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An overview of mammalian cell infection by *Trypanosoma cruzi*: Cellular and molecular basis

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Abstract

Trypanosoma cruzi, the agent of Chagas' disease, is an obligate intracellular parasite that infects many different cell types, through a series of events that involve the participation of both parasites and host cell signaling cascades and components. In order to infect mammalian cells, the parasite must attach the cell before to gain entry forming parasitophorous vacuoles from which escape to differentiate into amastigotes and replicate freely in the cytosol. After

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binary division, amastigotes differentiate back into trypomastigotes which are released after the host cell rupture. The basic features of the infection process of Trypanosoma cruzi have been known for nearly a century. However, various aspects of the infection mechanisms have been elucidated only recently, whilst others remain either controversial or unstudied. Several reviews have been published about different aspects of invasion process, in this chapter however, a general integral review of the whole infections process of trypomastigotes and amastigotes forms will be presented influenced by recent finding and specific questions that remain unresolved.

Introduction

Trypanosoma cruzi is the flagellated protozoan causative agent of Chagas' disease. The infection is transmitted to one of more than 100 mammalian species from different orders by one of more than 130 hemiptera of Reduviidae family; when the insect deposit on the host skin their feces containing metacyclic trypomastigotes during the blood meal (1, 2). Through the oral and ocular mucosa or lesion in the skin, the parasite invade host cells causing a chronic illness in humans, affecting 16 to 18 millions of individuals widespread from Southern United States to the Southern Argentine. Although less frequently, human to human transmission can also occur through exposure to contaminated blood during pregnancy or blood transfusion that together with organ transplantation are the main source of transmission in developed countries due to significant increases in immigration to the United States and Canada from endemic countries, estimating that approximately 100,000 to 650,000 people residing in USA may be infected with *T. cruzi.* This indicates the potential for the emergence of Chagas disease as a public health matter is in the USA (3, 4, 5, 6). The transmission naturally occur by a sylvatic cycle from the infected insect to wild vertebrate animals and back to the insect, while the transmission to humans dependent mainly by the so-called domestic cycle where the living conditions as the cohabitation of infected vectors that reside in the peridomestic habitat of mud-thatch houses in rural areas and infected domestic animals is important (7). Because the transmission of *T. cruzi* to human occurs predominantly via insect vectors, the members of Triatominae, a subfamily of Reduviidae, also known as *conenose bugs*, *kissing bugs* or *triatomines*, it has been broadly studied. The vector is widespread in America being *Triatoma infestant* in South America; *Rhodnius prolixus* in Venezuela, Colombia and Central America; *Triatoma dimidiata* in Ecuador, Northern Peru and Central America; *Rhodnius ecuadoriensis* in Northern Peru and *Phyllosoma* complex and *Triatoma dimidiata* in Mexico; the most common species responsible for transmission (8, 9, 10).

Improvements in housing conditions, vector control and screening of blood donor banks through the three Latin America initiatives (Southern cone, Andean cone and Central America cone Initiatives to control Chagas' disease) are making progress to decline the transmission of *Trypanosoma cruzi* to human in endemic countries. However, despite these important efforts, new cases are still found in some localities, especially in areas without vector-control measures. Thus, the concern remains about the continue transmission of the parasite by blood donation in countries as USA where no approved screening blood donation test is available, and the possibility of a reinfestation of homes by secondary sylvatic vectors, e.g. *Triatoma sordida*, which could compromise the long-term efficacy of vector control measures in Brazil and other Southern American countries (3, 11, 12, 13, 14, 15, 16). These conditions, taken together, have became an issue of great concern in Latin America and developed countries such as USA, where Chagas' disease is still, or is becoming an important public health problem; mainly considering that non effective drugs treatment for the chronic infection are available and the currents drugs treatment for the acute phase show serious and frequent side-effects and unsatisfactory cure rates. Moreover, this treatment is not always accessible to most patients in many endemic countries because the drugs have not been registered or their prices are too high.

