The Expression of Mannose Receptors in Skin Fibroblast and Their Involvement in *Leishmania (L.) amazonensis* Invasion

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**SUMMARY** *Leishmania* are protozoa that invade mononuclear phagocytes with the involvement of different ligand-receptor systems, including mannose receptors. Until now, scant data are available concerning the mechanisms that govern the infection of *Leishmania* in other host cell types such as fibroblasts. Our aim was to analyze the expression of mannose receptors in primary cultures of skin fibroblasts (SF) further characterizing their role during the invasion of promastigotes of *Leishmania (L.) amazonensis*. Both fluorescent, light, and electron microscopy assays revealed that SF have mannose receptors since they bound and internalized mannosylated ligands in addition to being positively labeled by fuc-BSA-FITC probes. D-mannose competition assays revealed the participation of mannose receptors during the parasite association with SF presenting upregulated receptor expression during the initial steps of the infection. After longer periods of *Leishmania*:fibroblasts contact, the modulation noted in the host mannose receptors was reverted concomitantly to the infection control, suggesting that the parasites were required for the alteration maintenance and providing evidences that the SF may display microbicidal mechanisms to control the *Leishmania* infection. (J Histochem Cytochem 53:35–44, 2005)

**KEY WORDS** skin fibroblasts mannose receptors *Leishmania (L.) amazonensis* transmission electron microscopy

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The trypanosomatid flagellates of the genus *Leishmania* cause leishmaniasis in man and several other mammalian species, being endemic in 88 countries from the four continents (reviewed in Cunningham 2002). The parasite has two developmental stages: the promastigotes, which are the extracellular flagellated forms transmitted by the insect vector, and the amastigotes that are intracellular multiplicative forms found in the mammalian hosts (Vannier-Santos et al. 2002). The disease, which is caused by the amastigote proliferation in host mammalian tissue, can be categorized into four main forms, including 1. visceral leishmaniasis, which is the most serious form being fatal when untreated; 2. cutaneous leishmaniasis that represent the most common form, causing many simple lesions on the skin that self-heal within a few months; 3. mucocutaneous leishmaniasis, which is related to the *L. braziliensis* infection, leading to skin ulcers that spread causing massive destruction of the tissues, and last; 4. diffuse cutaneous leishmaniasis that produces disseminated and chronic skin lesions like those of lepromatous leprosy (Bañuls et al. 1999; TDR 2002).

It is known that the parasites invade phagocytic cells of the vertebrate host with the involvement of different ligand-receptor systems, including the mannose receptors (MR) (Blackwell et al. 1985; Palatnik-de-Sousa et al. 1993; de Almeida et al. 2003). Inside the phagolysosomal compartment, the promastigotes differentiate to amastigotes and multiply until the lyses of infected cell, releasing large amounts of amastigotes, which can be taken up by other host cells. The life cycle is completed by the bite of the female phlebotomine sand fly when it takes the infecting blood meal containing amastigotes that again differentiate
The macrophage mannose receptors are C-type lectin, transmembrane glycoproteins (175 kD), which belong to a multilectin receptor protein family displaying eight carbohydrate recognition domains (CRDs) (Wileman et al. 1986; Taylor et al. 1990; East and Isacke 2002). The receptor is expressed on the surface of several cellular types and mediates the binding and internalization of mannosylated glycoproteins (Stahl et al. 1976; Lane et al. 1998; Leteux et al. 2000; Linehan et al. 2000; Lee et al. 2002) as well as participates in the endocytosis of different pathogens enriched with mannose residues at their surface such as *Trypanosoma cruzi* (Soeiro et al. 1999), *Mycobacterium tuberculosis* (Noorman et al. 1997), *Candida albicans* (Stahl 1990) and *Leishmania donovani* (Wilson and Pearson 1988).

