A Cardiomyocyte Mannose Receptor System is Involved in *Trypanosoma cruzi* Invasion and is Down-Modulated after Infection

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**ABSTRACT.** Mannosyl binding sites were detected “in vitro” on cardiomyocytes (CM) surface using horseradish peroxidase (HRP) as the ligand. Binding assays revealed a specific recognition system, which was time- and concentration-dependent. The binding required physiological pH and was inhibited by EDTA and trypsin treatments. HRP binding was reduced by pre-incubations with low concentrations of D-mannose. Ultrastructural analysis of the endocyotic process was followed using HRP coupled to colloidal gold particles (HRP-Au). The tracer was found within caveolae characterizing early steps of the receptor-mediated endocytosis. The addition of 10 mM D-mannose to the interaction medium blocked *Trypanosoma cruzi* uptake by CM. The labeling of CM with a subsaturating concentration of HRP-Au before their infection showed, by ultrastructural studies, that its association with trypomastigote forms occurred frequently near to HRP-gold particles that could also be seen to comprise the parasitophorous vacuole. After infection of CM with *T. cruzi*, a considerable reduction on HRP binding was noticed. Binding was almost completely restored by treating the infected cultures with the trypanocidal drug Nifurtimox. Our “in vitro” findings suggest that cardiomyocyte’s mannose receptors localized at the sarcolemma mediates *T. cruzi* recognition and can be down-modulated by parasite infection.

**Key words:** Mannose receptor/cardiomyocyte/horseradish peroxidase/*Trypanosoma cruzi*

Many lectin-like molecules with different carbohydrate specificities have been described in eukaryotic cells (5, 18, 43), including smooth and skeletal muscle cells (21, 38). Membrane-associated mannose binding receptor (MR) is a 175 kDa transmembrane glycoprotein, C-type lectin, that is expressed in the surface of several cell types including terminally differentiated tissue macrophages, subsets of vascular and lymphatic endothelial cells, and human dendritic cells (6, 39). MR belongs to a family of multilectin receptor proteins and is responsible for clearing glycoproteins terminating in mannose, fucose, and N-acetylglucosamine residues. It also plays a role in host defense as it recognizes the patterns of sugars that adorn the surface and cell walls of a wide range of infectious agents (e.g. bacteria, fungi, yeasts, and protzoa) mediating their phagocytosis and providing a link between innate and adaptive immunity (35, 39). Soluble mannose receptors (mannose binding proteins — MBP) have been isolated from rabbits, rodents and human sera (13), and are known to bind and inactivate lysosomal enzymes which have escaped into the blood (30). It has also been implicated in the innate immune response as it recognizes and distinguishes oligosaccharide patterns that decorate a range of pathogens and virally infected cells from self-glycoproteins (13, 30).

Skeletal and cardiomyocyte muscle cells are two important cell targets for *Trypanosoma cruzi* infection, the aetiological agent of Chagas’ disease. There are several reports concerning the role of carbohydrate-lectin specific recognition systems during *T. cruzi*-host cell interaction (2, 3). The invasive and non-proliferative
trypomastigote form of *T. cruzi* must enter the host cells to then differentiate into amastigotes and proliferate. When the host cells are plentiful with intracellular parasites, amastigotes differentiate again into trypomastigotes, which are the main forms released when the infected cell disrupts. Chronic myocarditis is one of the most frequent disturbances in symptomatic chagasic patients (17). Mannose residues have been implicated as ligands during amastigote and trypomastigote host cell invasion, and since they participate during the parasite internalization process, are also localized at the cardiomyocyte’s parasitophorous vacuole (2, 3, 7, 19).

Horseradish peroxidase (HRP), a glycoprotein devoid of phosphorylated carbohydrates and rich in mannose and N-acetylgalcosamine groups, has been used as a current label for histochemical analysis of mannosyl binding sites in frozen and fixed sections from different tissues and pathogenic parasites (10, 36, 37). The purpose of the present investigation was to characterize cardiomyocyte mannose receptors “in vitro” using HRP as ligand, and analyzing its expression during the infection with *T. cruzi*.

**Materials and Methods**

**Reagents**

The following reagents were purchased from Sigma (St. Louis, MO, USA): horseradish peroxidase (HRP), D-mannose, mannann, a-galactose, a-lactose, b-galactose, b-lactose, a-methyl mannoside, L-fucose, N-acetylgalcosamine and paraformaldehyde (PFA). Bovine serum albumin (BSA) was purchased from GIBCO (Long Island, NY, USA). Nifurtimox was a gift from Dr. Solange L. de Castro (Instituto Oswaldo Cruz, FIOCRUZ, Rio de Janeiro, Brazil).

