Molecular basis of mammalian cell invasion
by *Trypanosoma cruzi*

NOBUKO YOSHIDA

Departamento de Microbiologia, Imunologia e Parasitologia, Escola Paulista de Medicina
Universidade Federal de São Paulo, R. Botucatu, 862 – 04023-062 São Paulo, SP, Brasil

ABSTRACT

Establishment of infection by *Trypanosoma cruzi*, the agent of Chagas´ disease, depends on a series of events involving interactions of diverse parasite molecules with host components. Here we focus on the mechanisms of target cell invasion by metacyclic trypomastigotes (MT) and mammalian tissue culture trypomastigotes (TCT). During MT or TCT internalization, signal transduction pathways are activated both in the parasite and the target cell, leading to Ca^{2+} mobilization. For cell adhesion, MT engage surface glycoproteins, such as gp82 and gp35/50, which are Ca^{2+} signal-inducing molecules. In *T. cruzi* isolates that enter host cells in gp82-mediated manner, parasite protein tyrosine kinase as well as phospholipase C are activated, and Ca^{2+} is released from IP_3-sensitive stores, whereas in *T. cruzi* isolates that attach to target cells mainly through gp35/50, the signaling pathway involving adenylate cyclase appears to be stimulated, with Ca^{2+} release from acidocalciosomes. In addition, *T. cruzi* isolate-dependent inhibitory signals, mediated by MT-specific gp90, may be triggered both in the host cell and the parasite. The repertoire of TCT molecules implicated in cell invasion includes surface glycoproteins of gp85 family, with members containing binding sites for laminin and cytokeratin 18, enzymes such as cruzipain, trans-sialidase, and an oligopeptidase B that generates a Ca^{2+}-agonist from a precursor molecule.

Key words: *Trypanosoma cruzi*, trypomastigotes, cell invasion, signal transduction, Ca^{2+} mobilization.

OVERVIEW

Trypanosoma cruzi is transmitted by insect vectors when these blood-sucking triatomines deposit on the skin their feces containing MT, the infective forms of the parasite. Through the ocular mucosa or lesions in the skin, the parasites find their way to invade host cells. Metacyclic forms may enter mammalian hosts also by oral route. According to Coura et al. (2002), more than half of the acute cases of Chagas disease recorded between 1968 and 2000 in Brazilian Amazon can be attributed to microepidemics of orally transmitted infection from contaminated food. The potential sources of contamination are whole tri-atomine insects or their feces containing infective parasites. Hoft (1996) has shown that MT are efficient in establishing infection in mice when given orally. They enter the gastric mucosal epithelium, transform into amastigotes and replicate intracellularly (Hoft et al. 1996). Trypomastigotes emerge from infected cells, circulate in the bloodstream and disseminate to diverse organs and tissues. These circulating parasites can be transmitted by blood transfusion or congenitally. Following the acute phase,

E-mail: nyoshida@ecb.epm.br

in which the parasites go through multiple rounds of cell invasion and intracellular replication, their proliferation is controlled by the host immune response.

Mammalian cell invasion by *T. cruzi* has been extensively studied in vitro by using MT cultured in liquid media and TCT, as counterparts of insect-borne and bloodstream parasites respectively. Different *T. cruzi* isolates and a variety of cell types, mostly cells that are not professional phagocytes, have been used (Table I). The picture emerging from these studies is that *T. cruzi* penetration into host cells is a multi-step process involving various parasite and host cell molecules that, in a concerted series of events, leads to intracellular Ca^{2+} mobilization in both cells (Docampo et al. 1996, Burleigh and Andrews 1998, Yoshida 2003). Attachment of trypomastigotes is receptor-mediated and is restricted to cell surface domains (Schenkman et al. 1991a). For instance, parasites bind to and enter HeLa cells at their edges and invade polarized MDCK cells through their basolateral domains (Mortara 1991, Schenkman et al. 1988a). To invade mammalian cells, MT and TCT engage distinct sets of surface molecules that differentially interact with host components. The MT-specific glycoprotein gp82 binds to gastric mucin (Neira et al. 2003) whereas members of the TCT gp85 family have been shown to bind components of extracelullar matrix, such as fibronectin and laminin (Ouaissi et al. 1986a, Giordano et al. 1994). Although MT gp82 and TCT gp85 display differential adhesive properties and interact with different receptors on target cells, they are related molecules. They have considerable sequence identity (40-60%) and are included in the *T. cruzi* gp85/sialidase superfamily (Araya et al. 1994, Colli and Alves 1999). Mucin-like glycoproteins constitute another group of molecules implicated in host cell invasion that are differentially expressed in MT and TCT. Mucins from MT are protease-resistant gp35/50 molecules (Mortara et al. 1992, Schenkman et al. 1993a), whereas TCT mucins are larger molecules, migrating in SDS-PAGE as diffuse bands between 70 and 200 kDa (Almeida et al. 1994, Schenkman et al. 1991b).