Although most analysis concerning *Leishmania* invasion has been done using macrophages as host cells due to their high in vivo infection rates, some studies clearly show the infection of other cellular types by the parasites of the *Leishmania* genus in both in vivo (Zuckerman 1953) as well as in vitro assays (Belle 1958; Lewis 1974), including the infection of cultured human skin fibroblasts (Chang 1978; Schwartzman and Pearson 1985). Since fibroblasts are localized in close proximity to the parasite inoculation area, they can represent a potential cell target for *Leishmania* early infection and spreading as already observed by others in hamster (Zuckerman 1953) and canine infections (Herwas Rodriguez 1996). Our present aim is to characterize the mannose-receptor expression in primary cultures of skin fibroblasts, examining its possible role in *Leishmania* (L.) *amazonensis* infection.

**Materials and Methods**

**Reagents**

The following reagents were purchased from Sigma Chemical Co. (St. Louis, MO): horseradish peroxidase (HRP), d-mannose, α-L-fucopyranosyl-albumin-FITC and paraformaldehyde. Bovine serum albumin was purchased from Gibco (Long Island, NY). Collagenase was purchased from Worthington Biochemical Corporation (Lakewood, NJ). Poly/bed 812 was purchased from Polysciences, Inc. (Warington, PA).

**Parasites**

*Leishmania* (L.) *amazonensis* (MHOM/BR/77/LTB0016) isolated from a human case of cutaneous leishmaniasis was used in all experiments and was kindly supplied by Dr. Gabriel Grimaldi Jr. (Department of Immunology, Fundação Oswaldo Cruz, RJ, Brasil). Amastigotes were isolated from the lesions of heavily infected animals and maintained in NNN blood agar medium (Novy, McNeal and Nicolle) with an overlay of modified LIT medium (liver infusion-Trypticase) at 25°C (Jaffe et al. 1984). The promastigotes obtained on the 7th day of cultivation were harvested by centrifugation at 1500 × g for 10 min at 4°C and washed twice in 0.1 M phosphate-buffered saline (PBS) immediately before the experiments.

**Primary Cultures of Skin Fibroblasts**

Primary cultures of skin fibroblasts (SF) were obtained by the dissociation of mouse skin embryos. The tissues were dissected, minced and incubated for 30 min at 37°C in a dissociation solution containing 0.03% collagenase diluted in Dulbecco’s modified Eagle medium (DMEM). After three dissociation steps, the enzyme digestion was interrupted by adding 10% fetal bovine serum, the suspension was centrifuged and resuspended in DMEM supplemented with 5% fetal bovine serum, 1 mM L-glutamine plus 1000 U ml⁻¹ penicillin and 50 g ml⁻¹ streptomycin. Skin fibroblasts (10⁶ cells/well and 10⁴ cells/plate) were seeded in 10% fetal bovine serum, the suspension was centrifuged and resuspended in DMEM supplemented with 5% fetal bovine serum, 1 mM L-glutamin-treated 24-well culture plates (for light microscopy assays) or in 35-mm culture plates (for electron microscopy studies), respectively. The cultures were incubated at 37°C in a 5% CO₂ atmosphere and the medium replaced every 2 days. All procedures were carried out in accordance with the guidelines established by the FIOCRUZ Committee of Ethics for the Use of Animals, resolution 242/99.

**Parasite–Host Cell Infection Assays**

Promastigote forms of *Leishmania* (L.) *amazonensis* were resuspended in PBS to achieve a ratio of 10:1 parasite/host cell. After 2 hr of interaction at 34°C, the SF were rinsed with PBS to remove extracellular parasites, fixed or maintained for 24 hr/34°C. In some assays, the infection was followed for 7 days. For light microscopy assays, the SF were fixed with Bouin’s solution (75 ml 1.2% aqueous picric acid solution, 25 ml formalin (40% formaldehyde) and 5 ml glacial acetic acid) and stained with Giemsa. The percentage of skin fibroblasts containing associated parasites (parasite association rates) was determined by examining at least 400 randomly selected cells at ×63 magnification under a Zeiss Axiosplan microscope (Carl Zeiss Inc., Thornwood, NJ). The randomization permits the evaluation of the whole coverslips avoiding non-representative analysis of the cell culture infection. All the assays described here were run three to five times at least in duplicate, as described (Soeiro et al. 1999).