**Parasites**

Trypomastigote forms of *T. cruzi* Y and Dm 28c stocks were used throughout the experiments. Metacyclic forms of Dm 28c clone were obtained from a chemically defined differentiation medium (11). Bloodstream trypomastigote forms (Y strain) were harvested by heart puncture from *T. cruzi* infected Swiss mice at the parasitaemia peak day, as previously described (23).

**Cell cultures**

Primary cultures of embryonic cardiomyocytes (CM) were obtained following the method previously described (26). Fibroblast-free CM cultures were obtained by differential plating of cells on gelatin-coated culture plates. Peritoneal mouse macrophages (MO) were cultivated as described elsewhere (4).

**Parasite interaction**

After 24 h of plating, CM or MO cultures were infected with bloodstream or metacyclic forms of *T. cruzi* with parasite:cell ratio of 10:1. After 24 h of interaction at 37°C, the cultures were washed with Dulbecco’s modified medium and the kinetic of infection followed up to 72 h. The assays with metacyclic forms were performed in absence of serum. In some experiments, 24 h-infected cultures were treated for another 24 h with 50 μM Nifurtimox. Parasite association was measured by ELISA or by direct quantification by light microscopy as described elsewhere (22, 33).

**Enzyme binding assay (EBA)**

Mannosyl binding sites were detected by an enzyme binding assay (EBA) using HRP as the mannosylated ligand which was developed by its own enzymatic activity. CM or MO cultures were washed with 0.1 M phosphate-buffered saline (PBS) pH 7.2, and incubated at 4 or 37°C for different periods of time, with variable HRP concentrations diluted in PBS containing 5 mM calcium and 1% bovine serum albumin (BSA). Cell-free BSA coated wells were used as negative control for HRP binding. At the end of the binding assays, the cultures were washed with cold PBS and fixed for 1 h at 4°C with 2% paraformaldehyde (PFA). In some experiments, uninfected and infected cultures were fixed in 2% PFA before saturation with 1% BSA for 1 h at 37°C, then washed and incubated with HRP. Sucessive washings with PBS removed free HRP. The peroxidase activity was revealed by incubating the plates for 20 min at 37°C with 0.01% 3,3',5'-tetramethylbenzidine in 0.1 M citrate buffer pH 4.0, containing 50 μl of a 3% H₂O₂ solution. Optical density (O.D.) was read in an ELISA automatic reader (Titertek Plus-ICN/Flow, Costa Mesa, CA, USA). Nonspecific binding was monitored using 1% BSA coated wells as controls. Competition assays were performed before the addition of HRP, by incubating the cells for 20 min at 37°C with different concentrations (3.9 to 100 mM) of the following carbohydrates: D-mannose, a-galactose, b-galactose, b-lactose, D-fucose, L-fucose, or with N-acetylgalcosamine. Trypsin and EDTA effects were analyzed by treating the cells for 15 min at 4°C with 0.01% trypsin and/or 5 mM EDTA after HRP binding. The trypsin action was quenched by the addition of 20% of serum.

**Evaluation of *T. cruzi*-host cell infection by ELISA assay**

ELISA tests were performed as described by Luz et al. (22). Briefly, infected and non-infected cultures were fixed for 1 h with 2% PFA, washed with PBS and preincubated for 1 h at room temperature with normal mouse serum to avoid nonspecific reactivity. The cultures were then washed with PBS containing 0.05% Tween 20 and incubated for 2 h at 37°C with sera from chronic human chagasic patients previously absorbed onto CM cultures. After washing, the cells were in-
incubated for 1 h at 37°C with anti-human IgG-peroxidase conjugated.

**Effects of carbohydrates during T. cruzi-cardiomyocytes interaction**

Trypomastigote forms and CM were allowed to interact for 24 h at 37°C in the presence or absence of 10 mM D-mannose, α-galactose or β-galactose. The interaction occurred in the absence of serum and the infection rates were measured directly by counting the infected cells at the light microscopy level as described elsewhere (33).

All quantitative experiments described above were performed in triplicate and were run at least 3 times. Student’s *t* test was applied to ascertain the statistical significance of the observed data.

