Trypanosoma cruzi-cardiomyocytes: New Contributions Regarding a Better Understanding of this Interaction

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The present paper summarizes new approaches regarding the progress done to the understanding of the interaction of Trypanosoma cruzi*-cardiomyocytes. Mannose receptors localized at the surface of heart muscle cell are involved in binding and uptake of the parasite. One of the most striking events in the parasite-heart muscle cells interaction is the disruption of the actin cytoskeleton. We have investigated the regulation of the actin mRNA during the cytopathology induced in myocardial cells by the parasite.* T. cruzi *invasion increases calcium resting levels in cardiomyocytes. We have previously shown that* Ca^{2+} *ATPase of the sarcoplasmic reticulum (SERCA) is involved in the invasion of* T. cruzi *in cardiomyocytes. Treating the cells with thapsigargin, a drug that binds to all SERCA ATPases and causes depletion of intracellular calcium stores, we found a 75% inhibition in the* T. cruzi*-cardiomyocytes invasion.*

Key words*: Trypanosoma cruzi* - cardiomyocytes - calcium - SERCA ATPases - mannose receptor - mRNA regulation

Cardiomyocytes (CM) are the main targets for *Trypanosoma cruzi* infection in Chagas disease. It is generally accepted that calcium ions play an important role throughout the contraction-relaxation cycle of muscle cells. Furthermore, the existing literature shows the importance of calcium ions during the interaction of *T. cruzi* with different cell types (Tardieux et al. 1994, Moreno et al. 1994, Burleigh & Andrews 1995, Yoshida 1997, Garzoni et al. 1998, Caler et al. 1998). Sarcoplasmic reticulum (SR) performs calcium sequestration by a calcium stimulated ATPase (SERCA). Thapsigargin is a tumor promoting sesquiterpene lactone that binds itself to all SERCA ATPases leading to irreversible inhibition of their activity. When this drug was used during the interaction of *T. cruzi*, heart muscle cells showed a marked inhibition of the infection (Silva & Meirelles 1998). When invasive forms of *T. cruzi*-amastigote and trypomastigote enter in contact with the surface of host cells they induce a special endocytic process which leds to the formation of a vacuole known as parasitophorous vacuole (PV). It has been described that during the formation of the PV some host cell plasma membrane components will be part of the membrane of the PV, but other components will not be found in the membrane which lines the PV. The involvement of glycoconjugates in this invasion have shown that a number of carbohydrates – such as galactose, N-acetyl galactosamine, N-acetyl glucosamine, mannose and sialic acid – components of glycoproteins and glycolipids present on the surface of both parasite and/or host cell may participate in the interaction process (Andrews & Coli 1981, Crane & Dvorak 1982, Vilalta & Kierzenbaum 1983, 1985, Zingales & Coli 1985, Meirelles et al. 1986, Piras et al. 1987, Ouassi 1988, Vermelho et al. 1992, Barbosa & Meirelles 1992, Schenkman & Eichinger 1993, Vermelho & Meirelles 1994, Soeiro et al. 1995). Mannosyl residues and their counter-receptors play an important role during the interaction of *T. cruzi* and host cells. They have been implicated as ligands during host cell invasion and have been localized at the PV membrane of macrophages and cardiomyocytes (Barbosa & Meirelles 1992, Araujo-Jorge et al. 1993). We analysed the mannose receptor (MR) in primary cultures of heart muscle cells and during

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its interaction with *T. cruzi* (Soeiro et al. 1999). The actin cytoskeleton plays an important role in many cellular processes including the parasite invasion in mammalian cells. We have described alterations in the cytoskeleton organization in the cardiomyocytes *T. cruzi*-infected (Pereira et al. 1993). We have investigate the regulation of the actin mRNA during the cytopathology induced in myocardial cells by the parasite.

MANNOSE RECEPTORS AT THE SURFACE OF CARDIOMYOCYTES

Carbohydrate-protein interactions play an important role in a large variety of biological and pathological events. The mannose receptor (MR) is able to recognize a wide range of Gram-negative and Gram-positive bacteria, mycobacteria, yeast and parasites. Previously, it was thought that its expression was restricted to tissue macrophage but now it is known that it is expressed in other cells as subsets of endothelial cells, smooth muscle cells, retinal pigment epithelium, kidney mesangial cells, myeloid cells and Kaposi sarcoma cells (reviewed in Stahl & Ezekovitz 1998). We analysed the mannose receptors in primary cultures of cardiomyocytes (CM) from mouse embryos for studies of parasite-cell interaction (Meirelles et al. 1986, Soeiro et al. 1997). Horseradish peroxidase (HRP) was used as ligand to localize the MR. HRP is a glycoprotein devoid of phosphorylated carbohydrates and rich in mannose and N-acetylglucosamine groups. The role of mannosyl residues and MR during this interaction was tested by carbohydrate addition during CM-parasite uptake. Infection rates were analysed by the addition of 10 or 50 mM of D-mannose during the infection for 24 hr at 37°C in Eagle's medium without serum. After host cell-parasite interaction, cultures were fixed in Bouin, stained with Giemsa and the percentage of infected CM was calculated. Ultrastructural studies for MR detection were also performed using HRP coupled to colloidal gold particles (HRP-Au) as probe. Non-infected CM were first incubated incubated with HRP-Au for 30 min at 4°C and then the temperature was elevated to 37°C and followed for 1 and 4 hr. Some cultures were incubated with HRP-Au for 30 min at 4°C, washed and infected for 24 hr at 37°C with metacyclic trypomastigote forms.