Chagasic disease

The sickness presents very variable clinical manifestations exhibiting an acute phase followed by a chronic phase separated by an indeterminate period during which the patient is relatively asymptomatic. The acute phase is characterized by a swelling at the site of parasite entry in the skin (*chagoma*) or the conjunctiva or eyelid (*Romana's sign*) in addition to high blood parasitemia with non-specific symptoms, e.g. fever, malaise, edema and/or enlarged liver or spleen that not always warranty hospital visit, and therefore, anti-parasitic treatment is often not initiated. If untreated, the manifestations of the acute disease resolve spontaneously within 4 to 8 weeks in approximately 90% of infected individuals. About half of these patients will never develop chronic lesions and a direct progression from the acute phase to a clinical form of Chagas' disease only occurs in 5 to 10% patients (17). In less of 5% of acute patients, sudden death due to congestive heart failure associated with myocarditis or meningoencephalitis may occur, but the majority of patients enter an indeterminate phase detected only by the presence of *T. cruzi* specific antibodies because the absence of clinical signs of cardiac abnormalities. About 30 to 40 % of patients in the indeterminate period progress to the chronic phase which the most important clinical attention focuses on cardiac and digestive illness, although Chagas' disease may affect a variety of organs.

Clinical differentiation of cardiac or digestive forms of Chagas' disease from other causes of cardiac disease or visceral dilatation is not always possible. Between 10 to 30 years after initial infection, an estimated 30 to 40 % of chronic cases show chagasic cardiomyopathy in which once congestive heart failure supervenes and the life expectancy is reduced to a few years (18, 19, 20). The clinical manifestations of chagasic cardiomyopathy are congestive heart failure, thromboembolism in brain, limbs or lungs, ventricular fibrillation, arrhythmias and cardiomegaly with hypertrophy and dilation of the chambers and aneurism, particularly in the apical portion of the left ventricle, which may rupture (21). Necropsy and biopsy histological studies reveal focal tissue fibrosis, inflammation, and hypertrophy of cardiac fibers of chagasic heart disease. Persistent low-grade myocardial parasitism and autoimmune aggression due to *T. cruzi* antigenic mimicry are considered the most relevant pathogenetic mechanisms of chronic myocarditis (22, 23, 24, 25, 26, 27, 28). Meanwhile, the digestive forms of the disease lead to megaesophagus and/or megacolon in approximately 30 % of chronic cases, of which 20-50% also present with an associated cardiopathy. These patients develop gastrointestinal disorders as dilations of esophagus and colon, secondary to lesions of the enteric nervous system. The clinical manifestations of chagasic megaesophagus are dysphagia mainly for dry, solid, and cold food (reason why the patients are generally ingesting large quantities of water to make deglutition easier), odynophagia, hiccup, ptyalism, regurgitation (which can produce pneumonitis as a result of regurgitation and aspiration of food particularly during sleep), irritative esophagitis, salivary gland hypertrophy, weight loss, cachexia (in severe cases), signs of rupture of esophagus and it may be associate with an increased incidence of cancer of the esophagus. The patients with chronic chagasic megacolon present severe constipation for a few days to 2-3 months and abdominal pain frequently associated with episodes of bowel obstruction, asymmetric distension of abdomen, meteorism, signs of intestinal occlusion and sigmoid volvulus (29).

Even when it has been suggested that the occurrence of a variable clinical symptoms of acute episodes, symptom-less infection or severe cardiovascular or gastrointestinal chronic disease could be associated with some parasites and/or host factors, a correlation among the genetic parasite polymorphism, the degree of parasitemia or the level of antibodies with the different clinical forms or the severity of the lesions in humans has not been established yet.

Life cycle

The intracellular protozoan *T. cruzi* undergoes a complex biphasic life cycle, comprised four distinct developmental stages, two in the Reduviid beetle vector (epimastigotes and metacyclic trypomastigotes) and two in the

mammalian host (amastigotes and blood trypomastigotes). The triatomine became infected by taking up the non-dividing but infectious circulating trypomastigotes during the blood meal. After moving to the midgut and hindgut the trypomastigotes differentiate to epimastigotes in the insect gut lumen reproducing asexually by binary fission before to migrate along the hindgut and rectal where they attach hydrophobically to the waxy cuticle by means of their flagella and cAMP mediated process. Then, they transform to metacyclic trypomastigotes and remain in the rectal lumen until the triatomine infected takes its next blood meal (30, 31, 32, 33, 34). The trypomastigotes released together with the insect faeces enter to the host through the bite wound, or mucosal membrane such as the conjunctiva and once incorporated to the mammalian blood, the parasite penetrate non-phagocytic and phagocytic cells, typically around the wound site. Here, it initiates its intracellular replicative cycle, taking from 3 to 5 days, during which differentiates into an amastigote and initiates its replication in the cytoplasm of the infected cell. Ultimately, the amastigotes develop into non-dividing bloodstream trypomastigotes, which can either, initiate another round of infection to propagate it to different organs or be taken up by the Reduviid vector during a blood meal completing the life cycle.