**Transmission Electron Microscopy Analysis of Mannose Receptors in SF**

The expression of mannose receptors in skin fibroblasts was analyzed by two different ultrastructural approaches using the HRP as mannosylated probe (Soeiro et al. 1999). HRP was coupled to 15 or 10 nm colloidal gold particles (HRP-Au) according to Goodman et al. (1979). In the first approach, SF were incubated for 30 min/4°C with HRP-Au in the presence of 5 mM calcium chloride, washed to remove unbound ligands and then chased for 24 h/37°C. After the incubations, the cultures were fixed for 1 hr/4°C with 2.5% glutaraldehyde (GA) in 0.1 M sodium cacodylate buffer and postfixed with 1% osmium tetroxide diluted in the same buffer. The monolayer was then peeled off from the plastic
dish was in the dehydrating buffer, harvested and centrifuged. The pellet was dehydrated in a graded series of acetone and embedded in Lowicryl resin. Sections were hydrated in PBS buffer and incubated for 30 min in 50 mM ammonium chloride. After washing with 0.1 M PBS 3% BSA, the sections were incubated for 10 min in a blocking solution containing 1% BSA 0.2% Tween 20 and then incubated for 1 hr at room temperature with 50 μg/ml HRP-Au. In both protocols, the grids were stained with uranyl acetate and lead citrate and finally observed at Zeiss EM 10C Transmission Electron Microscope. We performed competition assays by incubating the samples with HRP-Au in the presence of 250 mM d-mannose.

The Endocytotic Activity of Mannose Receptors in SF

The ability of skin fibroblasts to incorporate large mannosylated particles was further investigated by using zymosan A particles (Zy) as probe. For light microscopy studies, uninfected cultures were formerly incubated for 10 min–24 hr at 37°C with 10^6 Zy particles, washed and fixed with Bouin’s solution followed by staining with Giemsa. The analysis of skin fibroblasts with internalized Zy particles was performed using a Zeiss photomicroscope (Zeiss Inc., Thornwood, NY). Fluorescent studies were conducted by staining Zy for 30 min/37°C with low doses of Concanavalin-A-TRITC (5 μg/ml), exhaustively washed and then incubated for 10 min and 24 hr/37°C with the skin fibroblasts. After the incubations, the SF were washed to remove unbound Con-A-treated Zy and fixed for 20 min/4°C with 2% PFA diluted in 0.1 M PBS. The DNA was stained with 10 μg/ml 4,6-diamidino-2-phenylindole (DAPI) to enable the visualization of SF nuclei, the coverslips were mounted over the sections with 2.5% 1,4-diazabicyclo-(2.2.2)-octane (DABCO) and examined immediately using a Zeiss photomicroscope equipped with epifluorescence. All the assays described here were run two to four times at least in duplicate.

d-Mannose Competition Assays

To ascertain the participation of mannose receptors during the Leishmania invasion, SF were incubated for 30 min with increasing doses of d-mannose (0–500 mM) and then allowed to interact for 2 hr/34°C with the promastigotes (parasite:host cell ratio of 10:1) diluted in serum-free medium. After interaction, the SF were washed with PBS, fixed with Bouin’s solution, stained with Giemsa and the parasite association rates measured as described above.

Statistical Analysis

Student’s t-test was used to determine whether differences between means in the d-mannose competition assays were significant. A p value <0.05 was considered significant. The data are representative of three to five experiments run in duplicate.

