

MEMBRANE RAFTS AND LYSOSOMES DURING *Trypanosoma cruzi* INVASION IN PRIMARY CARDIOMYOCYTE

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Trypanosoma cruzi, etiological agent of Chagas' disease, can invade several types of cells including fibroblasts, myocytes and Schwann cells. It has already been described the importance of molecules and proteins shed by the parasite [1], as well as the participation of host cell receptors [2,3,4] in parasite attachment to and internalization in host cells. The state-of-art of the interaction between *T. cruzi* and host cells includes the involvement of membrane rafts, sphingolipid and cholesterol enriched plasma membrane domains, in parasite cell adhesion and intracellular trafficking [5,6].

The main goal of this work was to evaluate if cholesterol depletion would interfere equally in *T. cruzi* entry into fibroblast cell lineage and primary murine cardiomyocytes, as well as its influence in lysosomal recruitment and fusion during host cell infection.

Our results suggested that cholesterol depletion, mediated by M β CD (Methyl-beta cyclodextrin), led to a reduction in *T. cruzi* cell invasion. In addition to that we verified that this impairment was not due to an isolated effect of the drug since incubation with HyCD (hydroxypropyl- gamma cyclodexytrin), a similar cyclodextrin with less affinity for cholesterol, did not significantly diminished parasite entry in both cell types used. Although we have shown differences in cellular invasion after cyclodextrin treatment we did not detect statistical differences regarding lysosomal recruitment and fusion in view of the high heterogeneity found inside treated groups. This data suggests two possible and non-excluding explanations for the role displayed by cholesterol during *T. cruzi* cell invasion. 1. When *T. cruzi* attaches to cholesterol enriched regions it may entry cells through membrane invagination followed by lysosomal fusion in a process different from the fusion at parasite adhesion site. 2. Cholesterol depletion dysregulates lysosome exocytosis phenomena leading to random fusion at plasma membrane. If a lysosome fuses at the attachment site of *T. cruzi* it can enter in host cell. However, its chances of internalization are lower than if that fusion had occurred in a regulated way as in control cells.

Acknowledgements: CNPq and WHO for financial support.

References:

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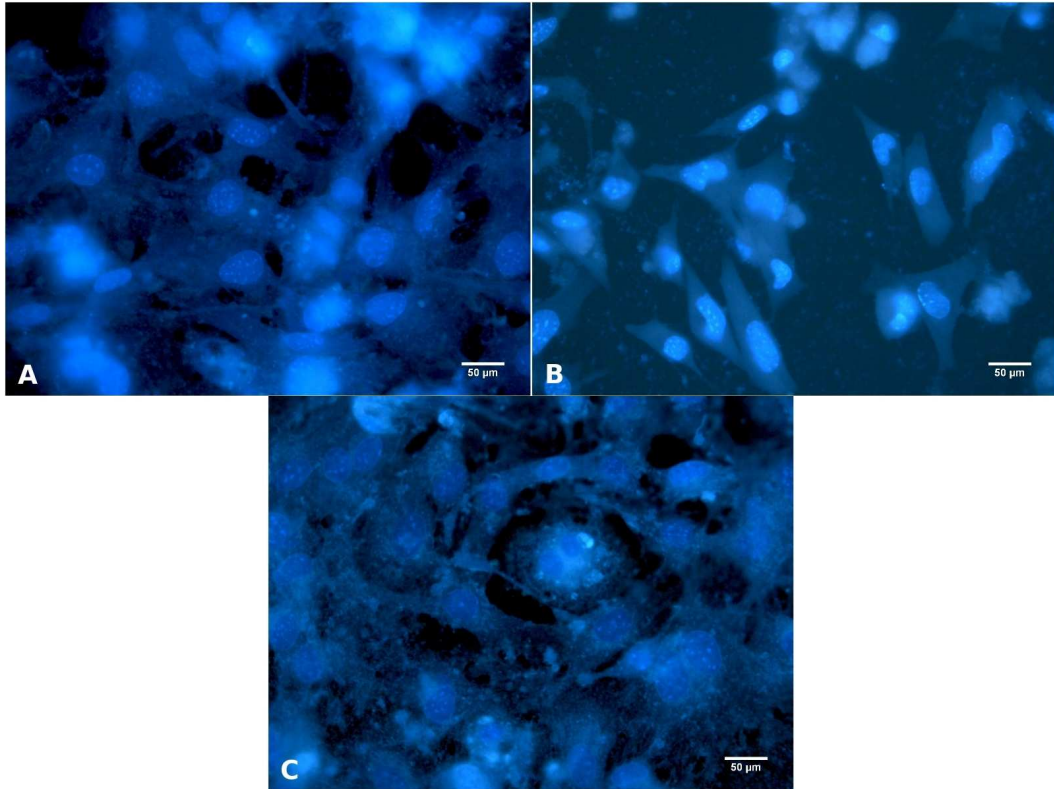


Figure 1 – Filipin III labeling after cholesterol depletion and replenishment. The blue stained regions show cholesterol labeling and the round structures are nuclei of murine cardiomyocytes labeled with DAPI. A) Cholesterol labeling of control cells. B) Cholesterol labeling of cells after depletion with M β CD 15 mM. C) Cholesterol labeling after depletion followed by replenishment with 0.05 mM of water-soluble cholesterol. Scale bar = 50 μ m.

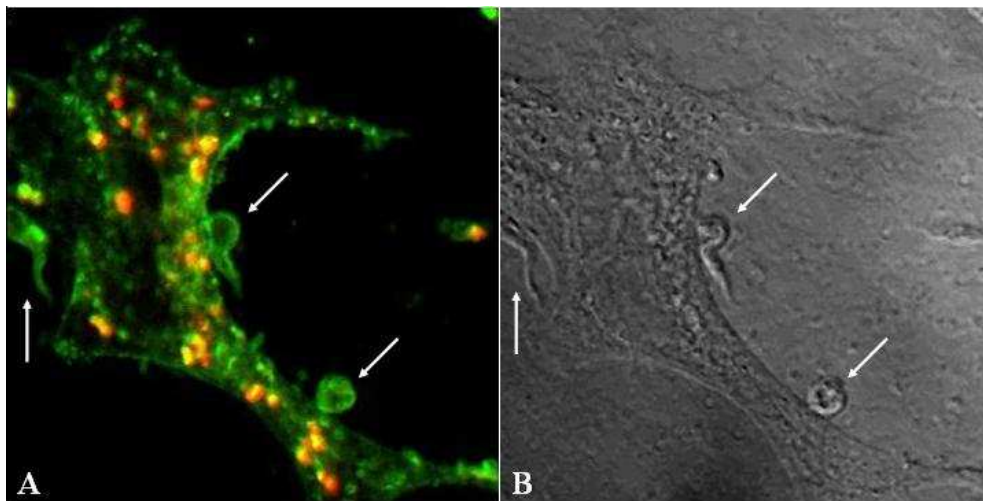


Figure 2 – Co-localization of lysosomal marker LAMP I (red), membrane rafts (green) and *T. cruzi* (bright field) observed in this image captured by confocal microscope A) *T. cruzi*, indicated by arrows, are co-localized with membrane rafts. We can also see co-localization with *T. cruzi* and LAMP I (yellow spots near attachment site of the parasite in its host cell B) Bright field image of *T. cruzi* trypomastigotes indicated by arrows. Scale bar = 50 μ m .