The presence of amastigotes in the circulation and the extra-cellular space between heart muscle cells has suggested an alternative sub-cycle probably as a result of the premature lysis of infected cells releasing intra-cellular amastigotes (35, 36, 37, 38) or by the extra-cellular differentiation of trypomastigotes obtaining the extra-cellular amastigotes-like forms (39) in which are able to invade professional and nonprofessional cells and generate a productive infection (35, 37, 38, 40, 41, 42).

Infection process

Even that the infection process of *T. cruzi* has been described many years ago, the molecular mechanisms involved remains poorly understood. *T. cruzi* is an obligate intracellular parasite that infects diverse professional and nonprofessional mammalian cells by a complex process that appears to involve several discrete steps beginning with the **attachment** of the parasite to the host cell followed by its **internalization** through a parasitophorous vacuole, from which escape to **multiply** freely in the citosol before to **differentiate** newly to bloodstream trypomastigote to ultimately be **liberated** from the host cell (Figure 1).

Given that the pathology of Chagas' disease is carried out by the persistence of parasites in muscle tissue (43, 44) and the trypomastigotes were considered for a long time as the only infective forms, several efforts has been concentrated to elucidate how the trypomastigotes infect non-phagocytic cells. Nevertheless, the recently demonstration that amastigotes can infect mammalian

