

## *Trypanosoma cruzi* infection alters cardiac caveolin-3 expression

Adesse D<sup>1,2\*</sup>, Garzoni LR<sup>1</sup>, Spray DC<sup>2</sup>, Tanowitz HB<sup>3</sup>, Meirelles MN<sup>1</sup>

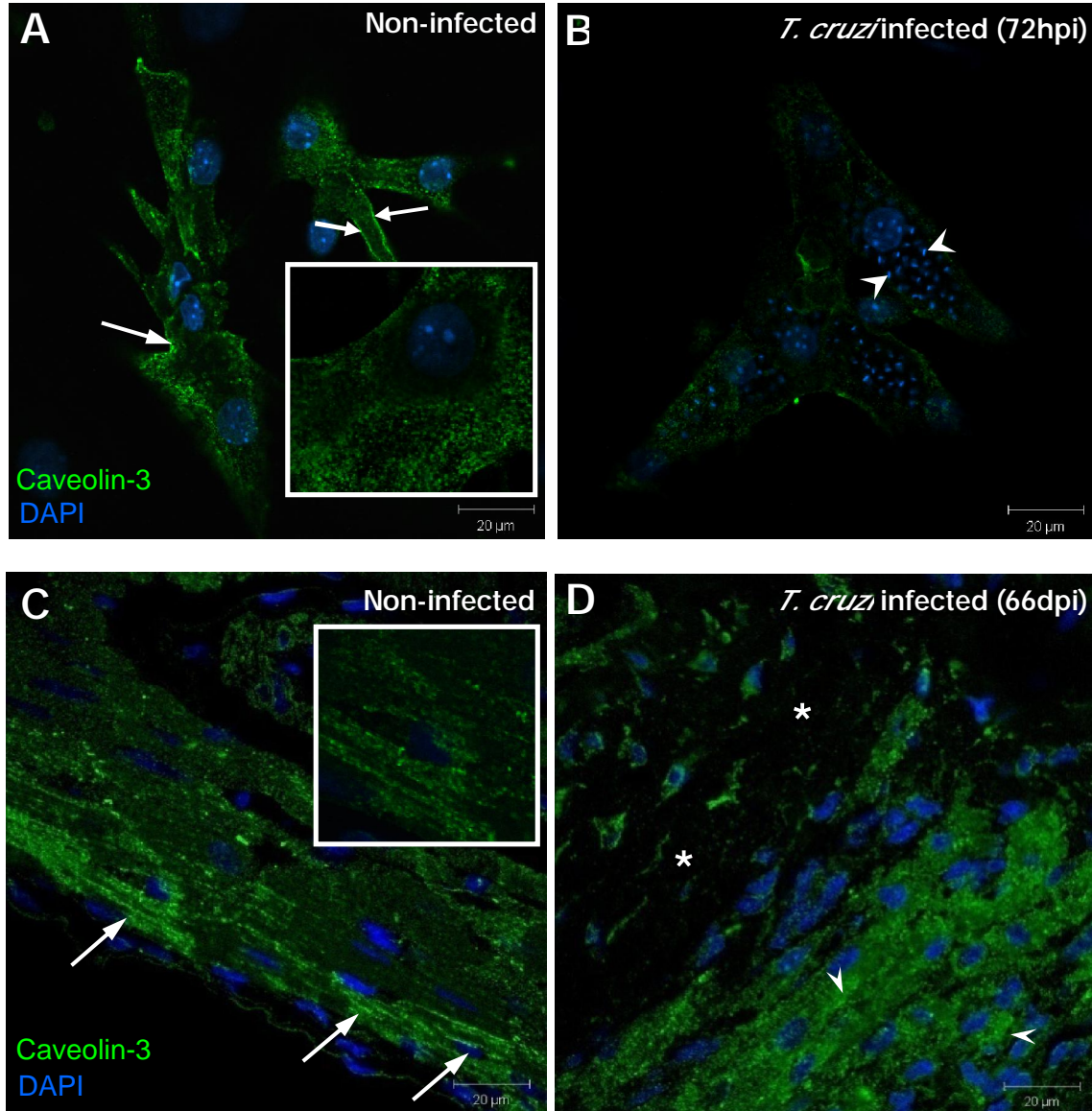
- 1) Laboratório de Ultraestrutura Celular, Instituto Oswaldo Cruz, Fiocruz (RJ)
- 2) Department of Neuroscience, Albert Einstein College of Medicine, Yeshiva University (NY)
- 3) Department of Pathology, Albert Einstein College of Medicine, Yeshiva University (NY)

