Chapter

How Does the Main Infective Stage of *T. cruzi* Enter and Avoid Degradation in Host Cells? A Description of the Pathways and Organelles Involved on These Processes

Emile Barrias, Lissa C. Reignault and Wanderley de Souza

Abstract

Trypanosoma cruzi, the etiological agent of Chagas disease, is an intracellular parasite that targets specific proteins of the host cell resulting in the generation of a unique parasitophorous vacuole (PV). As an intracellular parasite, *T. cruzi* interacts with cells from the mammalian host. Here we review aspects related with the binding of the main infective developmental stage (trypomastigote) to the host cell and its recognition by surface-exposed ligands/receptors. This process involves numerous signaling pathways and culminates in the entry of the parasite and modifications in both cells. The invasion of trypomastigotes occurs through multiple endocytic process, assembly of the PV, interaction of this vacuole with the endolysosomal system, lysis of the PV membrane, and multiplication of amastigotes within the cell in direct contact with host cell organelles.

Keywords: *Trypanosoma cruzi*, mammalian cells, host cell interaction, endocytic pathways, signaling, parasitophorous vacuole

1. Introduction

Trypanosoma cruzi, the etiological agent of Chagas disease, causes an anthropozoonosis discovered and characterized by Dr. Carlos Chagas in 1909 [1] and recognized by the World Health Organization (WHO) as one of the three most neglected tropical diseases of the world [2–4]. Nowadays, up to 8 million people are estimated to be infected with Chagas disease only in the Americas. Patients who do not receive treatment can develop severe cardiac debility and gastrointestinal organ dysfunction and may die, and 25 million are at risk of contracting the disease [3, 4]. Due to population migration and specific modes of transmission, Chagas disease is spreading beyond its natural geographical boundaries and becoming a global problem [5]. Although the protozoan has three major developmental stages, only two are capable of infecting mammals (trypomastigotes and amastigotes), and the trypomastigote

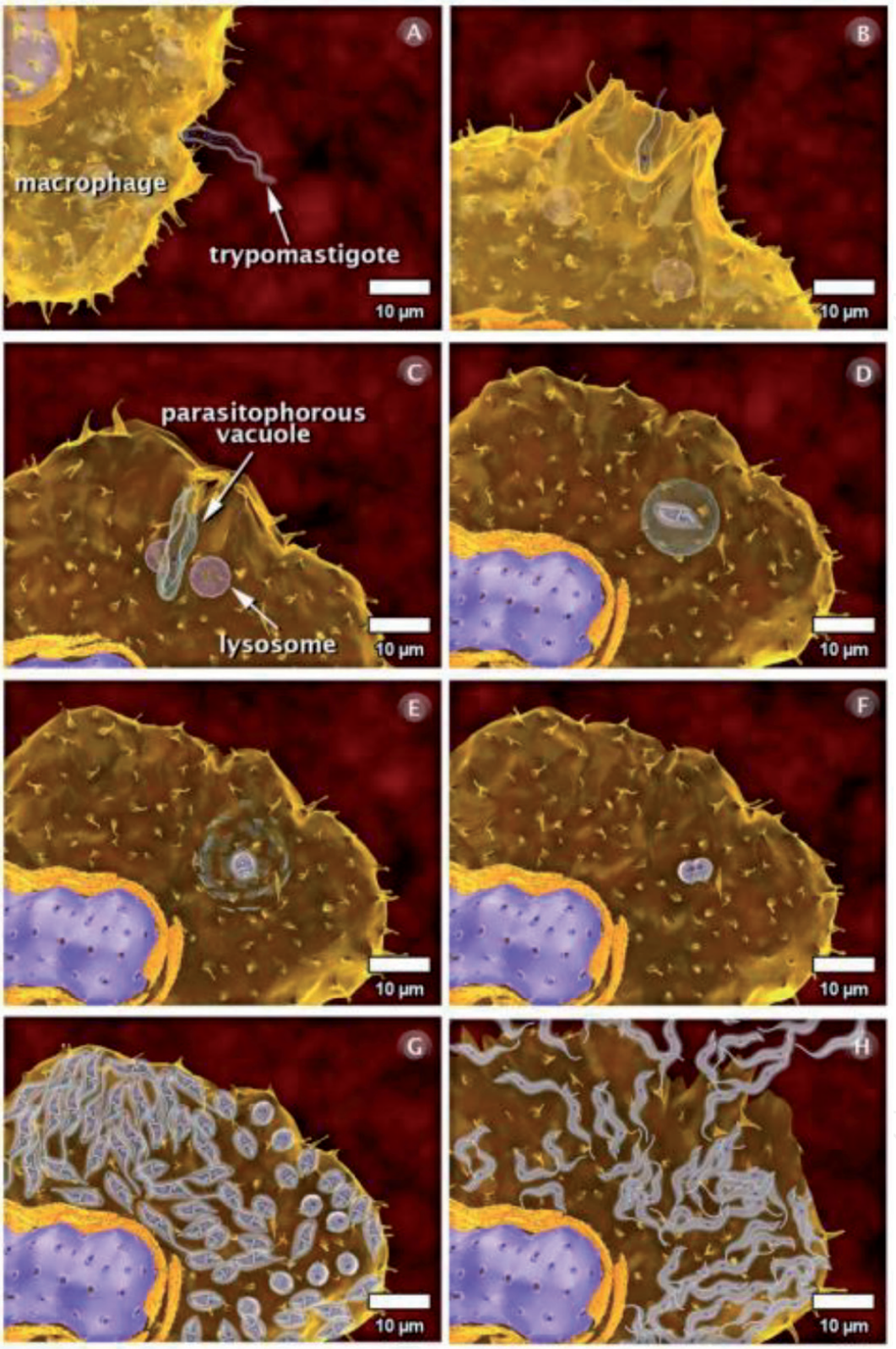

Figure 1.
Intracellular cycle of T. cruzi. (A) Attachment of the trypomastigote. (B) Internalization and recruitment and fusion of host cell lysosomes. (C) Parasitophorous vacuole is formed and lysosomal content is released. (D) Differentiation of trypomastigote to amastigotes. (E) Parasitophorous vacuole membrane disintegration. (F) Amastigote division into cytoplasm. (G) Differentiation of amastigotes into trypomastigotes. (H) Liberation of parasites into extracellular [12].

stage is the main *T. cruzi* vector [6]. Recent data indicate the existence of an infective epimastigote-like stage observed in axenic cultures as well as in the invertebrate host [7]. It is important to point out that before publication of this article, it was

assumed that replicating epimastigotes present in the insect gut are not infective to mammalian host. During the vector infection (caused by a hematophagous insect of the family Reduviidae), metacyclic trypomastigotes [8], which penetrate the vertebrate host (several mammals, including man), are released along with their excreta coming in contact with conjunctiva areas or through small lesions in the own site of the bite (favored by the itch caused after the insect's bite). In turn, metacyclic trypomastigotes are able to invade virtually all cell types in the vertebrate host, especially muscle cells, fibroblasts, and macrophages [6]. At this moment, the intracellular cycle of *T. cruzi* begins, where the firing of several signaling cascades culminates with the closure of the parasitophorous vacuole (PV) where the parasite is found [9, 10]. After the PV closure, the process of differentiation of the parasite from the trypomastigote stage to the amastigote stage begins. At the same time, fragmentation of the PV membrane takes place most probably due to the increased concentration of the Tc-Tox perforin-like protein produced by the parasite [11]. After the destruction of the vacuole, the parasite, in the process of differentiation, will be found in the cytoplasm of the host cell where it will initiate its multiplication and subsequent differentiation for trypomastigotes culminating in the rupture of the host cell (**Figure 1**) [13]. The whole process of formation of the parasitophorous vacuole until its rupture counts on the participation of several organelles of the host cell. Among these, the best characterized is the participation of host cell endosomes and lysosomes. It is the fusion of these organelles with the PV membrane that probably allows the increase or expansion of the PV. In addition, this process is also responsible for the generation of an acidic environment within the PV, which probably will potentiate the action of Tc-Tox and PV membrane fragmentation [13]. Wilkowsky and colleagues [14] have shown that early and late endosomes were critical for vacuole formation. In addition, other organelles responsible for the production of proteins and energy (endoplasmic reticulum (ER), Golgi complex (GC), and mitochondria) have also been observed during the initial infection process [11]. In this chapter we will discuss the available data on the process of parasite-host cell recognition, triggering of the internalization process, and biogenesis of the PV. A better understanding of all the processes may identify new potential targets to block parasite invasion and may constitute alternative ways to treat Chagas disease.

2. *T. cruzi* trypomastigote-host cell recognition

The trypomastigote stage of *T. cruzi* has the ability to invade both professional phagocytic cells and nonprofessional phagocytic mammalian cells either in vitro or in vivo [15–17]. The kinetics of the *T. cruzi*'s intracellular cycle varies according to the strain, including time duration (Y strain, e.g., has an intracellular cycle that is completed between 5 and 7 days), since the initial interaction with trypomastigotes, triggering a signaling cascade that culminates with the formation and closure of the PV where the parasite will be located for some time [18].

