRNA; Blue: macrophage nuclear DNA (DAPI); Green: microtubules of macrophages (phalloidin)

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Conflict of interest: We declare no conflict of interest.