MT SURFACE MOLECULES THAT MEDIATE BIDIRECTIONAL SIGNAL TRANSDUCTION AND HOST CELL INVASION

Studies with MT of 10 *T. cruzi* isolates, derived from different sources in distinct geographical regions, have revealed two groups of parasites that differentially express surface glycoproteins and display differential ability to invade mammalian cells in vitro (Fig. 1). The two groups are highly divergent. By molecular phylogenetic analysis, Briones et al. (1999) have found that the distance between the two *T. cruzi* lineages is larger than the distances among *Leishmania* spp.

MT of highly invasive isolates are deficient in gp90 and gp35/50, identified respectively by monoclonal antibodies (MAbs) 1G7 and 10D8, but express the variant forms of gp90 and gp35/50, identified by MAbs 5E7 and 2B10. MT of poorly invasive isolates express a gp90 molecule that is detectable by MAbs 1G7 and 5E7, and gp35/50 molecules recognized by both MAbs 10D8 and 2B10. Expression of gp82, which reacts with MAb 3F6, is ubiquitous among these isolates (Fig. 1).

Gp35/50, gp82 and gp90 bind to as yet undefined host cell receptors. The interaction of any of these molecules with its receptor triggers bidirectional signaling cascades. Whether the MT-target cell interaction results in productive infection depends on which surface molecule is predominantly engaged. To attach to and enter host cells, MT of highly invasive CL isolate, for instance, engage gp82 (Ramirez et al. 1993), which efficiently triggers Ca^{2+} signaling in MT and host cells (Ruiz et al. 1998), whereas MT of poorly invasive G isolate appear to rely mainly on gp35/50 for their internalization (Yoshida et al. 1989). Gp35/50 molecules are not as effective as gp82 in promoting invasion, due probably to their poor Ca^{2+} signal-inducing activity (Dorta et al. 1995). If the interaction is mediated by gp90, which is devoid of Ca^{2+} signaling activity, productive infection is precluded.

Extensive studies with MT of *T. cruzi* isolates CL and G, which represent the prototypes of highly

TABLE I

Mammalian cells and *T. cruzi* isolates used in invasion assays.

T. cruzi isolate or clone
CL, CL-14, Costalimai, Dm28, Dm28c, Dm30, F, MD, G, Guafitas, M226, MD, 569, 588, Silvio X-10/4, RA, Tulahun, Y

Mammalian cell type
Chinese hamster ovary (CHO) cell
Human carcinoma-derived epithelial HeLa cell
Human umbilical vein endothelial cell (HUVEC)
L_6E_9 myoblast
$LLC\text{-}MK_2$ cell
Madin-Darby canine kidney (MDCK) cell
Mouse 3T3 fibroblast,
My1Lu mink lung cell
Normal rat kidney (NRK) fibroblast
Primary canine cardiac myocyte
Vero cell derived from African green monkey fibroblast

and poorly invasive parasites, have shown that their infectivity is not related to the target cell type. Regardless of the mammalian cell type, the number of internalized parasites of CL isolate is always several fold higher than that of G isolate (Fig. 2A). These in vitro findings closely correlate to the observations in vivo. Regardless of the mouse strain or the route of parasite administration, MT of CL isolate produce high parasitemias, in contrast to MT of G isolate that invariably produce subpatent infection (Fig. 2B). Presumably the mechanisms of host cell entry acting in vitro prevail in vivo. Invasion of epithelial HeLa cells by MT may be equivalent to the invasion of gastric mucosal epithelium, upon oral infection. Likewise, MT entry into mouse peritoneal macrophages, whether the parasites are seeded onto cultured cells or are inoculated intraperitoneally, may be equivalent.