In order for the interaction process and consequent internalization of the parasite to occur, there must be a recognition between molecules present on the surface of both cells. These processes are complex and involve several adhesion molecules (**Figure 2**), signaling events, and proteolytic activities [10, 19]. Some of these molecules have been identified as participants in the adhesion and invasion processes, such as gp35/50 [20], gp82 (whose expression varies according to the *T. cruzi* strain analyzed) [21], and gp90 [22]. Both gp82 and gp90 are the main metacyclic stage-specific surface molecules and are extremely important to oral infection [21, 23]. However, while glycoprotein 90 is known as a negative modulator of metacyclic trypomastigote invasion [24, 25], gp82 binds to an unidentified receptor in host cell

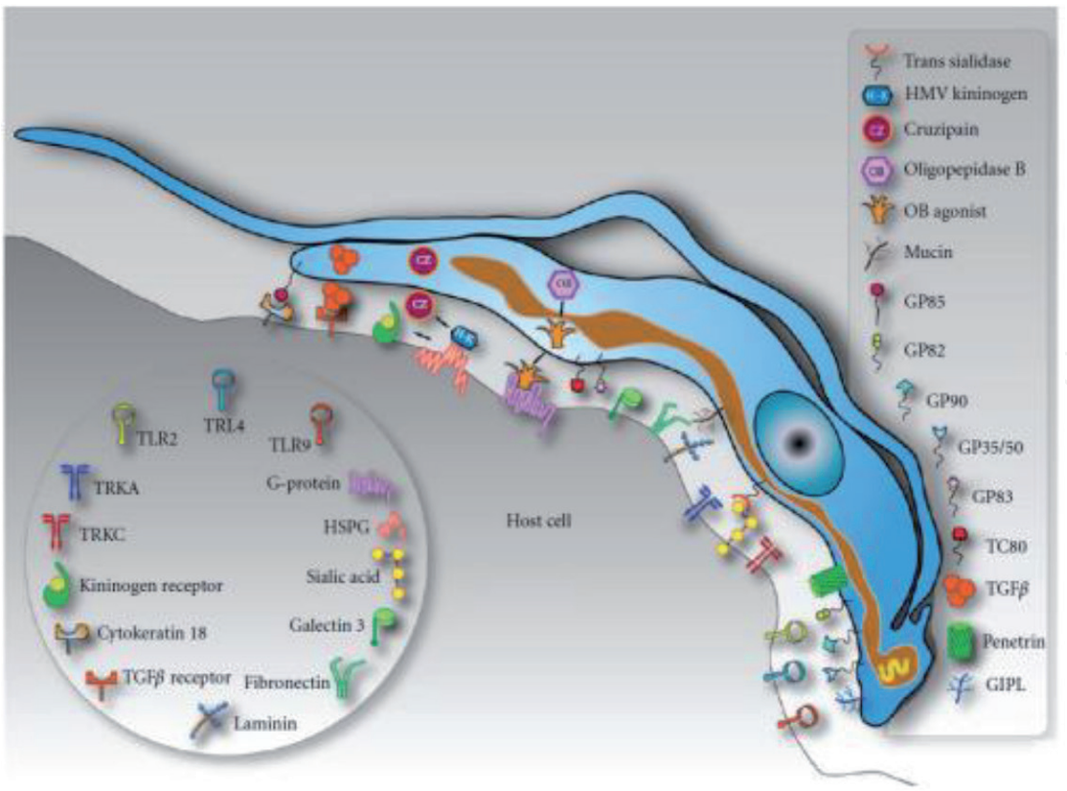

Figure 2.
Schematic model of molecules involved in T. cruzi-host cell interaction [10].

surface mediating a calcium-dependent signaling pathway that will be discussed below [20, 26]. Although the receptors presented in host cell surface that recognize gp82 and gp90 are not known, some data reinforce they are different since the target cell's interaction motif of both glycoproteins is distinct [26]. It is important to point out that these molecules are not present in tissue culture-derived trypomastigotes. At this stage, the molecules described as present in the membrane are gp85 (recognizes extracellular matrix), Ssp3, shed acute-phase antigen (SAPA) (trans-sialidase (TS)), oligopeptidases (serine proteases), and penetrin, which bind to heparin, heparan sulfate, and collagen [10].

Expression of a family of GPI-anchored glycoproteins, termed trans-sialidases/neuraminidases, present mainly on the surface of trypomastigotes is capable of modifying the exposure of surface glycoconjugates both in host cells and in the parasite itself. *T. cruzi* trans-sialidases (TS) are "shed" from the parasite membrane to the external medium through microvesicles. Microvesicles have a complex lipid bilayer and are responsible for carrying several molecules derived from the parasite, such as lipids, proteins, and nucleic acids [27–29]. Trypomastigote TS protein structure is formed by two major regions: an N-terminal catalytic region and a C-terminal region. C-terminal presents 12 amino acids repeated in tandem, named SAPA. TS can be active or inactive. Inactive TS are lectin-like proteins and are capable to bind SAcs and β-galactose [30]. Active TS are modified sialidases which, in addition to cleaving sialic acid, may transfer from sialoglycoconjugates of the host cell to β-galactose of *T. cruzi* glycoconjugates [31]. The TS gene family is complex, consisting of fourteen members divided into two groups: one translates TS present in stages present in mammalian hosts (trypomastigotes), and another translocates TS present in specific vector stages (epimastigotes). Although biochemical studies do not demonstrate the presence of TS at the amastigote stage, immunocytochemical assays have shown that TS can indeed be expressed in amastigotes [32–34]. Pereira et al. [35] observed that about 20–30% of cultured

trypomastigotes had trans-sialidases/neuraminidases on their surface and these parasites were more invasive than the population that did not express the enzyme. Expression of trans-sialidases in *T. cruzi* is directly related to infectivity/virulence of the parasite since proteome studies indicate that different strains exhibit significantly different amounts of TS and TS-containing microvesicles and that host cells incubated with larger quantities containing microvesicles of TS before infection with trypomastigotes will generate a greater infection [36]. Results regarding virulence had already been related to TS expression through the analysis of virulent and non-virulent strains by transcriptome. In this work it can be observed that the expression of TS-coding genes during the differentiation process from intracellular amastigote to trypomastigote (end of intracellular cycle) is much higher in the virulent than in the non-virulent strain. Associated with this the transfection of avirulent strain with gene allowing the constitutive expression of TS also makes the release of trypomastigotes faster [37]. The TS family also includes members that have no enzymatic activity but which may also be involved in recognition between the parasite and the host cell, such as gp85 [38]. Todeschini and colleagues [39] demonstrated that inactive enzymes of the TS family are sialic acid-binding proteins and terminal β-galactopyranose (βGalp) residues. In relation to gp85/trans-sialidase, San Francisco and colleagues [40] demonstrate that this protein plays a fundamental importance in invasion since its depletion causes a decrease in *T. cruzi* virulence. The same type of result was reported by Pascuale et al. [41] since inactive TS expression in trypomastigotes of a strain that does not express these TS (iTS null) allowed a better invasion and increase of the parasitic load in mice demonstrating that the inactive form may act alternatively or complementing the active TS in pathogenesis.

Sialic acid from the host cells has a crucial importance in intracellular cycle of *T. cruzi,* a parasite that does not have the ability to synthesize sialic acids. This molecule plays an important role in protecting *T. cruzi* from lysis by serum factors and also acting in interaction with host cell. Mucins and TS function as substrate and enzyme, respectively, and sialylated mucins are localized in microdomain regions of trypomastigotes [42]. During the interaction process involving macrophages, the presence of sialic acid on the surface of trypomastigotes hinders the invasion process, since the removal of these residues through the use of neuraminidase or their blockage through the use of periodic acid or lectins from *Limax flavus* or *Limulus polyphemus* increases adhesion and internalization rates. It is possible that the presence of desialylated parasites in macrophages may increase due to the recognition of galactose/N-acetyl galactosamine receptors present on the surface of macrophages [15, 16]. In relation to sialic acid present on the surface of macrophages, there has been an increase in the entry of trypomastigote forms in cells that expose this residue when compared to cells that do not expose galactose [15, 43]. The presence of sialic acid on the surface of trypomastigotes does not yet have a fully known function though it is believed that sialic acid helps adhesion and penetration into non-phagocytic cells [34, 44].