**Ultrastructural analysis**

HRP coupled to 15 nm colloidal gold particles (HRP-Au) were prepared according to Frans (14). Non-infected cultures were first incubated for 30 min at 4°C with HRP-Au in the presence of 5 mM calcium chloride and then the temperature was elevated to 37°C and followed for 1 and 4 hrs.

Some cultures were incubated with a non-saturating concentration of HRP-Au (50 μg/ml) at 4°C, washed and infected for 24 hrs at 37°C with metacyclic trypomastigote forms. Negative controls of HRP binding were prepared by co-incubating the cells with HRP and 500 mM D-mannose. The cells were fixed for 1 hr at 4°C with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer and then post-fixed with 1% osmium tetroxide in the same buffer. The monolayer that peeled off the plastic dish in the washing buffer was harvested and centrifuged. The pellet was dehydrated in a graded series of acetone and embedded in Epon. Sections were stained with uranyl acetate and lead citrate and observed with a Zeiss EM 10C transmission electron microscope.

**Results**

**HRP binding to cardiomyocytes is receptor-mediated via mannose residues**

Uptake of HRP by CM has been already used to trace lysosomal compartments and phagolysosomal fusion in *T. cruzi*-infected cells (26). To assess whether HRP binding to CM was receptor-mediated, we studied its specificity, as well as its time- and dose-dependence. A kinetic assay was performed by incubating CM with 50 μg/ml HRP at 4°C up to 60 min. The results showed that the binding was time dependent and attained maximal levels after 45 min (Fig. 1a). It was also observed that the binding was dependent on pH, reaching maximum values at pH 7.0 (Fig. 1b). CM were then incubated for 45 min with HRP with concentrations ranging from 0.078 to 5 mg/ml at pH 7.0 at 4 and 37°C. Binding at 4°C increased with HRP concentration (Fig. 1c), with both CM and MÖ attaining maximal levels at 1 mg/ml. No significant HRP binding to BSA-coated control wells could be noticed. Binding of HRP at 37°C to previously fixed cells resulted in a similar pattern as for 4°C (data not shown), but with a higher non-specific binding to BSA-coated wells. Endogenous peroxidase activity measured by the incubation of H2O2 with omission of exogenous HRP was nearly undetectable in CM (data not shown).

We next examined the sensitivity of HRP binding to divalent cation chelation and trypsin treatment. Experiments were conducted at 4°C with a low concentration of HRP (50 μg/ml). HRP binding was inhibited (70%) by EDTA treatment (Fig. 1d), as compared to binding in the presence of calcium chloride. To examine the peptidic nature of the receptor, the cultures were treated with trypsin, resulting in around 30% loss of maximal HRP binding (Fig. 1d). This loss was more pronounced when the cardiomyocytes were treated with a solution containing EDTA plus trypsin after the HRP incubation (Fig. 1d).

HRP binding to CM was further examined after previous incubation of cardiomyocyte with different carbohydrates for 20 min at 37°C. Pretreatment of CM with D-mannose, N-acetylglucosamine, L-fucose and α-galactose before the incubation with HRP resulted in inhibition of HRP binding. No inhibition could be detected using D-fucose, α-lactose, β-galactose or β-lactose (data not shown). D-mannose and L-fucose were the carbohydrates that more efficiently inhibited CM, the former showing a dose-dependent effect resulting in 50% of inhibition with 6 mM (Fig. 2a).

**HRP bound to the CMRR leads to ligand endocytosis**

Our previous data concerning the endocytic pathway of anionic groups, using cationized ferritin as a probe, showed that CM endocytosis is an active process and that ferritin particles were considerably internalized only after 3 h of incubation at 37°C (32). We have thus followed for 1 and 4 h at 37°C the HRP receptor-mediated binding and endocytosis using HRP-Au as probe. After 1 h chase, gold particles were localized in specialized areas of the membrane such as caveolae (Fig. 3a), as well as near and inside coated and uncoated invaginations and small vesicles (Fig. 3b). After 4 h of HRP-Au incubation, gold labeling was observed in small and large endosomes displaying different electron densities (Fig. 3c). Under the same conditions, a stronger labeling on the plasma membrane and a larger number of labeled vesicles were noticed in peritoneal macrophages after 1 h of incubation at 37°C (data not shown). Control assays were performed by the addi-
Fig. 1. Effects of time, pH, variable concentration, EDTA and trypsin treatments on HRP binding to CM. Cell-free BSA coated wells were used as negative controls. All samples were incubated in PBS containing 1% BSA and 5 mM CaCl\(_2\) as described in Materials and Methods. (a) Time course and (b) pH dependency of 50 µg/ml HRP binding to CM at 4°C. (c) The binding for 45 min at 4°C with increasing HRP concentrations on CM and MΦ cultures. (d) The effect of 5 mM EDTA or/and 0.01% trypsin treatments on HRP (50 µg/ml) binding to CM at 4°C.
tion of 500 mM D-mannose during the HRP-Au incubation, which completely abolished the cell labeling (data not shown). These results showed that cardiomyocytes possess a mannosyl binding site localized in the caveolae, the so-called cardiomyocyte mannose receptor (CMMR).