One interesting observation is that MT of CL and G isolates are morphologically different (Fig. 2C). As compared to CL isolate, parasites of G isolate are shorter and the kinetoplast is more proximal to the posterior end. Isolate-dependent morphological variations are also observed in blood trypomastigotes and the different morphologies apparently denote physiological differences (Brener 1973). Slender forms appear to penetrate host cells better than the stout forms (Brener 1969). In some *T. cruzi* isolates, slender trypomastigotes are prevalent during the first days of infection, whereas in other isolates stout forms predominate during the entire infection in mice (Brener 1973).

GP82, THE CA^{2+} SIGNAL-INDUCING MOLECULE
THAT PROMOTES CELL INVASION OF HIGHLY
INVASIVE *T. cruzi* ISOLATES

The role of the metacyclic stage-specific surface molecule gp82 in mammalian cell invasion was first determined by inhibition of MT internalization using MAb 3F6 or the purified native glycoprotein (Ramirez et al. 1993). Subsequent studies indicated that gp82 promotes MT entry into host cells by inducing the activation of signaling cascades and Ca^{2+} mobilization in both cells (Ruiz et al. 1998, Yoshida et al. 2000). Recent studies with *T. cruzi* clone CL-14, which is poorly infective, have further reinforced the role played by gp82. When compared to the parental CL isolate, the sole difference
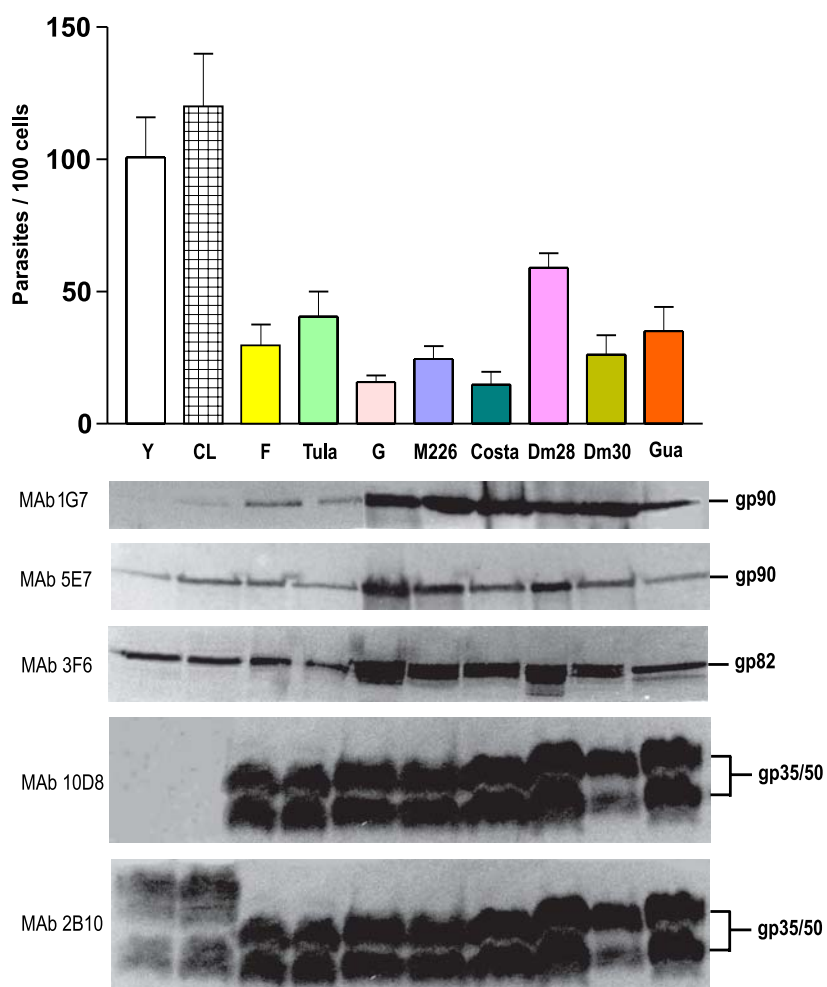
Fig. 1 – Mammalian cell invasion by MT of different *T. cruzi* isolates and profile of surface molecules. In the upper panel is shown the infectivity of MT of the indicated isolates, expressed as the number of internalized parasites per 100 cells, upon incubation with HeLa cells for 3h at 37°C. The values correspond to the means ± SD of five experiments performed in duplicates, in which at least 500 Giemsa-stained cells were counted. Shown in the lower panel are the profiles of surface molecules identified by monoclonal antibodies directed to gp90, gp82 and gp35/50.

in the surface profile of clone CL-14 MT was the deficient expression of gp82 (Atayde et al. 2004).