It is currently discussed that any class of molecules exposed on the surface of mammalian cells has a great receptor potential for molecules exposed on the surface of *T. cruzi* [19]. Most of the receptor classes have carbohydrates in their composition, such as galactose, mannose, sialic acid residues [19, 44, 45], and lectin-type proteins such as galectins (binding to carbohydrate residues present on the surface of the parasite). Pineda et al. [46] described binding data from different human galectins (gal-1, gal-3, gal-4, gal-7, and gal-8) to different strains of *T. cruzi* belonging to the six different strains (DTUs). It has been observed that all galectins bind preferentially to the infective stages (amastigotes and trypomastigotes) and that many can promote higher rates of adhesion and infection to host cells and higher

rates of infection to mice. In relation to galectin-1, it was observed that the presence of this glycoprotein in human and murine cardiomyocytes is able to prevent infection with trypomastigotes, one more data that goes against the modulating role of galectin in the process of internalization of trypomastigotes [47].

Galectin-3, a protein abundant in the cytoplasm of epithelial cells and macrophages, has also been described as a participant in the immune response and infection processes (in addition to the recognition process between the host and *T. cruzi*) [48, 49]. In addition, it has also been demonstrated that galectin-3 accumulates in both the parasite and phagosome entry regions and in tubules and vesicles that would derive from the endosomal system, thus suggesting that this protein is also an excellent marker of the lysis process of the PV containing this parasite [50]. The absence of galectin-3, in addition to increasing intracellular replication in vitro, is able to increase parasitemia in vivo by decreasing the secretion of pro-inflammatory cytokines and increasing cardiac fibrosis [51], which confirms the important role of this glycoprotein in the pathogenesis of Chagas disease.

In addition to all the molecules already mentioned, they also act as endothelin-1 receptors and bradykinin receptors. Both are used by trypomastigotes in the invasion of cardiovascular cells, being very important in the pathogenesis of Chagas disease [52]. Cytokeratin 18, fibronectin, laminin, and integrin are also recognized by Tc-85, forming a bridge between the parasite and the host cell [19, 53, 54]. Besides, a novel family of *T. cruzi* surface membrane proteins (designated as TcSMP) was detected in parasite surface and plays some role on host cell invasion by *T. cruzi* [54].

3. Mechanisms of internalization of *Trypanosoma cruzi* trypomastigotes in host cells

The mechanisms that lead to the internalization of trypomastigotes appear to be different when one considers the cell type where the internalization will occur. Morphological evidence shows that the parasite invades the host cell by an endocytic process that culminates in the formation of a PV. In cells of the immune system such as macrophages, which are specialized in phagocytosis events, we observe that the process of internalization occurs in two distinct ways. Dvorak and Schumunis [55] initially suggested that trypomastigotes forced the membrane of the host cell in an event where there is energy expenditure by the two cells involved. Nogueira and Cohn [56] observed the formation of projections on the surface of the host cell, which leads to a classical phagocytic process.

In cells considered as nonprofessional phagocytic, such as epithelial cells and fibroblasts, there appears to be a process of internalization where the parasite is the agent of penetration [33]. This process was confirmed by Martins et al. [57], where it was shown that metacyclic trypomastigotes (G and CL strains) require ATP to invade nonprofessional phagocytic cells. In these cell types, two different strategies are known to be involved in the invasion process and formation of the PV: one dependent on lysosomes and another one independent of lysosomes. The lysosomal-dependent pathway, first described by Tardieux et al. [58], was well characterized in nonprofessional phagocytic cell lines. Signals triggered by the recognition between *T. cruzi* and the host cell lead to the recruitment of lysosomes to the parasite's entry site, which would actively participate in the invasion process [33]. In addition, lysosome exocytosis would depend (1) on the performance of microtubules [8] and (2) on the regulation of host cell cytoplasmic Ca^{2+} levels [15]. This process of lysosomal-dependent invasion would occur in about 20% of the parasites [59]. The fusion of the lysosomes with the plasma membrane would thus end up donating the membrane

for the formation of the PV in a calcium-dependent process. In addition, a similar Ca^{2+}-dependent lysosomal exocytosis mechanism was observed during injury and repair of the plasma membrane [60]. Tam et al. [61] demonstrated that the mechanism of injury-dependent endocytosis is directly related to the secretion of a specific lysosomal enzyme and acid sphingomyelinase (ASM). ASM would cleave sphingomyelin, an abundant sphingolipid in the outer leaflet of the plasma membrane [62], forming ceramide, which is internalized by the cell [63–65]. Trypomastigotes are able to take advantage of this mechanism by inducing the formation of these vesicles rich in ceramides, thus facilitating the invasion process [66]. Over the years, this entry mechanism involving plasma membrane repair pathways has been extensively studied by several groups. The participation of lysosomal proteins known as Lamp1 and Lamp2 has been shown to be essential, since the knockout of both proteins decreases the entry of trypomastigotes. This decrease in entry is not accompanied by inhibition of lysosome exocytosis to the repair region as well as phenotypic modification of the host cell or generated PV. It is believed that the decrease in parasite entry is due to problems in caveolin-mediated endocytosis and in calcium efflux [67]. Considering that there are two distinct pools of lysosomes (cortical and internal) in mammalian cells, Hissa and Andrade used cardiomyocytes and observed, through the use of specific inhibitors, that trypomastigotes enter into this cell type mainly recruiting lysosomes from the more internal cell layer (perinuclear) [52]. Some molecules known as SAPs are secreted by microvesicles which are recognized by host cells and promote an efflux of perinuclear lysosomes. These molecules probably act together with gp82, activating Ca^{2+} pathway and promoting *T. cruzi* internalization [68]. Another molecule capable to recruit lysosomes to entry site is oligopeptidase also involved in Ca^{2+} efflux [69]. Using non-phagocytic cells it is described that the entry of metacyclic trypomastigotes is predominantly due to recruitment of lysosomes, whereas entry of cultured trypomastigotes (from the same CL strain) does not involve the participation of lysosomes [70]. Recently, Rodrigues et al. [26] observed that infection of host cells by metacyclic trypomastigotes is associated with lysosome spreading and presence of gp90 (metacyclic trypomastigote surface glycoprotein). Strains expressing low amount of surface gp90 are able to recruit more lysosomes to the site of infection giving rise to a more successful infection.

In all other mechanisms used by the parasites, there would be no recruitment and exocytosis of lysosomes to the entry site, which are classified as lysosomal-independent mechanisms. In these pathways, there is an invagination of the plasma membrane of the host cell with the consequent formation of a PV without the initial presence of lysosomal markers. In 2002, Wilkowsky et al. [14] demonstrated the existence of this lysosomal-independent pathway for the invasion of *T. cruzi* into two nonprofessional phagocytic cell lines. Using Hela and CHO cells transfected with Rab5, Rab7, or dinamine-GFP, the presence of these endosomal markers in the newly formed vacuole was observed, indicating that some vacuoles fuse first with early and late endosomes and not with lysosomes. Later these events were quantified, and 50% of the parasitophorous vacuoles formed used the plasma membrane of the host cell but were enriched with PI3-kinase action products and negative for endosomal markers; 20% of the other trypomastigote-containing vacuoles were positive for EEA-1 (Rab5 effector and marker of initial endosomes) and Rab5, and approximately 20% of the vacuoles were positive for LAMP-1 (lysosomal marker). Vacuoles from these pathways are matured by the gradual fusion of early endosomes as well as of lysosomes, which allows the complete formation of the PV [16, 19, 71].
Both models of interaction are illustrated in **Figure 3**. In addition to the endolysosomal system, the cytoskeleton and autophagic processes were also related to

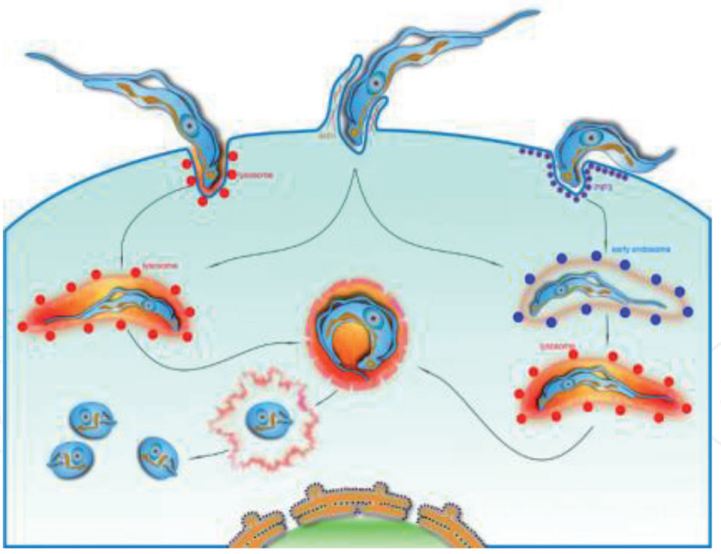

Figure 3.
Schematic model of T. cruzi internalization and parasitophorous vacuole formation using different entry pathways [10].

the complex formation process of *T. cruzi* parasitophorous vacuoles, but there is still much controversy about these involvements. The first time that a compound known to interfere with actin polymerization inhibitor was used with the aim of studying the entry of *T cruzi* was reported by Nogueira and Cohn [56]. Subsequently, Meirelles et al. [72] using the same compound demonstrated that the treatment of chicken macrophages with cytochalasin prevented the entry of the parasite without, however, preventing adhesion. This work was the first to split the entry's phase of *T. cruzi* (adhesion and internalization). On the other hand, using another cytochalasin (cytochalasin D—an actin filament polymerization inhibitor) as a tool, it was observed that the infection rate of the parasite was not significantly altered [33]. Subsequently the Tardieux group [58] demonstrated that invasion of *T. cruzi* into nonprofessional phagocytic cells is significantly enhanced by the depolymerization of the host cell actin cytoskeleton. The rapid reorganization of actin occurs as a response to the trypomastigote stage, suggesting that the direct reorganization of the actin cytoskeleton is a critical step for the entry process [18]. The reduction in the entry process was again observed in other studies, indicating that this divergence may be related to the observed interaction time [73].