The addition of D-mannose to the interaction medium impaired the parasite uptake by the CM, being the concentration of 10mM proved to be the most effective. The ultrastructural studies showed, in non-infected CM, the tracer over the sarcolemma, near or inside uncoated vesicles, in caveolae and also in endocytic vesicles. In infected CM, gold particles were seen at the site of parasite-sarcolemma association and also inside the parasitophorous vacuole containing parasite. These results indicated that the mannose receptors localized at the surface of CM are involved in the binding and uptake of *T. cruzi* (Soeiro et al. 1999).

T. CRUZI INFECTION AFFECTS mRNA REGULATION IN CARDIOMYOCYTES

Our in *vitro* system of cardiac cell cultures allowed to study biological and molecular events in the *T. cruzi*-CM. When this parasite invades and develops its intracellular cycle within heart cells, many alterations occur resulting in cytoskeleton disruption and myofibrillar breakdown in regions where the intracellular parasites are located, followed by the formation of actin polygons (Pereira et al. 1993). Then, we hypothesized that the regulation of actin mRNA would be, in the same way, affected. Our aim was to understand the molecular basis for the regulation of cytoskeleton proteins mRNA during CM differentiation and also after their infection with *T. cruzi*. Total cellular RNA was isolated from normal and *T. cruzi* infected CM using Tri-Reagent (Molecular Research Center, Inc.) Oligonucleotides probes were made to 3'-untranslated region of both actin mRNAs. Agarose gel electrophoresis and Northern Blotting analysis were carried out using standard procedures. GAPDH mRNA probe was used as internal standard.

The analysis of isoactin mRNAs expression during cardiomyogenesis *in vitro* showed a decrease in both cytoplasmic isoforms (β and γ) mRNAs concomitant with an increase in α-cardiac actin mRNA level. *T. cruzi* infected cells revealed a reduction of 50% in the α-cardiac actin mRNA expression with 72 hr of infection. In contrast, β-actin mRNA increased 47% after 48 hr of infection. *In situ* hybridization demonstrated that α-cardiac, γ and β cytoplasmatic actin were located in different compartments during cell myogenesis which corroborates with the concept that most mRNA are addressed to the functional protein compartment. Non-muscle cells displayed β-actin mRNA signal at the cell perifery, while α-cardiac actin mRNA had a perinuclear distribution in myocytes. After *T. cruzi* infection, β-actin mRNA was translocated from the periphery to the perinuclear region in highly infected cells. The changes observed over the distinct pattern of isoactin mRNAs expression during cardiac myogenesis were induced by *T. cruzi* infection. These results provide evidence that *T. cruzi* affects actin mRNA regulation.

THE ROLE OF CALCIUM IONS DURING THE INTERACTION OF _T. CRUZI_-CARDIOMYOCYTES

T. cruzi trypomastigote forms invade a variety of mammaliam cells triggering transient increases

in the $[Ca^{2+}]_i$ an effect not observed with the non infective epimastigote stage (Tardieux et al. 1994, Moreno et al. 1994, Burleigh & Andrews 1995, Dorta et al. 1995, Caler et al. 1998). According to Tardieux et al. (1994), the Ca^{2+} transients induced in host cells would promote the invasion by recruiting the lysosomes at the site of parasite entry. The Ca^{2+} signalling activity from *T. cruzi* is soluble and linked to the activity of a parasite serine hydrolase, the oligopeptidase B (Burleigh & Andrews 1995, Caler et al. 1998). Also, the finding of elevated $[Ca^{2+}]_i$ in trypomastigote forms during the process of cell invasion (Dorta et al. 1995, Moreno et al. 1994) suggests that the parasite posssesses the ability to control its own Ca^{2+} levels, possibly through regulation of influx or mobilization from intracellular stores. Treatments which decrease or elevate cytoplasmic Ca^{2+} (Yakubu et al. 1994) showed that a decrease or increase in *T. cruzi* $[Ca^{2+}]_i$ modifies infectivity downward or upward, respectively.

To study the role of calcium ions in the invasion of cultured cardiac myocytes we used fura-2, a dual ratiometric fluorescent Ca^{2+} indicator. For fura-2, significants shifts are observed in the excitation spectra, but not in the emission spectra. Ca^{2+} free and Ca^{2+} -bound forms of ratiometric indicators are characterized by spectral peaks at different wavelenghts, and intensity measurements can be made at two different wavelenghs and a ratio can be obtained. Cardiomyocytes were incubated with fura-2-AM, 4 mg/ml in Eagle medium for 20 min, then rinsed and maintained in Eagle's medium. They were analyzed with an inverted Carl Zeiss microscope – Attofluor digital fluorescence ratio imaging system. Profiles of fluorescence intensity were obtained before and after the interaction with *T. cruzi*. Fura-2 monitored the intracellular calcium in normal cardiomyocytes for 1hr; for interaction studies after the observation of normal cells, trypomastigotes forms of *T. cruzi* were added to the cultures. Our results showed a significant increase in intracellular calcium levels during the invasion process. We observed transients Ca^{2+} increases when the parasite only touched the cell, but did not adhere. When the parasite was seen adhered to the cell, a constant increase in Ca^{2+} was registered (Garzoni et al. 1998).

We have previously shown that Ca^{2+} ATPase of the SERCA was involved in the process of invasion of cardiomyocytes by *T. cruzi* (Silva & Meirelles 1998). Treating cardiomyocytes with 2, 4 and 6 μM of thapsigargin, during 3 and 6 hr, a drug that depletes intracellular calcium stores, we found a statistically significant inhibition -75% - in the *T.cruzi*-cardiomyocytes invasion. Further studies are being done to investigate how the CM excitation-contraction is affected during *T. cruzi* infection.

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