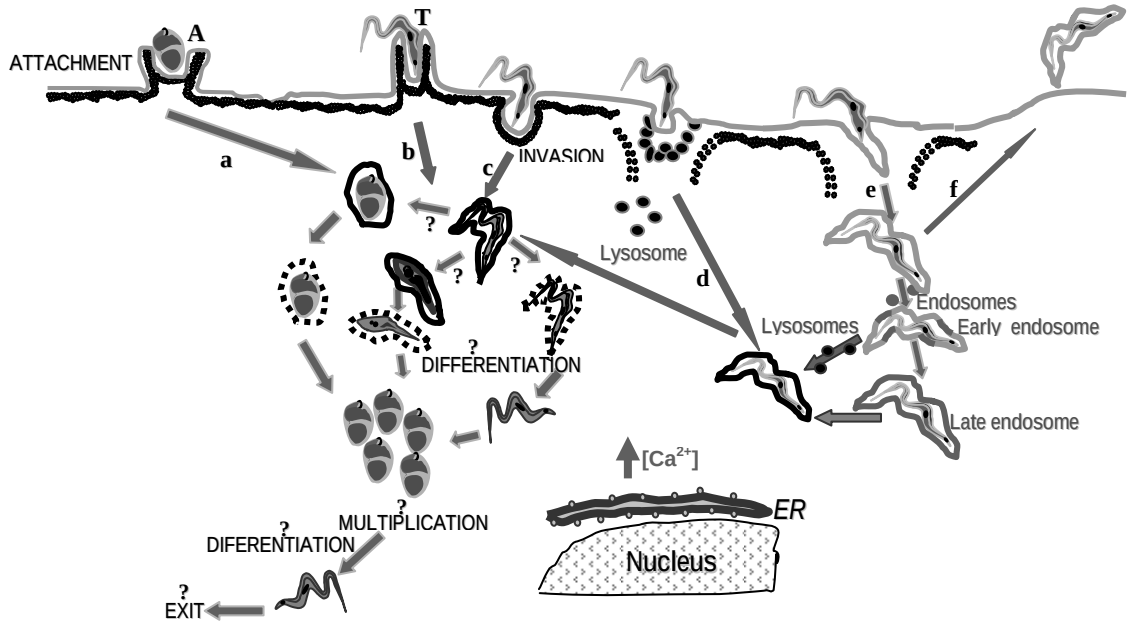

Figure 1. Schematic diagram of trypomastigotes (T) and amastigotes (A) cell infection process to non-professional phagocytic cells. Both stages utilizes multiple molecules to attach and invade mammalian cells that detonate diverse bidirectional signal transduction pathways, leading Ca^{2+} mobilization dependent internalization (40, 50-70, 115, 116). After attachment T invade the host cells through different possible mechanisms still in controversy and study: a phagocytic-like process that involve the formation of filopodium-like projections which dependent on actin filament assembly (b), an actin-dependent endocytic process with out formation of filopodium-like extensions (c), a lysosomes-dependent process (reported in some cases as dependent and other independent of actin microfilaments) (d), a plasma membrane-derived process that shows to be actin-independent in the recently internalized trypomastigotes but actin-dependent during the early endosomal derived vacuole and lysosomes fusion (e) (71-85). The continue actin microfilament disorganization can lead to promote the plasma-membrane-coated trypomastigotes exit (f) (84, 85). After invasion the parasite escape form the parasitophorous vacuole (being still unknown if it does as T, A or intermediary form), differentiate before and after its multiplication in the cytoplasm and exit from the host cell by a mechanisms poorly understand or still unstudied (96-105). After attachment the amastigotes invade the host cells by an actin-dependent process (a) (48); however, very little is known about the rest of its whole infection-process comparatively with trypomastigotes.

cells with infectivities comparable to those of trypomastigotes has lead to an increasing number of studies intended to understand the molecular mechanism of amastigotes entry into host cells. However, so far the information available about amastigote infection process is comparatively scarcer than that for trypomastigotes.

The amastigotes as well as the trypomastigotes, are able to infect phagocytic and non phagocytic cells (41), and it has been suggested that both developmental stages use different intracellular trafficking compartments during their invasion process and also possibly for amastigotes maturation (45, 46). Thus, the two *T. cruzi* infective forms interact with different cell surface structures during invasion of HeLa cells. Whereas trypomastigotes preferentially invade at the edges, the amastigotes bind and entangle to surface microvilli of HeLa cells, suggesting that the interaction with different cell surface structures could be a result of the expression of different receptors at the cell membrane (42). Also, it has been suggested that amastigotes are capable of invading both HeLa and Vero cells to a much higher degree than trypomastigotes in which a cell microfilament or microtubule entry dependent process was observed in amastigote but not in trypomastigote (42, 47). Beside, differences between the actin-rich membrane extensions formed around both infective forms and target cells and a repertoire of host cell/extracellular matrix components that engage in the invasion process of *T. cruzi* forms are apparently cell type- and parasite form-dependent (48). Also, different kinetics of vacuole escape has been reported for trypomastigotes and extracellular amastigotes, suggesting that both infective forms probably engage stage-specific mechanisms to escape from the parasitophorous vacuole (49).

All these results together are consistent with the notion that each developmental form-host cell pair mobilizes specific interacting component.

Cell infection by metacyclic trypomastigotes

The infection process of trypomastigotes to mammalian cells begins with the attachment of the parasite to the host cell. Many glycoproteins that have been implicated in this interaction, thus suggesting that *T. cruzi* exploit multiple molecules to attach and invade mammalian cells instead of using a unique ligand-receptor interaction.

Metacyclic trypomastigotes (MT) and tissue culture trypomastigotes (TCT) apparently utilize different surface molecules for cell adhesion that detonate diverse signal transduction pathways in parasite and host cell, leading a Ca^{2+} mobilization-dependant internalization (50). Thus, MT utilize for cell adhesion the surface glycoproteins gp82 and gp 35/50, while TCT employ the gp 85 family.