In relation to the microtubules, their dynamics are important to facilitate *T. cruzi* invasion targeting lysosomes to entry site [57, 74]. It is also believed that the PV acts as a secondary center for the organization of microtubules, as regards the lysosomal fusion process at the parasite's entry site [74]. Besides a protein known as a CLASP1 (a microtubule plus-end tracking protein) is described as involved in the internalization of *T. cruzi* integrating actin pathway with microtubules and helping with the perinuclear localization of PV [75]. Microtubules are also responsible for carrying vesicles such as those positive for Vamp7 (essential for lysosomal fusion and retention of infection) to the entry site. This transport is dependent on the KIF 5 protein (a kinesin) [76]. More recently, Romano et al. [77] have shown that an autophagic protein, LC3, would also be present in the PV membrane, also demonstrating that the induction of autophagy in the host cell (pharmacological or physiological) interferes with the mechanism of trypomastigote invasion. Autophagy mechanism also plays a protective role against *T. cruzi* infection in mice by activation of a host immune response [78].

4. Endocytic mechanisms involved in trypomastigote infection

Many processes are already described as involved in the entry of *T. cruzi* trypomastigotes. As already discussed in this topic since 1972, Dvorak and Schumunis [55] had already described that the internalization of trypomastigotes in host cells could be by endocytic mechanisms. Endocytic processes are currently divided into different classes: clathrin-mediated endocytosis, endocytosis mediated by membrane microdomains (planar and caveolae), macropinocytosis, and phagocytosis. The first endocytic mechanism described as a participant in *T. cruzi* entry into host cells was phagocytosis. The participation of this mechanism was described by Nogueira and Cohn [56] through the treatment of several cell types, peritoneal macrophages, L929, HeLa, and embryo fibroblasts of calves with cytochalasin B (a drug that interferes with the extension of actin filaments). Afterward, Barbosa et al. [79] demonstrated by transmission electron microscopy that trypomastigotes are able to bind to cardiac muscle cells and induce the formation of extensive pseudopodia, a typical feature of phagocytic processes. Subsequently several reports showed that actin filaments are essential in other endocytic mechanisms, such as the macropinocytosis. Cytochalasins B and D are quite specific inhibitors of phagocytic activity. When the parasite is internalized via phagocytosis, there is internalization of CR3 receptors, β1 integrin, lysosomal membrane glycoproteins (Lpg), and Fc receptors (the latter appears only when trypomastigotes are opsonized). The participation of toll-2 receptors ("toll-like receptors 2"), as well as membrane components containing galactosyl, sialoconjugate, and glycoconjugate residues [80–82], is also demonstrated. In general phagocytosis is also a process that can be divided into different subtypes that are morphologically distinct. Using scanning electron microscopy, it was demonstrated that during internalization of trypomastigotes, the plasma membrane of peritoneal macrophages can cover the parasite in a juxtaposed way, with bilateral projections of plasma membrane forming a funnel-like structure that can follow the entire extent of the parasite's body, culminating in its total internalization (similar to what is described as classical phagocytosis). It has also been observed in the development of structures similar to the initial stages of trigger phagocytosis or macropinocytosis, in addition to the formation of structures described as coiled-type phagocytosis [19, 82, 83].

The participation of membrane rafts in the invasion of *T. cruzi* has also been demonstrated [84, 85]. Regions of membrane microdomains (rafts) are small, dynamic, cholesterol-rich membrane invagination regions, where sphingolipids, GM1 gangliosides, and caveolae (caveolae are a special type of membrane rafts) concentrate. These regions are known as signaling hotspots because they contain several proteins that can be deposited by triggering signaling cascades. This topic (signaling activation in *T. cruzi* entry process) will be discussed later in this chapter. More recently it has been demonstrated that *T. cruzi* could also use another endocytic mechanism in the invasion of its host cell: macropinocytosis. [86]. This endocytic pathway involves the internalization of large areas of plasma membrane along with significant amounts of extracellular fluid. It is important to point out the participation of dynamin as a key protein for the formation and consequent release of the early PV from the plasma membrane. Its inhibition using dynasore or its blockage through an overexpression of a dominant-negative mutant of dynamin inhibits the internalization of trypomastigotes, demonstrating that GTPase activity is also important [71, 86].

5. Signaling pathways involved in trypomastigote penetration

There are several external factors that can regulate different types of cellular responses. For these responses to occur, it is necessary that a conformational change of several proteins takes place, which means that they can now interact

with other molecules leading to their activation, transducing the signal and amplifying it. The conformational change is usually dependent on the action of protein kinases or protein phosphatases. The case of the process of interaction between *Trypanosoma cruzi* and host cells is not different since it is also an external process that will require an internal response. From this perspective, several studies have focused on the investigation of different pathways that coordinate the invasion of *T. cruzi* and that modulate the gene expression of the host cell in response to this process [85, 86].

T. cruzi seems to exploit an infinity of cell surface receptors, secondary messengers, and transcription factors of different pathways to ensure its invasion and survival [86–89]. Among the signaling events, the best studied is that which leads to a calcium release in the host cells. This calcium release is one of the main responsible for regulating the process of invasion of *T. cruzi* [9, 18]. Three different models have already been proposed by different groups as being responsible for the activation of this signaling pathway. Among the described models, two involve peptidases such as oligopeptidase B and cruzipain, and the third one involves a membrane glycoprotein called gp82. Although all three mechanisms activate calcium firing, none of them are correlated. The first proposed model is based on the activation of the serine peptidase called oligopeptidase B where this enzyme present in the trypomastigote cytosol cleaves an inactive precursor to generate an active calcium agonist that is released by the parasite and binds to the receptor present on the surface of the host cell. This receptor is coupled to G protein which stimulates phospholipase C activity generating inositol 3,4,5-trisphosphate which binds to its receptor releasing calcium [90]. The second mechanism, proposed by Scharfstein et al. [91], is based on the secretion of cruzipain through the flagellar pocket region. This protein binds to a kininogen molecule that is cleaved into short kinin molecules, which in turn bind to the bradykinin receptor by stimulating the release of calcium from IP3. Cruzipain is also capable to regulate arginase activity increasing *T. cruzi* survival inside the cell through an increase in the production of IL-10 and TGF-β [92]. The model based on the activation of the glycoprotein gp82 is known to be bidirectional since it has been shown that a peak of calcium is generated not only in the host cell but also in the parasite itself. In the case of activation from this glycoprotein, the receptor is not yet known [90].

As previously described one of the activated pathways is TGF-β, where molecules secreted by trypomastigotes stimulate TGF-β receptors and activate the transcription of genes regulated by this molecule [93]. Activation of this pathway is involved in Chagas disease fibrosis development [94–96]. TGF-β was first described as being activated through cruzipain, but it is now known that trypomastigotes are capable of exposing phosphatidylserine to the outer layer of the plasma membrane. This exposure would be responsible for triggering the TGF signaling pathway in macrophages, based on the phosphorylated Smad2 nuclear translocation, leading to inhibition of iNOS in infected macrophages. This event would favor intracellular survival of the parasite [97]. More recently, the mechanism used to favor this intracellular survival was proposal by Calvet and colleagues [98] using cardiomyocytes as host cell model. In these cells the TGF-β receptor (TβRII) is localized in cardiomyocyte's costameres, which are also rich in vinculin and associated with cytoskeleton (known as a signaling domain). Its activation potentiates Smad2 phosphorylation. When *T. cruzi* infection is established, the cytoskeleton is disorganized, disrupting TβRII striations and decreasing Smad2 phosphorylation making cardiomyocytes less responsive to exogenous TGF-β stimulation.