Gp82 is a glycoprotein containing N-linked oligosaccharides (Ramirez et al. 1993) that is anchored to MT plasma membrane by glycosylphosphatidylinositol (GPI) moiety (Cardoso de Almeida and Heise 1993). The identity of amino acid sequences of gp82 deduced from cDNA clones of G and CL metacyclic forms is 97.9%, and 100%

as regards the central domain containing the mammalian cell binding site (Ruiz 1998). Using synthetic peptides and truncated recombinant gp82 constructs, Manque et al. (2000) found that the cell binding site is contiguous to and partially overlaps the epitope for MAb 3F6, and appears to be conformational, being possibly formed by juxtaposition of two sequences separated in the linear molecule by a hydrophobic stretch (Fig. 3).
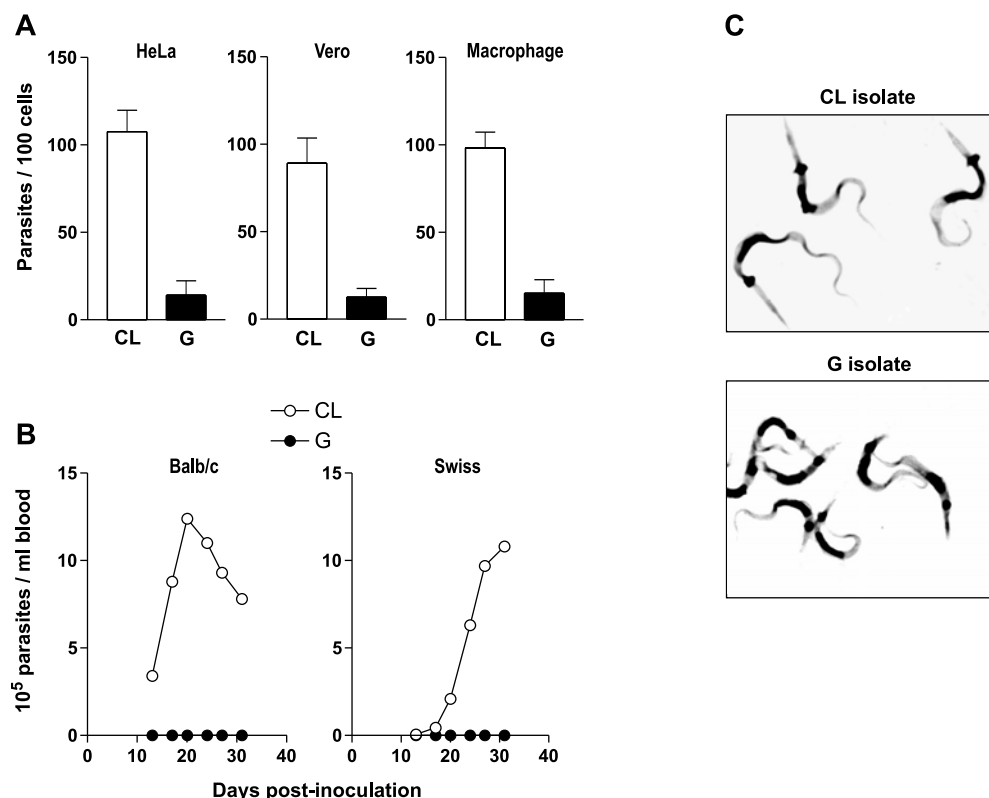
Fig. 2 – Differential in vitro and in vivo infectivity of *T. cruzi* isolates CL and G. A) MT infectivity upon incubation of parasites for 3h with different cell types (HeLa, Vero or mouse peritoneal macrophages). The values, expressed as the number of internalized parasites per 100 cells, correspond to the means $\pm$ SD of 10 (HeLa), 8 (Vero) or 5 (macrophage) experiments performed in duplicates, in which at least 500 Giemsa-stained cells were counted. B) Course of infection upon inoculation of 4×10^5 MT by oral route into Balb/c mice, or by intraperitoneal route into outbred Swiss mice. Each data point corresponds to the mean parasitemia of 6 animals. C) Purified MT of CL and G isolates stained with Giemsa. Note that MT of CL isolate are longer and the kinetoplast is located more distal to the posterior end, as compared to G isolate.