Analysis of Mannose Receptors by Neoglycoprotein Binding

To analyze the expression of mannose receptors through fluorescent approaches, skin fibroblasts were allowed to interact for 2, 24, 48 and 72 hr/34°C with L. (L.) amazonensis promastigote forms (parasite:host cell ratio of 10:1). After washing, both uninfected and Leishmania-infected SF were immediately fixed for 20 min/4°C in 2% PFA. The cultures were then incubated for 1 hr/37°C with 50 μg/ml α-L-fucopyranosyl-albumin-FITC (fuc-BSA-FITC), a tracer for mannose receptors (Stahl et al. 1976; Avrameas et al. 1996; Lane et al. 1998). After washing with PBS, the infected and Leishmania-infected cultures were further incubated with DAPI for visualization of SF and parasites nuclei and kinetoplast of the parasites, mounted with DABCO and examined as described above. In all experiments, optimal lectin or neoglycoprotein concentrations were determined by preliminary titration assays. To assure labeling specificity, some assays were performed with the addition of 250 mM d-mannose.

Results

The Expression of Mannose Receptors in SF and Their Endocytotic Activity

The expression of mannose receptors in skin fibroblasts was initially investigated by fluorescent microscopy assays using related neoglycoproteins. Our data showed that fuc-BSA-FITC bound to the mannose receptors present in skin fibroblasts, which displayed a fluorescent labeling throughout the cell surface (Figures 3A and 3B). The expression of mannose receptors in skin fibroblasts was further characterized by following the binding and internalization of HRP-Au particles, another well-known mannosylated ligand (Shepherd et al. 1994; Marzolo et al. 1999; Soeiro et al. 1999) by transmission electron microscopy. The tracer could be localized at the surface of SF after their incubation for 30 min/4°C (Figure 1A) as well as during the labeling of the ultrathin sections with HRP-Au (Figure 1B). Following the internalization at 37°C, we found the tracer both within intracellular vesicles in close proximity to the cell surface (Figure 1C inset) and inside perinuclear endosomes, which displayed different sizes and electron density (Figure 1C). The competition assays performed by the addition of d-mannose residues blocked the labeling and confirmed the specificity of the HRP-Au labeling toward the mannose receptors (Figure 1B inset). To further examine the endocytotic activity of SF through mannose receptors, the cells were incubated with Zy, a large mannosylated particle commonly used in endocytotic assays (Soeiro et al. 2002). Our results showed that skin fibroblasts are able to internalize Zy particles, which could be noticed by both fluorescent (Figures 1D and 1E) and light microscopy studies (data not shown). As noted during the incubation of fibroblasts with HRP-Au, the Zy particles were localized in both endosomes close to the cell surface (Figure 1E) as well as close to the nucleus of the SF.
The Role of Mannose Receptors during the Invasion of Skin Fibroblasts by Promastigotes of *L. (L.) amazonensis*

To investigate the involvement of mannose receptors during the interaction of SF with *L. (L.) amazonensis*, competition assays were done employing different doses of D-mannose. Our data showed that the addition of D-mannose in the interaction medium leads to a dose-dependent inhibition in the parasite association rates, reaching maximum levels of 78% inhibition with 500 mM (Figure 2). The analysis of the inhibition rates revealed statistical significance (*p*<0.05) in all time points, except with the dose of 63 mM.

(Figure 1D), possibly corresponding to early and late compartments, respectively.

**Figure 1** Transmission electron microscopy analysis showing mannose receptors at the surface of skin fibroblasts during incubation with HRP-Au both at 4°C (A) as well as during the incubations with ultrathin sections (B). After incubation for 24 hr at 37°C, the internalized HRP-Au was noted within endosomes localized near the plasma membrane (C, inset) and around the nucleus, displaying different electron densities (C). Competition assays performed with the addition of D-mannose blocked the HRP-Au labeling (B inset). Bar = 0.25 μm. Fluorescent micrographs showing the double labeling of ConA-TRITC labeled Zy internalized by SF (arrow) and DAPI, which stains the cell’s nuclei (D,E). Note the presence of Zy particles within endosomes localized near the nucleus (D). Bar = 2 μm. N, nuclei; ER, endoplasmatic reticulum. *Fibroblast nucleus stained with DAPI.*
Leishmania *Invasion by Fibroblast Mannose Receptor*