Phosphorylation of protein tyrosine kinases is an important step in the regulation of a variety of eukaryotic cell signaling pathways [99]. In professional phagocytes, the entry of *T. cruzi* into macrophages is inhibited by treatment with

genistein, a tyrosine kinase inhibitor [79]. It has also been shown that trypomastigotes of the Y strain stimulate tyrosine phosphorylation of a large number of proteins [100]. Pretreatment of fibroblasts with genistein does not inhibit entry of the parasite [18], suggesting different roles for protein tyrosine kinases in *T. cruzi* invasion of the Y strain in professional phagocytic cells or nonprofessional phagocytic cells. In cardiomyocytes and cardiac fibroblasts, tyrosine kinases appear to be essential to infection. Tyrosine kinase C (TrkC) is recognized by *T. cruzi* parasite-derived neurotrophic factor (PDNF) through neurotrophin receptor culminating in the entry of trypomastigotes into cardiac cells, while TrkA activation by the same ligand in the same cell types leads to a decrease in oxidative stress [101]. In this same sense of protection from the *T. cruzi* infection, other signaling pathways are also activated as, for example, the pathways of Erk11/Erk2 and Jak/STATs [102]. Other protein kinases also participate in *T. cruzi* invasion in host cells, such as protein kinase C (PKC), MAP kinases, and phosphatidylinositol 3-kinases (PI3-K) [86, 88, 97]. Recently the regions known as membrane microdomains have been described as signaling platforms. These regions are capable of recruiting a wide range of proteins involved in signal transduction processes. These proteins may include tyrosine kinase receptors and protein kinases such as PI3 kinase, protein kinase C, Src kinase (Lyn and Fyn) family proteins, FAK, bradykinin receptors, GTP (Rac, Rho, and Ras), and adapter proteins (Vav, Sos, and Shc). Some of these molecules have already had their share in the process of invasion of *T. cruzi* elucidated, while others have not. Proteins such as those from the Src kinases family (responsible for ITAM phosphorylation, which is essential for initiating the signal transduction cascade that triggers pathogen growth) and adapter proteins such as Vav, although not demonstrated as participants in this process, have been described by Vieira et al. [88] as possible phosphorylated proteins during the *T. cruzi* invasion in macrophages. Signaling pathways described as involved in macropinocytosis pathway (Pak1 and PKC pathways) are important to the intracellular development of infection [19]. More recently, Wnt signaling also has been shown to be an important pathway to immunomodulatory functions during *T. cruzi* infection, regulating the control of parasite replication. Activation of Wnt pathway is important to avoid a production of pro-inflammatory cytokines and indoleamine 2,3-dioxygenase activity by *T. cruzi*, helping to control the infection [103].

6. Organelles involved in parasitophorous vacuole formation

After the internalization process, *T. cruzi* resides obligatorily, and temporarily, inside a parasitophorous vacuole [10]. Over the years, several groups have described that the formation of the vacuole membrane is a complex process related to the participation of numerous molecules, depending also on the type of host cell involved in the process, i.e., phagocytic lines or nonprofessional phagocytic lines. As regards the formation of the PV membrane, it has been previously discussed in this text that several components of the host cell have already been described as components of the cytoskeleton [19, 73, 104, 105]. The organelle known as endoplasmic reticulum (ER) has been described in the literature as a donor membrane for phagosome formation, binding directly to the base of this phagosome in formation [106]. Recently, our group demonstrated by electron tomography (followed by 3-D reconstruction) and fluorescence videomicroscopy that the endoplasmic reticulum (ER) participates in the process of formation of the vacuole from initial moments until its complete maturation. The participation or not of ER in this process may be directly related to the input mechanism used by the parasite at the time of the invasion [104].

The Golgi complex (CG) plays a central role in eukaryotic cells, serving as an intermediary and bidirectional axis of protein and lipid trafficking in the endomembrane system [107]. In the case of *T. cruzi*, Carvalho et al. [82] described that after long infection times, there would be no change in GC distribution, remaining in the perinuclear region. Recently, transmission electron microscopy showed an intimate proximity between the CG and the parasite-containing vacuole. These data are of extreme relevance due to the function of GC in eukaryotic cells, i.e., because the membrane of the PV containing the parasite is still in the process of formation, requiring lipids and proteins from the host cell that would aid its closure as well as its expansion. As regards mitochondria, apparently there is no direct link between this organelle and the vacuole [104].

7. Disorganization of the parasitophorous vacuole and the endolysosomal system

As discussed above trypomastigotes of *T. cruzi* use different molecules and mechanisms to invade a host cell. Regardless of the chosen mechanism (participation of plasma membrane components or lysosomes at the site of invasion), the parasite will be located inside a vacuole.

Inside the PV the trypomastigotes release the enzyme trans-sialidase/neuraminidase, which is responsible for the removal of the sialic acid residues from the vacuole membrane. This removal makes it sensitive to the action of another enzyme, homologous to factor 9 of human complement, Tc-Tox. The lysis of the vacuole membrane by the action of Tc-Tox may be associated with the formation of pores in this membrane, which, together with the secretion by the trans-sialidase/neuraminidase parasite, will lead to the complete fragmentation of the PV membrane [35, 108].

Despite the attempt to determine the exact moment of the *T. cruzi* exit process from the parasitophorous vacuole, this step has not yet been well elucidated. Since 1989, however, our group has been trying to chart the paths necessary for this discovery. Initially Carvalho and De Souza [109] demonstrated by transmission electron microscopy that the lysing process of the PV membrane begins to occur in the first 2 hours of interaction of the trypomastigote stage in peritoneal macrophages, already suggesting the occurrence of fusion of lysosomes, after entry of the parasites. More recently, Reignault and colleagues [50] demonstrate that galectin-3 decorates *T. cruzi* vacuole acting as an important marker to be used also for the study of parasitic vacuole lysis of *T. cruzi*, as it also undergoes a process of disorganization with consequent exit of the parasite into the cytoplasm of the host cell. Transcriptome studies have shown an extensive remodeling of the intracellular *T. cruzi* in the first 4 hours of trypomastigote invasion (until the parasitophorous vacuole disintegration), and these modifications can be associated with a regulation of the initial step of host cell invasion [110]. Some modifications occurring in intracellular differentiation processes (trypomastigotes to amastigotes and subsequently to trypomastigotes again) may be involved in the process of destruction of PV. One of the modifications that seems to be involved with this process is the increase in the enzymatic activity of glutamine synthase during the amastigogenesis process. This enzyme is normally involved in the process of handling excess ammonia, and given the fact that the main energy source of the intracellular forms of *T. cruzi* is from amino acids, the ammonium generation is high. Marim et al. [111] observed that the activity blockage of this enzyme impair the progression of the intracellular cycle (amastigotes did not differentiate for trypomastigotes). In this case the role of the enzyme is to regulate the intracellular pH by controlling the content of

intravacuolar ammonium (generated by the consumption of amino acids in this reduced space). It is this acidification process followed by the pH control that allows the enzymatic activity that culminates in the release of parasite in the cytoplasmic environment allowing the infection to continue [112].

8. Concluding remarks

Host cell invasion and parasite internalization are important steps in the evolution of parasitism by several pathogens. These processes present at least two important advantages: protection against the host immune response and access to a microenvironment rich in metabolic products. Substantial progress has been made in understanding the roles of proteins in infection and invasion by *T. cruzi*. Host cell intracellular signaling can combat the infection; but it can also favor parasite entry. Parasites hijack the host immune response, phagocytosis, ECM, and antiparasitic proteins for their own survival, replication, and immune evasion purposes. The complex networks are interconnected and require extensive study to identify intracellular rearrangements that facilitate parasite internalization. A multidisciplinary approach is necessary to a better understanding of parasite-host interaction and will be critical to better understand Chagas disease physiopathology, diagnosis, and treatment.

Acknowledgements

We thank all authors who work with this theme and for the contribution during the last years.

Conflict of interest

The authors declare "no conflict of interest."

Author details

Emile Barrias[1*], Lissa C. Reignault[2] and Wanderley de Souza[2]

1 National Institute of Metrology, Quality and Technology (INMETRO), Duque de Caxias, Rio de Janeiro, Brazil

2 Federal University of Rio de Janeiro (UFRJ), Rio de Janaeiro, Brazil

*Address all correspondence to: esbarrias@inmetro.gov.br

References

[1] Chagas C. Nova tripanozomíaze humana. Memórias do Instituto Oswaldo Cruz. 1909;**1**:11-80

[2] Hotez PJ, Molyneux DH, Fenwick A, Kumaresan J, Sachs SE, Sachs JD, et al. Control of neglected tropical diseases. The New England Journal of Medicine. 2007;**357**:1018-1027

[3] WHO. 2019. Available from: https://www.who.int/chagas/en/

[4] Tzizik DM, Borchardt RA. Chagas disease: An underrecognized diagnosis. Journal of the American Academy of PAs. 2018;**31**:30-33. DOI: 10.1097/01.JAA.0000547749.92933.6a

[5] Pérez-Molina JA, Molina I. Chagas disease. Lancet. 2018;**391**:82-94

[6] De Souza W. Basic cell biology of *Trypanosoma cruzi*. Current Pharmaceutical Design. 2002;**8**:211-231

[7] Kessler RL, Contreras VT, Marliére NP, Aparecida Guarneri A, Villamizar Silva LH, Mazzarotto G, et al. Recently differentiated epimastigotes from *Trypanosoma cruzi* are infective to the mammalian host. Molecular Microbiology. 2017;**104**:712-736