The central domain of MT gp82 shares 60-65% identity with the C-terminal region of TCT glycoproteins of gp85 family. However, within the sequences containing the cell binding site and the epitope for MAb 3F6, there are significant differences between MT and TCT molecules, and these include substitutions of acidic amino acids for uncharged or positively charged residues, substitutions of uncharged residues for lysine or arginine, and substitutions of residues with polar side chains for those with nonpolar side chains and vice-versa.

Binding of gp82 triggers in host cells a transient increase in intracellular Ca^{2+} concentration, in the same manner as the soluble extracts of MT (Fig. 4). Non infective epimastigotes do not induce Ca^{2+} signaling (Tardieux et al. 1994, Dorta et al. 1995) unless they are transfected with *T. cruzi* expression vector carrying gp82 cDNA (Manque et al. 2003). Gp82 triggers Ca^{2+} response in mammalian cells susceptible to *T. cruzi* infection, such as HeLa and Vero cells, but not in *T. cruzi*-resistant human leukemic K562 cells.

The kinetics of Ca^{2+} mobilization in MT is distinct from that observed in host cells and can be triggered by soluble extracts of HeLa cells (Fig. 4), but not of K562 cells, in a manner inhibitable by native or recombinant gp82. Upon binding to host cells, gp82 relays a signal to the parasite that re-
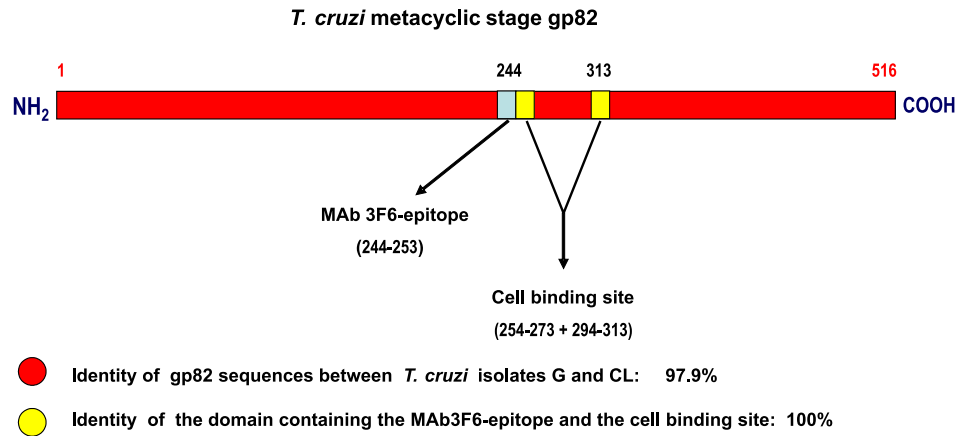
T. cruzi metacyclic stage gp82