The CMMR mediates T. cruzi recognition and is down-modulated after-infection

The effect of carbohydrates during T. cruzi invasion process was evaluated by adding D-mannose, α- or β-galactose to the interaction medium. During the 24 h of parasite-cell interaction, D-mannose significantly reduced parasite uptake by CM (p<0.03), reaching values higher than 70% of internalization inhibition (Fig. 4a). α-galactose also inhibited infection in 3 out

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**Fig. 2.** Competition assays were performed by incubating CM with different carbohydrates for 20 min at 37°C followed by the addition of 50 μg/ml HRP: L-fucose, D-mannose, N-Acetylglicosamine and α-galactose efficiently inhibited HRP binding to CM surface.

**Fig. 3.** Ultrastructural analysis of HRP-Au binding to cardiomyocytes. CM was incubated 30 min at 4°C with a non-saturated concentration of HRP-Au particles and was further incubated at 37°C for different time period. After 1 hour at 37°C, the HRP-Au particles (arrowheads) were localized (a) inside caveolae (cv), (b) in uncoated invaginations and (b) within small endosomes (ed). After 4 h, the tracer was found (c) inside large endosomes (le) with different sizes and electrodensities. a. ×57,200; b. ×92,400; c. ×41,600. re: endoplasmic reticulum.
of 4 experiments (Fig. 4a), but it was not statistically significant (p<0.09). Addition of β-galactose (Fig. 4a) did not significantly change the infection levels (p<0.3).

As the specific ligand for CMMR (D-mannose) impaired T. cruzi invasion, we next studied some ultra-structural aspects of the role of CMMR during the two successive steps of the parasite-host cell interaction: adhesion and internalization. During adhesion, HRP-Au particles were concentrated at the site of the parasite's early (Fig. 5a) and later adhesion process (Fig. 5b), mediating their association to the CM surface. During internalization, the tracer was detected in the invagination of the host cell membrane that will compose the parasitophorous vacuolar membrane (Fig. 5c), and afterwards, together with the intracellular parasites inside the parasitophorous vacuole (Fig. 5d).

After characterizing the cardiomyocyte mannose receptor and its involvement in T. cruzi invasion, we extended our studies to analyse its expression after T. cruzi infection. If CMMR was interiorized as a mediator of parasite invasion, HRP binding should be reduced in infected cultures. We noticed that after 24 h of infection with 10 parasites/cell (Y strain), HRP binding was reduced around 25%, and decreased even more after 48 h of infection, concomitant with the parasite load increase (Fig. 4b). However, parasite infection significantly impaired HRP binding only after 48 h, displaying a decrease (p<0.05) of around 40%. The infection of macrophages for 48 h also displayed similar results (Table I). Interaction of cardiomyocytes with metacyclic forms of Dm 28c clone, which results in a higher infection of the cultures, showed a significant inhibition of the HRP binding even after 24 hours of infection (Table I). The loss of HRP binding that was noticed after infection with T. cruzi was parasite-dependent since the receptor activity was recovered after intracellular parasite killing by Nifurtimox treatment (Table I). This approach led to a recovery both in MR expression on CM and macrophage cultures, despite the fact that the chagasic serum could still detect cytoplasmatic parasite antigen (Table I). Although the parasite