[8] Garcia ES, Castro DP, Figueiredo MB, Azambuja P. Immune homeostasis to microorganisms in the guts of triatomines (Reduviidae)—A review. Memórias do Instituto Oswaldo Cruz. 2010;**105**:605-610

[9] Rodriguez A, Samoff E, Riolt MG, Andrews NA. Host cell invasion by trypanosomes requires lysosomes and microtubule/kinesin-mediated transport. The Journal of Cell Biology. 1996;**134**:349-362

[10] de Souza W, Carvalho TMU, Barrias ES. Review on *Trypanosoma cruzi*: Host cell interaction. International Journal of Cell Biolog. 2010;**pii**:295394. DOI: 10.1155/2010/295394

[11] Andrews NW. From lysosomes into the cytosol: The intracellular pathway of *Trypanosoma cruzi*. Brazilian Journal of Medical and Biological Research. 1994;**27**:471-475

[12] Teixeira DE, Benchimol M, Crepaldi PH, de Souza W. Interactive multimedia to teach the life cycle of *Trypanosoma cruzi*, the causative agent of Chagas disease. PLoS Neglected Tropical Diseases. 2012;**6**(8):e1749

[13] De Souza W, Carvalho TMU. Active penetration of *Trypanosoma cruzi* into host cells: Historical considerations and current concepts. Frontiers in Immunology. 2013;**4**:1-3

[14] Wilkowsky SE, Barbieri MA, Stahl PD, Isola ELD. Regulation of *Trypanosoma cruzi* invasion of nonphagocytic cells by the endocytically active GTPases dynamin, Rab5, and Rab7. Biochemical and Biophysical Research Communications. 2002;**291**:516-521

[15] Araújo-Jorge TC. The biology of *Trypanosoma cruzi*—Macrophage interaction. Memórias do Instituto Oswaldo Cruz. 1989;**84**:441-462

[16] Araújo-Jorge TC, Barbosa HS, Meirelles MN. *Trypanosoma cruzi* recognition by macrophages and muscle cells: Perspectives after 15-year study. Memórias do Instituto Oswaldo Cruz. 1982;**87**(suppl 5):43-56

[17] Burleigh BA, Andrews N. The mechanisms of *Trypanosoma cruzi* invasion in mammalian cells. Annual Review of Microbiology. 1995;**49**:175-200

[18] Rodriguez A, Riolt MG, Ora A, Andrews NA. A trypanosome-soluble

factor induces PI3 formation, intracellular Ca^{2+} mobilization and microfilament rearrangement in host cells. The Journal of Cell Biology. 1995;**129**:1263-1273

[19] Barrias ES, De Carvalho TM, De Souza W. *Trypanosoma cruzi*: Entry into mammalian host cells and parasitophorous vacuole formation. Frontiers in Immunology. 2013;**1**:184-186

[20] Ruiz RC, Favoreto S, Dorta ML, Oshiro MEM, Ferreira AT, Manque PM, et al. Infectivity of *Trypanosoma cruzi* strains is associated with differential expression of surface glycoproteins with differential Ca^{2+} signaling activity. The Biochemical Journal. 1998;**330**:505-511

[21] Neira I, Silva FA, Cortez M, Yoshida N. Involvement of *Trypanosoma cruzi* metacyclic trypomastigote surface molecule gp82 in adhesion to gastric mucin and invasion of epithelial cells. Infection and Immunity. 2003;**71**:557-561

[22] Franco FR, Paranhos-Bacallà GS, Yamauchi LM, Yoshida N, Da Silveira JF. Characterization of a cDNA clone encoding the carboxy-terminal domain of a 90-kilodalton surface antigen of *Trypanosoma cruzi* metacyclic trypomastigotes. Infection and Immunity. 1993;**61**(10):4196-4201

[23] Cortez C, Martins RM, Alves RM, Silva RC, Bilches LC, Macedo S, et al. Differential infectivity by the oral route of *Trypanosoma cruzi* lineages derived from Y strain. PLoS Neglected Tropical Diseases. 2012;**6**(10):e1804

[24] Nogueira NF, Gonzalez MS, Gomes JE, De Souza W, Garcia ES, Azambuja P, et al. *Trypanosoma cruzi*: Involvement of glycoinositolphospholipids in the attachment to the luminal midgut surface of Rhodnius prolixus. Experimental Parasitology. 2007;**116**:120-128

[25] Malaga S, Yoshida N. Targeted reduction in expression of *Trypanosoma cruzi* surface metacyclic trypomastigote surface molecule gp82 in adhesion to gastric mucin. Microbiology. 2001;**4**(11):701-711

[26] Rodrigues JPF, Sant'ana GHT, Juliano MA, Yoshida N. Inhibition of host cell lysosome spreading by *Trypanosoma cruzi* metacyclic stage-specific surface molecule gp90 downregulates parasite invasion. Infection and Immunity. 2017;**85**(9):pii: e00302-pii: e00317

[27] Torrecilhas AC, Schumacher RI, Alves MJ, Colli W. Vesicles as carriers of virulence factors in parasitic protozoan diseases. Microbes and Infection. 2012;**14**(15):1465-1474

[28] Garcia-Silva MR, Cabrera-Cabrera F, Das Neves RF, Souto-Padron T, De Souza W, Cayota A. Gene expression changes induced by *Trypanosoma cruzi* shed microvesicles in mammalian host cells: Relevance of tRNA-derived halves. BioMed Research International. 2014;**2014**:305239

[29] De Souza W, Barrias ES. Exosomes in the pathogenic protozoan *Trypanosoma cruzi*. International Journal of Pathology and Clinical Research. 2017;**3**:1-9

[30] Parodi AJ, Pollevick GD, Mautner M, Buschiazzo A, Sanchez DO, Frasch AC. Identification of the gene(s) coding for the trans-sialidase of *Trypanosoma cruzi*. The EMBO Journal. 1992;**11**:1705-1710

[31] Previato JO, Andrade AF, Pessolani MC, Mendonça-Previato L. Incorporation of sialic acid into *Trypanosoma cruzi* macromolecules. A proposal for a new metabolic route. Molecular and Biochemical Parasitology. 1985;**16**(1):85-96

[32] Souto-Padrón T, Hath G, De Souza W. Immunocytochemical localization

of neuraminidase in *Trypanosoma cruzi*. Infection and Immunity. 1990;**58**:586-592

[33] Schenkman S, Jiang MS, Hart GW, Nussenzweig V. A novel cell surface trans-sialidase of *Trypanosoma cruzi* generates a stage-specific epitope required for invasion of mammalian cells. Cell. 1991;**65**:1117-1125

[34] Schenkman S, Eichinger D. *Trypanosoma cruzi* trans-sialidase and cell invasion. Parasitology Today. 1993;**9**:218-222

[35] Pereira ME, Zhang K, Gong Y, Herrera EM, Ming M. Invasive phenotype of *Trypanosoma cruzi* restricted to a population expressing trans-sialidase. Infection and Immunity. 1996;**64**:3884-3892

[36] Ribeiro KS, Vasconcellos CI, Soares RP, Mendes MT, Ellis CC, Aguilera-Flores M, et al. Proteomic analysis reveals different composition of extracellular vesicles released by two *Trypanosoma cruzi* strains associated with their distinct interaction with host cells. Journal of Extracellular Vesicles. 2018;**7**:1463779

[37] Belew AT, Junqueira C, Rodrigues-Luiz GF, Valente BM, Oliveira AER, Polidoro RB, et al. Comparative transcriptome profiling of virulent and non-virulent *Trypanosoma cruzi* underlines the role of surface proteins during infection. PLoS Pathogens. 2017;**13**(12):e1006767

[38] Alves MJ, Abuin G, Kuwajima VY, Colli W. Partial inhibition of trypomastigote entry into cultured mammalian cells by monoclonal antibodies against a surface glycoprotein of *Trypanosoma cruzi*. Molecular and Biochemical Parasitology. 1986;**21**:75-82

[39] Todeschini AR, Dias WB, Girard MF, Wieruszeski J, Mendonça-Previato L, Previato JO. Enzymatically inactive trans-sialidase from *T. cruzi* infection. The Journal of Biological Chemistry. 2004;**275**:32182-32186

[40] San Francisco J, Barría I, Gutiérrez B, Neira I, Muñoz C, Sagua H, et al. Decreased cruzipain and gp85/trans-sialidase family protein expression contributes to loss of *Trypanosoma cruzi* trypomastigote virulence. Microbes and Infection. 2017;**19**: 55-61

[41] Pascuale CA, Burgos JM, Postan M, Lantos AB, Bertelli A, Campetella O, et al. Inactive trans-sialidase expression in iTS-null *Trypanosoma cruzi* generates virulent trypomastigotes. Frontiers in Cellular and Infection Microbiology. 2017;**4**(7):430

[42] Lantos AB, Carlevaro G, Araoz B, Ruiz Diaz P, Camara Mde L, Buscaglia CA, et al. Sialic acid glycobiology unveils *Trypanosoma cruzi* trypomastigote membrane physiology. PLoS Pathogens. 2016;**12**(4):e1005559

[43] Araujo-Jorge TC, De Souza W. Effect of carbohydrates, periodate and enzymes in the process of endocytosis of *Trypanosoma cruzi*. Acta Tropica. 1984;**41**:17-28

[44] Alves MJ, Colli W. *Trypanosoma cruzi*: Adhesion to the host cell and intracellular survival. IUBMB Life. 2007;**59**:274-279

[45] Pinazo M, Thomas M, Bustamante J, Almeida I, Lopez M, Gascon J. Biomarkers of therapeutic responses in chronic Chagas disease: State of the art and future perspectives. Memórias do Instituto Oswaldo Cruz. 2015;**110**:422-432

[46] Pineda MA, Cuervo H, Fresno M, Soto M, Bonay P. Lack of galectin-3 prevents cardiac fibrosis and effective immune responses in a murine model of *Trypanosoma cruzi* infection. The Journal of Infectious Diseases.