The gp82 is a MT surface molecule specialized for adhesion and the subsequent parasite penetration into epithelial cells (51). The gp-82-mediated binding to the host cell induces a bidirectional activation of signal transduction pathways which leads to intracellular Ca^{2+} mobilization (52, 53). Although gp82 present high homology with other members of TCT gp85-transilidase super-family (54, 55) both glycoproteins display different adhesive properties

and interact with different target cells. The gp82 bind to gastric mucin whereas members of the gp85 family bind to components of the extracellular matrix such as fibronectin and laminin (56, 57). Also, the binding of gp82 triggers a transient increase of intracellular Ca^{2+} concentration via activation of PTK, PLC and IP3 in host cells, in the same way than the soluble extract of MT, a behavior not observed in the no-infective epimastigotes unless they are transfected with gp82 coding sequence (53, 58). Like gp82, gp35/50 bind to target cells in a receptor-mediated manner, being the receptor still unknown, this triggers the bi-directional Ca^{2+} flux but in a lower degree than gp82 does (52). Gp35/50 mucins, the main acceptors of sialic acid in trans-sialidase (TS)-mediated reaction (59), are expressed in metacyclic forms and epimastigotes of the all South American *T. cruzi* isolates examined, in which strain-dependent size polymorphisms were observed (60). The role of mucin like gp35/50 molecules in mammalian cell invasion was suggested by using monoclonal antibody or the purified native glycoprotein (61,62). The treatment of MT with bacterial neuroaminidase that removes sialic acid from gp35/50 increased the parasite infectivity, which suggests that sialyl residues are not required for MT invasion (63). Also, the capacity to bind to the host cells and trigger the intracellular Ca^{2+} was higher in desialylated gp35/50 as compared to its sialylated counterpart (63).

MT specific glycoprotein of 90 kDa is another molecule related with the attachment and infectivity of *T. cruzi*. The interaction of gp90 with its receptor apparently trigger an inhibitory pathway as a result of the induction of signaling cascades acting as negative regulators (64, 65) that mediate the activation of MT tyrosine phosphatases which counteracts the activation of PTK by dephosphorylating p175 (58). The gp90 is a GPI anchored protein that binds to the host cell in an receptor-mediated manner but, contrary to what happen with gp82 and gp35/50, it does not trigger host Ca^{2+} signal or parasite Ca^{2+} mobilization upon binding to gp90 antibodies, in contrast to what happens upon interaction with antibodies directed to gp82 or gp35/50 (52).

Ortega Barria and Pereira (66) reported a protein of 60 kDa, called penetrina that bind selectively to heparin, heparin sulfate and collagen and promotes adhesion and invasion of tissue culture trypomastigotes to fibroblasts.

It is known that the sialic acid monosacaride participate in the interaction process, but *T. cruzi* is not capable of synthesize it. Nevertheless the parasite expresses Transialidase (TS) that is an enzyme that transfers the sialic acid from host sources to β-galactosyl residues in the surface of the parasite (67). The TS is secreted and localized on the surface of TCT, possible acting together during the interaction with the host cell. The role of TS in mammalian cell invasion is not clear. The use of polyclonal antibodies that block TS activity of TCT resulted in the enhancement of host cells invasion, which suggest that the enzyme negatively modulates *T. cruzi* infection (68, 69).

However, contradictory results were also found when comparing TCT TS^+ that were highly invasive with TCT TS^- parasite that were extremely inefficient in invading epithelial cells and fibroblasts (70). Moreover, the same authors showed that a small amount of TS in the supernatant of non-penetrating TS^- trypomastigotes changed them back to a penetrating phenotype. Beside that, TS can function as a counter-receptor for TCT binding to α2,3-sialyl host cell receptors, then after binding through another molecules, secreted TS may transfer sialic acid from the mammalian cell membrane to the parasite mucin, thus disrupting the binding of sialoadhesins and allowing the detachment of the parasite so it find a new binding site in order to proceed its internalization (59).