The Mannose Receptors Are Regulated during the Infection of SF by Promastigotes of *L. (L.) amazonensis*

Finally, we analyzed the expression of mannose receptors in skin fibroblasts infected by *Leishmania*. Our results using fuc-BSA-FITC probes showed that after interaction for 2–48 hr with promastigote forms, a higher expression of mannose receptors was noted both at the surface of the infected SF as well as within intracellular vesicles (Figures 3C, 3E and 3G) as compared with uninfected cells (Figure 3A). The addition of 250 mM D-mannose during the incubation with the fuc-BSA-FITC blocked completely the labeling (data not shown). The up-regulation of mannose receptors was reverted after 72 hr of interaction (Figure 3I) in parallel to the reduced intracellular parasite load visualized by the DAPI staining (Figure 3J). The ultrastructural analysis of *Leishmania*-infected SF after 72 hr of parasite:host cell contact showed intracellular parasites with important morphological damages including intense vacuolization and kinetoplast alterations (arrowhead) in addition to a large amount of cellular debris within the phagolysoosomal vacuole that lodges the parasites (Figure 4).

**Discussion**

To successfully invade the host cells, the promastigotes of *Leishmania* species must first interact with molecules present at the surface of the host cells both directly and/or indirectly through their opsonization by host soluble molecules (Guy and Belosevic 1993; Kelleher et al. 1995; Handman and Bullen 2002). In vitro data described the participation of different receptor-ligand systems during the internalization of promastigotes by macrophages, including 1. mannose receptors (Wilson and Pearson 1986, 1988); 2. complement receptors type 1 (CR1) and type 3 (CR3) (Da Silva et al. 1989; Russell and Talamas-Rohana 1989; Kedzierski et al. 2004), receptor for advanced glycosylation end products (Mosser et al. 1987); and 3. receptors for extracellular matrix molecules, including fibronectin (Rizvi et al. 1988; Russell and Talamas-Rohana 1989) and heparan sulfate (Butcher et al. 1992).

![Graph showing competition assays showing the participation of mannose receptors during the association of promastigotes with skin fibroblasts.](Image)

**Figure 2** Competition assays showing the participation of mannose receptors during the association of promastigotes with skin fibroblasts. The assays were realized by adding increasing doses of D-mannose (0–500 μM) in the interaction medium. The graphic revealed a dose-dependent inhibition in the percentage of host cells lodges the parasites (Figure 4).

Although macrophages are considered the major host cells for *Leishmania* infection in vivo, the infection of other phagocytes has been reported both in vivo as well as in vitro, including neutrophils (Laskay et al. 2003) and dendritic cells (Colmenares et al. 2004; Prina et al. 2004). Regarding the infection of non-professional phagocytic cells, Belle (1958) described the infection of epithelial cells by *L. (L.) donovani* in vitro and later Lewis (1974) demonstrated the ability of *L. (L.) mexicana* parasites to invade MDCK epithelial cells. Likewise, the infection of fibroblasts by *Leishmania* species has been reported both in vivo (Zuckerman 1953; Hervas Rodriguez 1996; Tarrantino et al. 2001) and in vitro studies (Chang 1978; Schwartzman and Pearson 1985; Corte-Real et al. 1995).