2015;**212**:1160-1171. DOI: 10.1093/infdis/jiv185

[47] Benatar AF, García GA, Bua J, Cerliani JP, Postan M, Tasso LM, et al. Galectin-1 prevents infection and damage induced by *Trypanosoma cruzi* on cardiac cells. PLoS Neglected Tropical Diseases. 2015;**9**(10):e0004148

[48] Souza BSF, Silva KN, Silva DN, Rocha VPC, Paredes BD, Azevedo CM. Galectin-3 knockdown impairs survival, migration, and immunomodulatory actions of mesenchymal stromal cells in a mouse model of Chagas disease cardiomyopathy. Stem Cells International. 2017: 3282656

[49] Kleshchenko Y, Moody T, Furtak VA, Ochieng J, Lima M, Villalta F. Human galectin-3 promotes *Trypanosoma cruzi* adhesion to human coronary artery smooth muscle cells. Infection and Immunity. 2004;**72**:6717-6721

[50] Reignault LC, Barrias ES, Soares Medeiros LC, De Souza W, De Carvalho TM. Structures containing galectin-3 are recruited to the parasitophorous vacuole containing *Trypanosoma cruzi* in mouse peritoneal macrophages. Parasitology Research. 2014;**113**:2323-2333

[51] Da Silva AA, Teixeira TL, Teixeira SC, Machado FC, Dos Santos MA, Tomiosso TC, et al. Galectin-3: A friend but not a foe during *Trypanosoma cruzi* experimental infection. Frontiers in Cellular and Infection Microbiology. 2017;**7**:463

[52] Hissa B, Andrade LDE. *Trypanosoma cruzi* uses a specific subset of host cell lysosomes for cell invasion. Parasitology International. 2015;**64**:135-138

[53] Magdesian MH, Tonelli RR, Fessel MR, Silveira MS, Schumacher RI, Linden R, et al. A conserved domain of the gp85/trans-sialidase family activates host cell extracellular signal-regulated kinase and facilitates *Trypanosoma cruzi* infection. Experimental Cell Research. 2007;**313**:210-218

[54] Martins NO, Souza RT, Cordero EM, Maldonado DC, Cortez C, Marini MM, et al. Molecular characterization of a novel family of *Trypanosoma cruzi* surface membrane proteins (TcSMP) involved in mammalian host cell invasion. PLoS Neglected Tropical Diseases. 2015;**9**(11):e0004216

[55] Dvorak JA, Schumunis GA. *Trypanosoma cruzi*: Interaction with mouse peritoneal macrophages. Experimental Parasitology. 1972;**32**:289-300

[56] Nogueira N, Cohn Z. *Trypanosoma cruzi*: Mechanism of entry and intracellular fate in mammalian cells. The Journal of Experimental Medicine. 1976;**143**:1402-1420

[57] Martins RM, Alves RM, Macedo S, Yoshida N. Starvation and rapamycin 18 differentially regulate host cell lysosome exocytosis and invasion by *Trypanosoma cruzi* metacyclic forms. Cellular Microbiology. 2011;**13**:943-954

[58] Tardieux I, Webster P, Ravesloot J, Boron W, Lunn JA, Heuser JE, et al. Lysosome recruitment and fusion are early events required for trypanosome invasion of mammalian cells. Cell. 1992;**71**:1117-1130

[59] Burleigh BA. Host cell signaling and *Trypanosoma cruzi* invasion: Do all roads lead to lysosomes? Science. 2005;**293**:36

[60] Reddy A, Caler EV, Andrews NW. Plasma membrane repair is mediated by Ca (2+)-regulated exocytosis of lysosomes. Cell. 2001;**106**:157-169

[61] Tam C, Idone V, Devlin C, Fernandes MC, Flannery A, He X, et al. Exocytosis of acid sphingomyelinase by wounded cells promotes endocytosis and plasma membrane

repair. The Journal of Cell Biology. 2010;**189**:1027-1038

[62] Koval M, Pagano RE. Intracellular transport and metabolism of sphingomyelin. Biochimica et Biophysica Acta. 1991;**1082**:113-125

[63] Gulbins E, Kolesnick R. Raft ceramide in molecular medicine. Oncogene. 2003;**22**:7070-7077

[64] Grassmé H, Riethmüller J, Gulbins E. Biological aspects of ceramide-enriched membrane domains. Progress in Lipid Research. 2007;**46**:161-170

[65] Trajkovic K, Hsu C, Chiantia S, Rajendran L, Wenzel D, Wieland F, et al. Ceramide triggers budding of exosome vesicles into multivesicular endosomes. Science. 2008;**319**:1244-1247

[66] Fernandes MC, Andrews NW. Host cell invasion by *Trypanosoma cruzi*: A unique strategy that promotes persistence. FEMS Microbiology Reviews. 2012;**36**:734-747

[67] Couto NF, Pedersane D, Rezende L, Dias PP, Corbani TL, Bentini LC, et al. LAMP-2 absence interferes with plasma membrane repair and decreases *T. cruzi* host cell invasion. PLoS Neglected Tropical Diseases. 2017;**11**(6):e0005657. DOI: 10.1371/journal.pntd.0005657

[68] Zanforlin T, Bayer-Santos E, Cortez C, Almeida IC, Yoshida N, Da Silveira JF. Molecular characterization of *Trypanosoma cruzi* SAP proteins with host-cell lysosome exocytosis-inducing activity required for parasite invasion. PLoS One. 2013;**8**(12):e83864

[69] Motta FN, Bastos IM, Faudry E, Ebel C, Lima MM, Neves D, et al. The *Trypanosoma cruzi* virulence factor oligopeptidase B (OPBTc) assembles into an active and stable dimer. PLoS One. 2012;**7**(1):e30431

[70] Cortez C, Real F, Yoshida N. Lysosome biogenesis/scattering increases host cell susceptibility to invasion by *Trypanosoma cruzi* metacyclic forms and resistance to tissue culture trypomastigotes. Cellular Microbiology. 2016;**18**:748-760

[71] Woosley AM, Sunwoo L, Petersen CA, Brachmann SM, Cantley LC, Burleigh BA. Novel PI3-kinase-dependent mechanisms of trypanosome invasion and vacuole maturation. Journal of Cell Science. 2003;**116**:3611-3622

[72] Meirelles MNL, Araújo-Jorge TC, De Souza W. Interaction of *Trypanosoma cruzi* with macrophages *in vitro*: Dissociation of the attachment and internalization phases by low temperature and cytochalasin B. Zeitschrift für Parasitenkunde. 1982;**68**:7-14

[73] Rosestolato CT, Dutra JDM, De Souza W, De Carvalho TM. Participation of host cell actin filaments during interaction of trypomastigotes forms of *Trypanosoma cruzi* with host cells. Cell Structure and Function. 2002;**27**:91-98

[74] Tyler KM, Luxton GW, Applewhite DA, Murphy SC, Engman DM. Responsive microtubule dynamics promote cell invasion by *Trypanosoma cruzi*. Cellular Microbiology. 2005;**7**:1579-1591

[75] Zhao X, Kumar P, Shah-Simpson S, Caradonna KL, Galjart N, Teygong C, et al. Host microtubule plus-end binding protein CLASP1 influences sequential steps in the *Trypanosoma cruzi* infection process. Cellular Microbiology. 2013;**15**:571-584

[76] Cueto JA, Vanrell MC, Salassa BN. Soluble N-ethylmaleimide-sensitive factor attachment protein receptors required during *Trypanosoma cruzi* parasitophorous vacuole development. Cellular Microbiology. 2017;**19**:e12713

[77] Romano PS, Cueto JA, Cassassa AF, Vanrell MC, Gotlieb RA, Colombo MI. Molecular and cellular mechanisms involved in the *Trypanosoma cruzi* host cell interplay. IUBMB Life. 2012;**64**:387-396