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**Fig. 4.** The participation of CMMR during *T. cruzi*-cardiomyocytes interaction (trypomastigotes from Y strain, host cell:parasite ratio of 1:10). (a) The effect of 100 mM D-mannose, α-galactose or β-galactose on *T. cruzi* invasion into cardiomyocytes for 24 h at 37°C. (b) Inhibition of HRP binding to *T. cruzi* infected-cardiomyocytes (expressed as a variation index in the left Y axis) and immune detection of *T. cruzi* infection in CM (expressed by the optical density in the right Y axis). CM were infected for different periods of time up to 96 h (X axis). After parasite contact, the cultures were washed, fixed and incubated with HRP, or with Chagasic sera. * Student’s t test was used to determine statistical significance (p<0.05).
Fig. 5. Ultrastructural evidences of CMMR participation during T. cruzi infection. CM were incubated for 30 min at 4°C with a non-saturated concentration of HRP-Au (50 μg/ml), washed to remove unbound ligand and infected for 24 h. A noticeable labeling of the CMMR (arrowheads) was observed concentrated at the site of the parasite (p) initial (a) and (b) later attachment. (c) HRP-Au particles were localized at the sarcolemma area that will compose the parasitophorous vacuole membrane (pvm). (d) Parasites were found inside sealed parasitophorous vacuole enriched with HRP-Au particles (arrowheads). a, ×26,000; b, ×32,500; c, ×33,000; d, ×41,000.
Table I. Effect of *T. cruzi* infection in cardiomyocyte mannose receptor expression

<table>
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<th>Assay #</th>
<th>Host Cell</th>
<th><em>T. cruzi</em></th>
<th>Time after infection</th>
<th>Nifurtimox Treatment</th>
<th>CMRR activity (% of maximal HRP binding)</th>
<th>Infection level: Chagasic serum binding (OD)*</th>
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<tr>
<td>1</td>
<td>CM</td>
<td>—</td>
<td>24 h</td>
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<tr>
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<td>0.77</td>
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<tr>
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<tr>
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* Student's *t* test was applied to ascertain the statistical significance (p < 0.05).

Discussion

In the present study we showed for the first time physiological and ultrastructural evidences for mannosyl binding sites expression at the sarcolemma of cardiomyocytes, the CMRR. HRP, a non-phosphorylated glycoprotein rich in mannose and N-acetylglucosamine residues was used for binding and internalization assays. This marker of MR has been used in different systems (31, 36, 37), including "in vitro" uptake by mouse innervated and denervated skeletal muscle (38).

Our EBA results showing time- and dose-dependence, and EDTA, D-mannose and pH sensitivity, are consistent with the requirements already established for mannose receptor binding in other systems (34, 36, 37, 41, 43). A MR from primary cultures of bovine airway smooth muscle cells has been characterized that shared functional and structural properties with macrophage MR (21). Higher binding levels observed at neutral pH are consistent with the results found on receptors involved in the delivery of ligands to acidic compartments (31). The HRP binding displayed higher background when CM were used with prior fixation, probably due to the ligand interacting with aldehydes exposed by the PFA fixation. These results indicated that HRP binding to CM was receptor-mediated, dose- and time-dependent, and hampered by acidic and alkaline pH.

In competition assays, specificity was clearly demonstrated by the previous saturation of the CMRR before the binding of HRP with specific carbohydrates for MR (D-mannose, L-fucose and N-acetylglucosamine). D-mannose and L-fucose were the most efficient, showing 50% inhibition with very low concentrations (6 mM) and displaying a dose-dependent inhibition profile. We also observed an inhibition with α-galactose, which is not a specific carbohydrate recognized by MR. Mouse macrophages have a lectin-like molecule with specificity for both D-mannose and D-galactose, presenting two binding sites, complementary to each of these sugar residues, and that the blockage of one of them interferes with the access to the other (18). Our results indicated that the CM receptor, which recognizes HRP, was a trypsin- and EDTA-sensitive glycoprotein, and that binding occurred via mannose residues of the HRP molecule, since it is devoid of α-galactose terminal residues.

Ultrastructural approaches allowed the analysis of the distribution and internalization pathway of CMRR using gold-labeled HRP. The tracer was internalized via both uncoated and coated areas, and was localized inside small endosomes, which were probably early endosomes due to their localization near to the sarcolemma. The fact that gold particles were mainly loaded in caveolae confirmed our EBA data concerning the specificity of a classical receptor mediated endocytosis (28). A number of different functions have been proposed for caveolae including signal transduction, calcium signaling or regulation, and non-clathrin dependent endocytosis and transcytosis (28). After longer incubation times, the mannosylated ligands were associated with late endosomes and lysosomes characterized by their perinuclear localization and electrondensity pattern. The localization of gold particles within such structures predicts that the ligand will be degraded by lysosomal hydrolases, since macrophage MR is known to be recycled back to the plasma membrane (34). Studies are in course to further characterize the intracellular pathway of the CMRR.

There was a significant increase in HRP labeling and internalization when peritoneal macrophages were employed, suggesting a higher receptor density and/or faster ligand internalization as compared to cardiomyo-
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...cytes.