Immediately after attachment, the trypomastigotes are internalized through a parasitophorous vacuole by different controversial mechanisms, still in debate, about the involvement of host cell actin cytoskeleton during the invasion process. Morphological studies have showed actin polymerization at the site of parasite invasion, although some parasites also penetrate by another process which not shows concentration of microfilaments around them (71, 72). It has been reported that the treatment of host cells with drugs as cytochalasin B or D, which inhibit phagocytosis because they disrupt actin microfilaments, inhibit the infection process (72, 73, 74). Others, however, show that the same treatment even increased parasite penetration (75, 76, 77). Also, over a decade ago, an atypical lysosome-mediated and actin-independent entry process was proposed. This process consists of the recruiting of the lysosome to the site of trypomastigotes entry and its fusion with the plasma membrane in a calcium-signaling dependent pathway (78, 79, 80, 81, 82). Recently, a lysosome-independent pathway was also described where the trypomastigotes invade through a host cell plasma membrane-derived vacuole, initially devoid of lisosomal markers that subsequently acquire endosomal and/or lisosomal markers as a result of vacuole maturation, which shows a mutually exclusive behavior with respect to lisosomal-dependent pathway (83). Therefore, studies in several different cell types using markers to distinguish the last two modes of entry demonstrated that 20% to 30% of invading or recently internalized parasites were associated with lysosomes; about 50% were contained in plasma membrane-dependent vacuoles and the 20% to 30% were found in vacuoles containing early endosomes markers. All these percentages became to more than 90% of parasites associated with lisosomal markers whiting the 60 minutes of internalization; probably, as a result of the following fusion with lysosomes during the vacuole maturation process mentioned above. Also, differential dependence to PI-3 kinase activity has been reported between the lysosomes dependent and independent pathways, in which the lysosome-mediate entry process is PI-3 kinase dependent while the plasma membrane-mediate entry is independent of this signaling pathway (83). Contradictory results were found when the role of host

cell actin cytoskeleton in the lysosomes dependent and independent pathways during the parasite invasion process were analyzed. Some reports showed that pretreatment of host cells with agents that disrupt actin cytoskeleton, facilitate lysosome-mediated entry (79, 82); however, other results report that cytochalasin D pretreatment of host cell hinders early association with lysosomes and enhances entry by a lysosome-independent route (84). Additionally, other interesting result indicated that the ability of trypomastigotes to associate with the endocytic compartments and their permanency inside the host cell is an actin-dependent and lysosome fusion-dependent process (84, 85).

The discrepancy reported in the experiments about actin cytoskeleton-dependent or independent invasion of *T. cruzi* described above was analyzed recently by Ferreira *et al* (86). Some assays were performed using metacyclic trypomastigotes of G and CL strains, which differ in infectivity and invade host cell using different surface molecules (gp35/50 and gp82 respectively), treated with drugs that disrupt F-actin or using co-infection assays with enteroinvasive *Escherichia coli* (that recruits host cell actin for internalization) or treated with drugs that interfere with mammalian cell signaling pathways. This analysis showed that both strains invade differentially the host cells through actin-dependent (G strain) or actin-independent (CL strain) routs, by engaging distinct surface molecules for attachment and triggering distinct signal transduction pathways. These results indicated that the host cell invasion mediated by gp35/50 was associated with signaling events that favor actin recruitment, while gp82-dependent invasion was associated with signaling pathways leading to disassembly of F-actin (86).

Al these results together suggested that the contradictory results about the actin cytoskeleton-dependence or independence role in the invasion process found so far, could be associated with some parasites and/or host cell factors or even with different invasion mechanisms that could take place differentially under diverse conditions or stimulus. However, more experiments will be necessary to establish a correlation and elucidate the molecular basis of these differences.

Like in the attachment, the parasite uses several molecules for cell invasion which also detonate diverse signal transduction pathways. Enzymes such as cruzipain and oligopeptidase B, which generate a Ca^{2+}-agonist from precursor molecules, are implicated in cell invasion process (87, 88). The cruzipain (gp57/51) is a secreted cystein proteinase active at pH 5-7.5, and it is expressed in all developmental forms of different *T. cruzi* isolates (89, 90). Experiments using inhibitors of cystein proteinases have suggested the involvement of cruzipain in host cell invasion and intracellular development (91, 92). The cruzipain of *T. cruzi* liberate active kinins, such as bradykinin from host kininogen, which bind to the host cell bradykinin (B_2R) receptors to stimulate IP3-mediated Ca^{2+} release (88). Oligopeptidase B is a TCT cytosolic