[78] Casassa AF, Vanrell MC, Colombo MI, Gottlieb RA, Romano PS. Autophagy plays a protective role against *Trypanosoma cruzi* infection in mice. Virulence. 2019;**10**:151-165

[79] Barbosa HS, Meirelles MNL. Evidence of participation of cytoskeleton of heart muscle cells during the invasion of *Trypanosoma cruzi*. Cell Structure and Function. 1995;**20**:275-284

[80] Hall BF, Furtado GC, Joiner KA. Characterization of host cell-derived membrane proteins of the vacuole surrounding different intracellular forms of *Trypanosoma cruzi* in J774 cells. Evidence for phagocyte receptor sorting during the early stages of parasite entry. Journal of Immunology. 1991;**147**:4313-4321

[81] Maganto-Garcia E, Punzon C, Terhorst C, Fresno M. Rab5 activation by toll-like receptor 2 is required for *Trypanosoma cruzi* internalization and replication in macrophages. Traffic. 2008;**9**:1299-1315

[82] Carvalho TMU, De Souza W, Coimbra E. Internalization of components of the host cell plasma membrane during infection by *Trypanosoma cruzi*. Memórias do Instituto Oswaldo Cruz. 1999;**94**:143-147

[83] Rittig MG, Scroppel K, Seack KH, Sander U, N'diaye EN, Parini I, et al. Coiling phagocytosis of trypanosomatids and fungal cells. Infection and Immunity. 1998;**66**:4331-4339

[84] Barrias ES, Dutra JM, De Souza W, Carvalho TMU. Participation of macrophage membrane rafts in *Trypanosoma cruzi* invasion process. Biochemical and Biophysical Research Communications. 2007;**363**:828-834

[85] Fernandes MC, Cortez M, Geraldo Yoneyama KA, Straus AH, Yoshida N, Mortara RA. Novel strategy in *Trypanosoma cruzi* cell invasion: Implication of cholesterol and host cell microdomains. International Journal for Parasitology. 2007;**37**:1431-1441

[86] Barrias ES, Reignault LC, De Souza W, Carvalho TM. *Trypanosoma cruzi* uses macropinocytosis as an additional entry pathway into mammalian host cell. Microbes and Infection. 2012;**14**:1340-1351

[87] Chuenkova MV, Furnari FB, Cavenee WK, Pereira MA. *Trypanosoma cruzi* trans-sialidase: A potent and specific survival factor for human schwann cells by means of phosphatidylinositol 3-kinase/akt signaling. Proceedings of the National Academy of Sciences. 2001;**98**:9936-9941

[88] Vieira MC, De Carvalho TMU, De Souza W. Effect of protein kinase inhibitors on the invasion process of macrophages by *Trypanososma cruzi*. Biochemical and Biophysical Research Communications. 1994;**203**:967-971

[89] Watanabe Costa R, Da Silveira JF, Bahia D. Interactions between *Trypanosoma cruzi* secreted proteins and host cell signaling pathways. Frontiers in Microbiology. 2016;**7**:388

[90] Burleigh BA, Woolsey AM. Cell signalling and *Trypanosoma cruzi* invasion. Cellular Microbiology. 2002;**4**:701-711

[91] Scharfstein J, Schmitz V, Morandi V, Capella MM, Lima AP, Morrot A, et al. Host cell invasion by *Trypanosoma cruzi* is potentiated by activation of bradykinin B(2) receptors. The

Journal of Experimental Medicine. 2000;**192**:1289-1300

[92] Ferrão PM, D'avila-Levy CM, Araujo-Jorge TC, Degrave WM, Gonçalves AS, Garzoni LC, et al. Cruzipain activates latent TGF-β from host cells during *T. cruzi* invasion. PLoS One. 2015;**10**(5):e0124832

[93] Hall B, Pereira M. Dual role for transforming growth factor β-dependent signaling in *Trypanosoma cruzi* infection of mammalian cells. Infection and Immunity. 2000;**68**:2077-2081

[94] Araújo-Jorge TC, Waghabi MC, Bailly S, Feige JJ. The TGF-β pathway as an emerging target for Chagas disease therapy. Clinical Pharmacology and Therapeutics. 2012;**92**:613-621

[95] Araújo-Jorge TC, Waghabi MC, Hasslocher-Moreno AM, Xavier SS, Higuchi MDL, Keramidas M, et al. Implication of transforming growth factor-beta1 in Chagas disease myocardiopathy. Journal of Infectious Diseases. 2002;**186**:1823-e3018

[96] Araújo-Jorge TC, Waghabi MC, Soeiro MDNC, Keramidas M, Bailly S, Feige JJ. Pivotal role for TGF-beta in infectious heart disease: The case of *Trypanosoma cruzi* infection and consequent chagasic myocardiopathy. Cytokine & Growth Factor Reviews. 2008;**19**:405-413

[97] Damatta RA, Seabra SH, Deolindo P, Arnholdt AC, Manhães L, Goldenberg S, et al. *Trypanosoma cruzi* exposes phosphatidylserine as an evasion mechanism. FEMS Microbiology Letters. 2007;**1**:29-33

[98] Calvet CM, Silva TA, de Melo TG, de Araújo-Jorge TC, Pereira MC. TGF-β receptor type II costameric localization in cardiomyocytes and host cell TGF-β response is disrupted by *Trypanosoma cruzi* infection. Parasitology. 2016;**143**(6):704-715

[99] Storz P, Toker A. 3-Phosphoinositide dependent kinase-1 (PDK-1) in PI 3-kinase signaling. Frontiers in Bioscience. 2002;**7**:886-902

[100] Ruta S, Plasman N, Zaffran Y, Capo C, Mege JL, Vray B. *Trypanosoma cruzi*-induced tyrosine phosphorylation in murine peritoneal macrophages. Parasitology Research. 1996;**82**:481-484

[101] Aridgides D, Salvador R, Pereiraperrin M. *Trypanosoma cruzi* highjacks TrkC to enter cardiomyocytes and cardiac fibroblasts while exploiting TrkA for cardioprotection against oxidative stress. Cellular Microbiology. 2013;**15**(8):1357-1366

[102] Stahl P, Schwarz RT, Debierre-Grockiego F, Meyer T. *Trypanosoma cruzi* parasites fight for control of the JAK-STAT pathway by disarming their host. JAK-STAT. 2014;**3**(4):e1012964

[103] Volpini X, Ambrosio L, Fozzatti L, Insfran C, Stempin C, Cervi L, et al. *Trypanosoma cruzi* exploits Wnt signaling pathway to promote its intracellular replication in macrophages. Frontiers in Immunology. 2018;**9**:859

[104] Reignault LC, Alcantra C, Barrias ES, de Souza W. 3D reconstruction of Trypanosoma cruzi-macrophage interaction shows the recruitment of host cell organelles towards the parasitophorous vacuole during its biogenesis. Journal of Structural Biology. 2019;**205**(2):133-146. DOI: 10.1016/j. jsb.2018.12.010

[105] Tardieux I, Nathanson MH, Andrews N. Role in host cell invasion of *Trypanosoma cruzi*-induced cytosolic free Ca^{2+} transients. The Journal of Experimental Medicine. 1994;**179**:1017-1022

[106] Huynh KK, Kay JG, Stow JL, Grinstein S. Fusion, fission, and secretion during phagocytosis. Physiology. 2007;**22**:366-372

[107] Mcdermott MI, Mousley CJ. Lipid transfer proteins and the tuning of compartmental identity in the Golgi apparatus. Chemistry and Physics of Lipids. 2016;**200**:42-61

[108] Rubin-de-Celis SS, Uemura H, Yoshida N, Schenkman S. Expression of trypomastigote trans-sialidase in metacyclic forms of *Trypanosoma cruzi* increases parasite escape from its parasitophorous vacuole. Cellular Microbiology. 2006;**8**:1888-1898

[109] Carvalho TMU, De Souza W. Early events related with the behaviour of *Trypanosoma cruzi* within an endocytic vacuole in mouse peritoneal macrophages. Cell Structure and Function. 1989;**14**:383-392

[110] Li Y, Shah-Simpson S, Okrah K, Belew AT, Choi J, Caradonna KL, et al. Transcriptome remodeling in *Trypanosoma cruzi* and human cells during intracellular infection. PLoS Pathogens. 2016;**12**(4):e1005511

[111] Crispim M, Damasceno FS, Hernández A, Barisón MJ, Pretto Sauter I, Souza Pavani R, et al. The glutamine synthetase of *Trypanosoma cruzi* is required for its resistance to ammonium accumulation and evasion of the parasitophorous vacuole during host-cell infection. PLoS Neglected Tropical Diseases. 2018;**12**(1):e0006170

[112] Ming M, Chuenkova M, Ortega-Barria E, Pereira ME. Mediation of *Trypanosoma cruzi* invasion by sialic acid on the host cell and trans-sialidase on the trypanosome. Molecular and Biochemical Parasitology. 1993;**59**:243-252