Except for lysosomal hydrolases, glycoconjugate carbohydrate chains of higher organisms do not present terminal mannose or glucose residues. In contrast, lower organisms, unable to process N-linked oligosaccharides possess abundant mannose, glucose, and other sugars on their surface. It has been shown that macrophage MR participate in the uptake of microorganisms such as *Leishmania donovani*, *Candida albicans* and *Saccharomyces cerevisiae* through their surface mannose residues (13, 16, 31, 44). Mannose residues are involved in the invasion of both trypomastigote and amastigote forms of *T. cruzi* (2, 7, 19, 20). The detection of mannosyl residues on trypomastigotes has already been described by the use of lecists (1, 29) and addition of mannose to the interaction medium inhibited the uptake of the bloodstream trypomastigotes by macrophages (2) just as we showed presently for cardiomyocytes.

The infection of CM by *T. cruzi* involves an adhesion of the parasite to the host cell's surface followed by its internalization (23). The presence of HRP-gold particles near the trypomastigote attachment site to CM also suggests that parasite mannosyl residues and CMMR can take part during the recognition process of *T. cruzi* and cardiomyocytes, as proposed previously (7). The localization of HRP-Au inside the parasitophorous vacuole could be: (a) the result of the participation of CMMR during its formation, or (b) due to the later fusion of endosomal compartments containing HRP-Au to the parasitophorous vacuole as described by Meirelles et al. (26). The former hypothesis was confirmed through the visualization of HRP-Au at the membrane that will form the parasitophorous vacuole. This membrane is highly selective since ubiquitous components of the plasma membrane are present (e.g. Con A, WGA and RCA binding sites), but others are excluded (e.g. anionic sites) (7, 24, 25). These results indicate the participation of CMMR during the adhesion and uptake of *T. cruzi* by cardiomyocytes, with the incorporation of this receptor to the vacuolar membrane during the formation of the parasitophorous vacuole.

Although the engagement of lecists and carbohydrates as a mechanism for the parasite uptake by the host cells has been postulated (2, 3), it was not clear whether this process could also modulate receptor expression on the host cell. Our present results showed for the first time that during *T. cruzi* infection in CM a reversible loss of CMMR activity occurs. This is probably due to the utilization of CMMR during the parasite uptake into CM since: (a) it was observed after 24 h of CM infection with the Dm 28c clone, which is known to infect CM very rapidly and at high rates (30-50% in 2 h, Contreras *et al.*, 1988), (b) it was observed after 24 h but was only significant after 48 h of CM infection by Y strain, that takes a longer time to invade CM and at lower levels (20% in 18 h, Meirelles *et al.*, 1986), (c) it is sensitive to Nifurtimox treatment, and (d) recovered after 72 h of infection, when amastigotes are dividing intracellularly. Furthermore, ultrastructural analysis localized the CMMR at the site of parasite attachment and later on, composing the parasitophorous vacuolar membrane. In a previous work we also showed that during the infection of CM by *T. cruzi*, a considerable loss in the host cell surface charge occurred, and that the higher alteration was observed when the CM were infected with parasites from the clone Dm 28c (33). As we usually found a higher loss of the HRP binding after 48 hours of *T. cruzi* infection, we believe that the CMMR modulation could occur both during the initial step of the parasite uptake and later on, as a consequence of its intracellular development.

The down-regulation of CMMR could be expected by analogy with macrophage mannose receptors expression known to be modulated by *Bacillus calmette-guérin*, *Leishmania donovani* and *Candida albicans* (9, 12, 15, 31). In the latter case this alteration was correlated to both tumor necrosis factor and nitric oxide and through the combination of the production of modulatory molecules and enhanced receptor degradation (15, 31). Other possibilities could explain the down-regulation of CMMR during *T. cruzi* infection such as decrease in receptor synthesis mediated by the parasite and/or host infected-cell factors or impairment of the transport to the plasma membrane of newly synthesized or recycled CMMR. Some experiments are underway in order to investigate these possibilities. Our present results suggest that internalization of *T. cruzi* is mediated, at least in part, by cardiomyocytes mannose receptors and that the infection impairs binding of mannosylated ligands to CMMR.

In summary, the present studies demonstrated for the first time the specific binding of HRP to a surface cardiomyocyte mannose receptor and provided data that it participates in *T. cruzi* invasion which in turn leads to a reversible down-modulation of these receptors.

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