serine peptidase of 80 kDa that cleaves an inactive precursor to generate an active Ca^{2+}-agonist that is released from the parasite. This agonist binds to pertussis-toxin sensitive G-protein-coupled receptor, activates phospholipase C (PLC) and generate inositol 3,4,5 triphosphate (IP3), which binds to its receptor and releases intracellular Ca^{2+} from endoplasmic reticulum stores (93). It was reported recently, that the protein family called SAP (serine, alanine and proline-rich protein) was also involved in the invasion of mammalian cells by metacyclic trypomastigotes. SAP binding to host cell induces activation of signal transduction pathways thus leading to intracellular Ca^{2+} mobilization necessary for parasite internalization. Although the deduced amino acid sequence of SAP contains two elements typical of several GPI-anchored surface molecules, these proteins were barely detected on the cell surface. Beside that a secrete SAP of approximately 55 kDa has been found, which indicates that this is the first shed *T. cruzi* molecule of known identity that directly triggers a Ca^{2+} response in host cell. Also, recombinant SAP exhibited an adhesive capacity toward mammalian cells, whose binding was dose dependent and saturable, thus indicating a possible ligand-receptor interaction (94). Finally, a cell surface (CKII) substrate of a protozoan parasite that is phosphorylated by human CKII that maybe is important for parasite invasion has been reported (95).

The residence inside the parasitophorous vacuole exposes the parasite to acidic pH that contrary to be a dangerous condition seems to be necessary to triggers differentiation of trypomastigotes to the intracellular amastigotes stages (96) and also to disrupt the parasitophorous vacuole (97, 98, 99), both essential steps for complete the parasite intracellular cycle. At this point of the process, it is still unknown if the parasite exit from the vacuole as trypomastigote, intermediate form, completely transformed amastigote or as in more than one of these possibilities. The combined activities of parasite lytic molecules (TcTox and LYT1) and transialidase, activated by the vacuole's acid pH, are believed to be crucial for trypomastigotes to escape into the host cytosol (97, 98, 100). One parasite factor likely to be involved was originally identified as a secreted acid stable hemolytic activity call TcTox that although expressed in all developmental forms, is most highly expressed in the infective trypomastigotes and amastigotes. These characteristics have taken to suggest that its pore-forming activity could play an important role in vacuole escape (97, 98). Unfortunately because of the instability of TcTox *in vitro* its amino acid sequence has not been determined nor gene cloned, consequently very little progress on unraveling the role of the protein has been reported. We have reported the genetic characterization of *LYT1* gene, which also shows a lytic activity in acid conditions and is most highly expressed in amastigotes. We demonstrated by mutational analysis, that the deletion of *LYT1* resulted in attenuation of infection, which was associated with diminished hemolytic activity. Also, we confirmed by the reintroduction of *LYT1* in null mutants, which restored the parasite infectivity, the critical role of

LYT1 in infection. Interestedly, the knock-out parasites exhibited accelerated in vitro development, which suggests that the diminished infectivity was not a result of the *LYT1* deficiency that affected the ability of the parasite to complete the life cycle (100). Additionally, we demonstrated that the diverse phenotypes associated with *LYT1* deficiencies was a result of alternative *trans* splicing, regulated differentially during the parasite life cycle, which produced three different *LYT1* mRNAs. Two of them encoded the full-length LYT1 protein and therefore contain a possible signal sequence and a third one encoded a truncated LYT1 protein which lacks a possible signal sequence. Therefore, it is possible that two forms of the protein are produced; one with the secreted form involved in pore-forming activity and possibly in vacuole escape and other nuclear or cytosolic responsible for suppression of stage transition (101). Another molecule that has also been implicated in vacuole exit is the *trans*- sialidase which is an enzyme that is used to obtain sialic acid from host glycoconjugates as it was mentioned above (59, 97, 98, 102, 103, 104). It has been reported that cell culture trypomastigotes that highly express and release TS exit earlier from the vacuole and differentiate into amastigotes more efficiently than axenic metacyclic trypomastigotes, even when both stages have the same invasion capacity to mammalian cells. These results were further demonstrated by transfection experiments which showed that the expression of TS on the surface of transfected metacyclic facilitated the parasite escape from the vacuole and differentiate more efficiently into amastigotes that non-transfected metacyclics or metacyclics expressing TS in their cytoplasm (105).