The MR are expressed on the surface of several cellular types, including mononuclear phagocytes, especially tissue macrophages (Shepherd et al. 1982; Basu et al. 1991), retina epithelial cells (Lane et al. 1998), dendritic and Langerhans cells (Noorman et al. 1997), tracheal smooth muscle cells, kidney mesangial cells and Kaposi sarcoma cells (reviewed in Leteux et al. 2000), fibroblasts (Straus 1983) and cardiomyocytes (Soeiro et al. 1999). It has been implicated in clearing glycoconjugates ending in mannose, l-fucose, glucose or N-acetyl-O-glucosamine residues (Lane et al. 1998; Linehan et al. 2000). MR also plays a role in host defense due to its ability to recognize the patterns of sugars usually present at the plasma membrane and cell walls of a wide range of infectious agents, such as bacteria, fungi, yeast, and protozoa, mediating their internalization and providing a possible link between innate and adaptive immunity (Linehan et al. 2000; Gordon 2002). Alternatively, the MR could also cooperate in inflammation processes by removing potential harmful enzymes found at the extracellular space and limiting tissue injury (Lane et al. 1998). A similar soluble receptor, the mannose-binding protein (MBP) (also named mannose-binding lectin) was isolated from the plasma of rabbits, rodents and human sera (reviewed in Ezekowitz and Stahl 1988). The soluble receptor can contribute to the clearance of lysosomal enzymes, which have escaped into the blood (Sastry and Ezekowitz 1993). It also neutralizes the invading mi-
Figure 3: Fluorescence microscopy analysis of mannose receptors expression in uninfected SF (A–B) and after their interaction with L. (L.) amazonensis promastigotes (C–J). Observe the gradual increase in the receptor expression after interaction for 2 (C), 24 (E) and 48 hr (G) as compared with uninfected SF (A). The up-regulation was reverted after 72 hr of infection (I) concomitantly to the reduced intracellular parasite load (J). The SF nuclei (N) and the parasites nuclei and kinetoplast (arrow) were evidenced in blue by DAPI staining (B,D,F,H and J) and the mannose receptors were stained in green by fuc-BSA-FITC (A,C,E,G and I). Bar = 5 μm.
croorganisms by binding to their cell-surface carbohydrates (mannose and N-acetyl-glucosamine residues) and activating MBP-associated serine proteases-1, -2 and -3 (Ezekowitz and Stahl 1988; Sastry and Ezekowitz 1993; Chen and Wallis 2004). Regarding the MR and Leishmania, it is known that promastigotes of L. (L.) donovani utilize mannose receptors during their invasion into macrophages (Blackwell 1985; Palatnik-de-Sousa et al. 1993; Chakraborty et al. 1998), down-regulating after infection (Basu et al. 1991). Furthermore, MBP binds to the surface of L. (L.) major and L. (L.) mexicana promastigotes possibly through the surface molecule lipophosphoglycan (LPG) (Green et al. 1994). The authors suggested that MBP could have the potential to opsonize the major developmental stages of Leishmania parasites, and provide a possible mechanism for the antibody-independent activation of complement on the parasite surface.

In the present work, we characterized the expression of MR in primary cultures of skin fibroblasts evaluating its role during the invasion by L. (L.) amazonensis promastigotes. Our data revealed that uninfected skin fibroblasts express mannosyl binding sites because 1. they bound a neoglycoprotein that specifically bind to mannose receptors (Avrameas et al. 1996); 2. they are able to internalize zymosan A, which are mannosylated particles frequently used in phagocytic assays (Speert and Silverstein 1985; Lombard et al. 1994); and 3. ultrastructural assays evidenced the association of HRP-Au to the SF surface, another mannosylated ligand commonly used for MR analysis (Shepherd et al. 1994; Marzolo et al. 1999; Soeiro et al. 2002). The addition of D-mannose efficiently blocked the binding of both HRP-Au and α-L-fucopyranosyl-albumin-FITC demonstrating that the ligands specifically bound to the MR. Our results corroborated previous cytochemical assays that demonstrated the presence of mannosyl binding sites in fibroblasts localized in liver (Straus 1981) and uterus (Straus 1983). The competence of live SF to internalized zymosan A and horseradish peroxidase, in addition to validating the MR activity, also revealed the phagocytic capacity of the SF as previously reported for other non-professional phagocytes (Soeiro et al. 2002). Actually, fibroblasts are denominated facultative phagocytes being able to internalize a variety of particles and pathogens both in vitro and in vivo (Rabinovitch 1970; Chang 1978; Dedet et al. 1983; Schwartzman and Pearson 1985; Côte-Real et al. 1995), although without the high phagocytic capacity of cells from the mononuclear phagocytic system (Van Furth 1970). Zymosan A is mainly composed of mannan and β-(1-3)-
glucan (Di Carlo and Fiore 1957). Since data reported in the literature showed that it can be ingested by mannose receptors (Speert and Silverstein 1985) via CR3 leading to O2− production (Le Cabec et al. 2000) and by β-glucan receptors (Reis e Sousa et al. 1993), we cannot discard the possibility that zymosan A internalization by fibroblasts may also involve β-glucan receptors, which have already been reported in murine macrophages and in other white blood cells (Goldman 1988; Brown and Gordon 2001). These receptors may be expressed in the fibroblasts and also contribute with the mannose receptors for zymosan A uptake in a similar manner to what was found for Langerhans cells (Reis e Sousa et al. 1993).