Caveolae are plasma membrane invaginations enriched in cholesterol, sphingolipids, and the marker protein caveolin (cav). Caveolae and cavs are implicated in transcytosis of macromolecules, cholesterol transport and signal transduction. Knock-out mice for cav-3 (muscle specific) develop progressive cardiomyopathy with ventricular wall thickening and increased levels of the extracellular signal-regulated kinase (ERK 1/2) [1], which is involved in cardiac remodeling. Infection with *Trypanosoma cruzi* leads to acute myocarditis and chronic cardiomyopathy. Acutely infected mice displayed decreased mRNA levels of cardiac caveolins and increased mRNA levels of ERK [2]. Interestingly, *T. cruzi* decreases phosphorylated ERK (pERK) in human fibroblasts, but the infection of endothelial and smooth muscle cells increases pERK expression [3]. Since cardiac myocytes are important target of *T. cruzi* in the *in vivo* infection, we studied the effect of the infection in caveolin-3 and pERK expression in cultured cardiac myocytes and hearts of chronically infected mice. Infected cultures displayed reduced caveolae and caveolin-3 abundance at 48 (74%) and 72 (51%) hours post infection (hpi) as revealed by electron and confocal microscopy and further confirmed by Western blot analyses. Cav-3 levels were also 50% decreased in the hearts of mice during the intermediate phase of the *in vivo* infection (66dpi) as revealed by confocal microscopy and western blot analysis. ERK phosphorylation was progressively increased during the *in vitro* infection starting at 2hpi and reaching a 3-fold increase at 72hpi. Likewise we observed a drastic (3.5-fold) increase in ERK phosphorylation in the hearts of infected mice when compared to age-matched controls. Our data indicate that infected cardiac myocytes contribute to increased pERK observed in the *in vivo* infection. Moreover, the decreased expression of muscle-specific caveolin-3 induced by *T. cruzi* may be involved in the development of the chagasic cardiomyopathy.

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### References:

- [1] Woodman SE, Park DS, Cohen AW, Cheung MW, Chandra M, Shirani J, Tang B, Jelicks LA, Kitsis RN, Christ GJ, Factor SM, Tanowitz HB, Lisanti MP. J Biol Chem. 2002 Oct 11;277(41):38988-97.
- [2] Nagajothi F, Desruisseaux M, Bouzahzah B, Weiss LM, Andrade Ddos S, Factor SM, Scherer PE, Albanese C, Lisanti MP, Tanowitz HB. Cell Cycle. 2006 Jan;5(1):107-12.
- [3] Mukherjee S, Huang H, Petkova SB, Albanese C, Pestell RG, Braunstein VL, Christ GJ, Wittner M, Lisanti MP, Berman JW, Weiss LM, Tanowitz HB. Infect Immun. 2004 Sep;72(9):5274-82.



**Figure 1: Caveolin-3 (Cav-3) expression is decreased after *T. cruzi* infection.** Cultured cardiac myocytes were infected for 3 days. **A:** Confocal Laser Scanning analysis of caveolin-3 distribution in uninfected cardiac myocytes, fixed and stained after 96 hours in culture cells presented abundant peripheral staining (white arrows). **B:** After 72 hours of infection, Cav-3 signal was reduced among highly parasitized cardiac myocytes, present mostly at cell periphery and with reduced presence of caveosomes. **Cav-3 in infected hearts.** Hearts obtained from infected (Brazil strain) and uninfected CD-1 mice were embedded in paraffin and processed for immunohistochemistry. Sections were stained with anti-Cav-3 antibody (green) and DAPI (blue) and observed by confocal microscopy. **C.** Uninfected cardiac tissue displayed intense Cav-3 staining of cardiac myocytes, especially in pericardial area with T-tubule patterns (arrows). **D.** This staining pattern was altered in the hearts of infected mice infected where areas of tissue damage were evident (\*). In other areas Cav-3 staining was observed mainly in the cytoplasm of cardiac myocytes (arrows). Bars=20μm.