After the parasite escape from the parasitophorous vacuole, it is liberated to multiply as amastigotes freely in the cytosol before to differentiate newly to bloodstream trypomastigote to ultimately be liberated from the host cell by mechanisms still uncovered. Although the parasite and host cell machinery involved in the attachment and internalization is reasonably well understood, diverse aspect and key questions about the parasite escape from the parasitophorous vacuole, its differentiation before and after its multiplication in the cytosol and its exit to the host cell remain poorly understand or unstudied.

Cell infection by amastigotes

As it was mentioned before the intra-cellular and extra-cellular amastigotes can invade, survive and sustain the life cycle of *T.cruzi*. Although intra-cellular and extra-cellular amastigotes share some biochemical and ultra-structural characteristics (39, 41, 106) and express similar antigenic stage-specific markers (39, 107, 108, 109, 110), they also show a polymorphic expression of some surface epitopes (110) and minor morphology differences such as that the intra-cellular amastigotes are larger and slightly more elongated than extra-cellular amastigotes (111).

Little it is known about the molecules involved in the adhesion and invasion process of amastigotes. The SA85-1 is one of the major surface glycoproteins expressed in amastigotes and trypomastigotes but only amastigotes express the mannose-binding protein ligand, as a result of the developmentally regulated glycosylation of SA85-1 and other related surface glycoproteins that control ligands expression. It has been suggested that mannose-binding protein opsonization may facilitate amastigotes entry into cells. In addition, an interaction between amastigotes and macrophages mannose receptor that contributes in the amastigote adhesion process has been reported (112, 113). Finally the involvement of Ssp-4 in the invasion has been suggested recently (114).

After attachment the amastigotes invade the host cell by an actin-dependent mechanism. Procopio *et al* (1998) demonstrated that when HeLa and Vero cells are pre-treated with cytochalasin D, the amastigotes invasion is inhibited which indicated that functional actin microfilaments of host cells are necessary for an efficient parasite internalization and suggested a parasite passive role in agreement with its intrinsic motionless nature. Also, the accumulation of cytoskeleton elements (α-actinin, gelsolin, vinculin, talin, tropomyosin and ABP_{280}) integrins or matrix elements in the actin-rich membrane extensions around invading amastigotes further support the participation of host cell cytoskeleton during the entry of amastigotes to non phagocytic cells (48).

Invasion studies of extra-cellular amastigotes of G (*T. cruzi* I) and CL (*T. cruzi* II) strains showed that the signaling pathways engaged during HeLa cell invasion by extra-cellular amastigotes are distinct of the corresponding metacyclic trypomastigotes stages (40, 115, 116). Fernandes *et al* (2006) showed that G and CL strain extra-cellular amastigotes engaged signal pathways that lead to an increase of cyclic adenosine monophosphate and Ca^{2+} mobilization from acidocalcisomes. Moreover, a protein tyrosine kinase activity appears to be associated with G but not with CL strain, a result that is consistent with the fact that HeLa cells extracts contained a protein tyrosine kinase activity that mediate the phosporylation of 87 and 175 kDa polypeptides of extra-cellular amastigotes from G but not from CL strais. These results showed that although substantial differences in the signal transduction pathways are observed during the invasion of either G or CL strain of metacyclic trypomastigotes, a little or no discriminative effect was observed in extra-cellular amastigotes thus indicating that amastigotes not only engage signaling pathways distinct to trypomastigotes but also are strains-independent (40, 115, 116).

Concluding remarks

Pathogenic microbes have evolved ways to subvert normal host-cell processes to create specialized niches to support their life cycle. Also, intracellular pathogens as *T. cruzi* circumvent the host immune system using

evasion strategies in order to survive, replicate, propagate and spread, developing ways to interfere with and influence host-cell pathways that can facilitate the infection process and the establishment of the disease. In order to achive this, *T. cruzi* must ensure the use of effectors molecule onto or into host cells for different purposes which include attachment, entry into cells, vacuole formation and disruption, differentiation, replication and exit from the host cell. Though many proteins are undoubtedly important for *T. cruzi* infection and successful completion of the life cycle, surprisingly few have been identified experimentally, and even when a considerable advance has been made toward understanding the mammalian cell invasion, little is known about the complete and detailed comprehension of the molecular mechanisms involved during the whole infection process of *T. cruzi.* This knowledge is necessary to understand the biologically important processes enabling the parasite to efficiently infect humans.

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