After characterizing the MR in skin fibroblasts, we next evaluated its role during the invasion of *L. (L.) amazonensis* promastigotes. Competition experiments conducted by the addition of increasing concentrations of D-mannose impaired the parasite association, showing a clear dose-dependent inhibition. These data suggest the participation of MR in the interaction of promastigotes and SF, in a similar way to the described data employing macrophages as host cells (Wilson and Pearson 1986, 1988; Chakraborty et al. 1998). However, as the interaction medium was depleted from serum sources, we cannot discard the possibility that other receptor-ligand systems cooperated as well during the invasion of SF by promastigotes, mostly in the presence of serum opsonins in analogy to the well-known invasion of promastigotes into macrophages.

Finally, as 1. the modulation of MR has been described in host cells infected with various microorganisms, including Bacillus Calmette-Guérin (Ezekowitz and Gordon 1982), *L. (L.) donovani* (Basu et al. 1991), *Candida albicans* (Shepherd et al. 1997; Gelderman et al. 1998), *Pneumocystis carinii* (Ezekowitz et al. 1991), *Trypanosoma cruzi* (Soeiro et al. 1999), and 2. our present data pointed to the participation of MR during the parasite invasion, and our next aim was to analyze the expression of MR in *Leishmania*-infected SF. Our results showed a gradual increase in the expression of MR in infected SF, reaching maximal expression after 48 hr of infection, with the labeling localized mostly at the cellular surface as well as intracellularly. The reversion of the up-regulation was evidenced after 72 hr of infection concomitantly to the presence of non-viable parasites and cellular debris visualized and confirmed by transmission electron microscopy, which was suggestive of abortive infection of *L. (L.) amazonensis* in SF. The mechanisms that govern the MR modulation in *Leishmania*-infected skin fibroblasts were not presently characterized, but some possibilities can be envisaged including alterations in the receptor degradation levels and/or transportation of recycled receptors (and/or newly synthesized) for the cellular surface and/or reduced MR synthesis mediated by the parasite and/or host-infected-cell factors. Our results demonstrated that the modulation in the expression of host mannose receptors induced by *Leishmania* invasion was reverted concomitantly to the loss of parasite viability, suggesting that the presence of viable parasites is required for the alteration maintenance noted in the MR.

The modulation of MR has been reported during other parasitic infections (Ezekowitz and Gordon 1982; Basu et al. 1991; Shepherd et al. 1997; Gelderman et al. 1998; Soeiro et al. 1999). Then the modulation of MR during the invasion of SF by *Leishmania* promastigotes could be expected by analogy with MR modulation by Bacillus Calmette-Guérin (Ezekowitz and Gordon, 1982), *L. (L.)* donovani (Basu et al. 1991), *Candida albicans* (Shepherd et al. 1997; Gelderman et al. 1998) and *T. cruzi* (Soeiro et al. 1999). At the present time, assays are underway to better investigate the factors involved in this up-regulation.

In summary, our present results pointed to the role of mannose receptor during the interaction of *L. (L.) amazonensis* promastigotes and skin fibroblasts in a similar way to the already reported system occurring during the invasion of macrophages by *Leishmania* promastigotes.

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