Fig. 3 – Schematic representation of *T. cruzi* gp82. The sequence deduced from a cDNA clone derived from MT of G isolate is represented, showing the epitope for MAb 3F6 and the host cell binding site, which were mapped using synthetic peptides and truncated recombinant constructs of gp82.
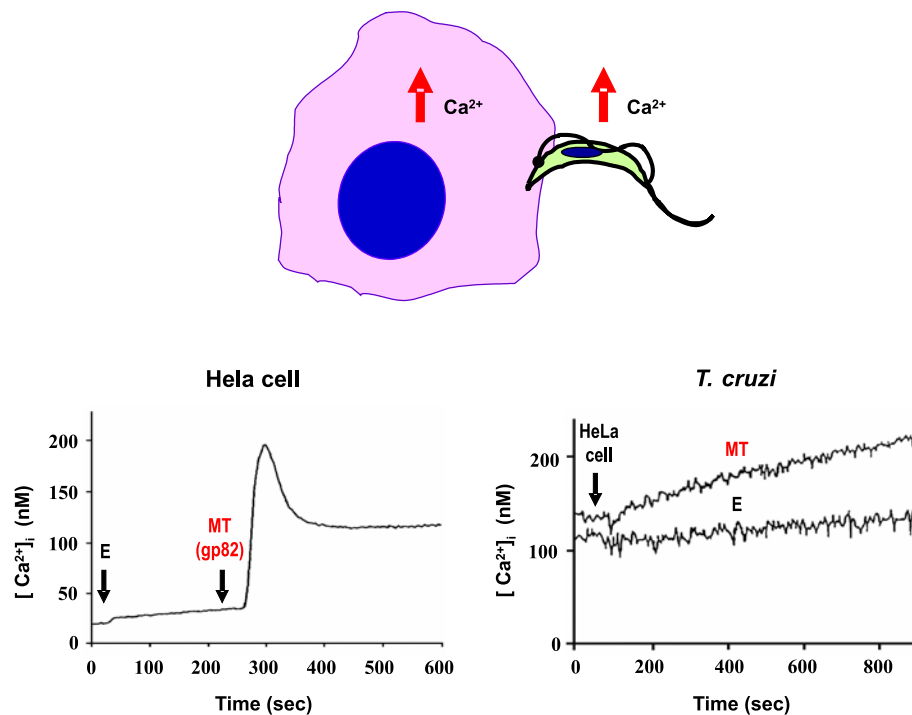
Fig. 4 – Ca^{2+} response induced by *T. cruzi*-host cell interaction. Transient intracellular Ca^{2+} mobilization triggered in HeLa cells upon addition, at the indicated time (arrow), of sonicated extract of MT or purified native gp82, is shown on the left. Note the lack of response by addition of non-infective epimastigote (E) extract. Shown on the right is the Ca^{2+} response induced in MT, but not in E, by sonicated HeLa cell extract.
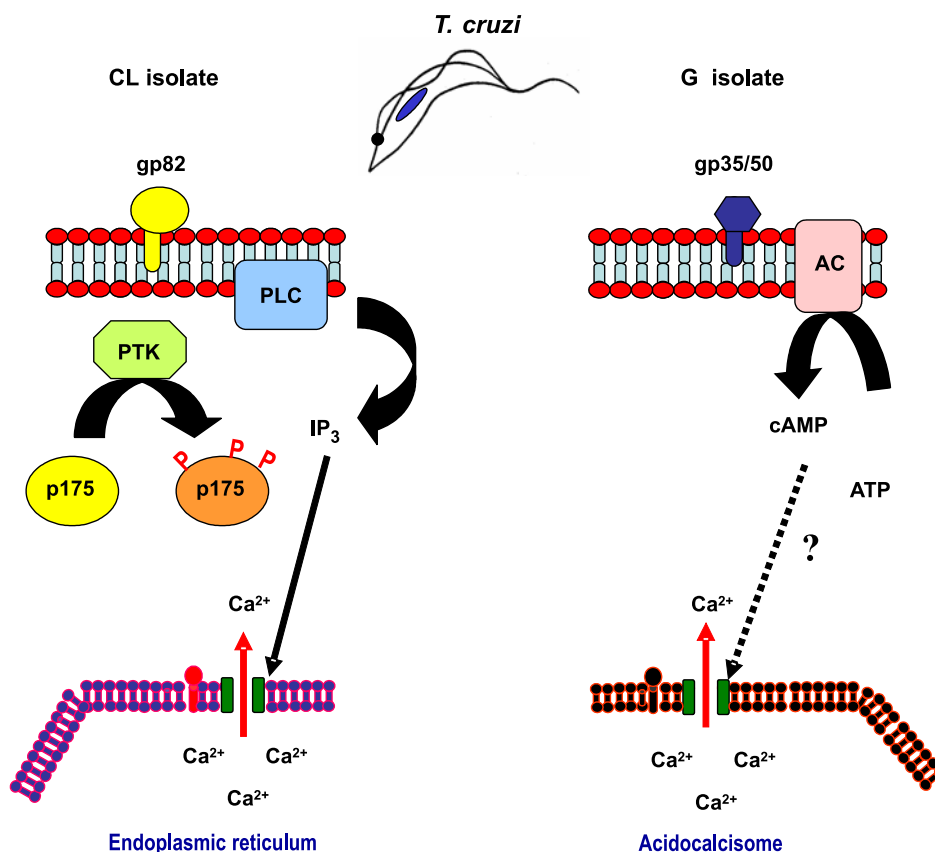
Fig. 5 – Activation of different signal transduction pathways in MT of phylogenetically distinct *T. cruzi* isolates during host cell invasion. In the highly invasive CL isolate, the recognition of the surface molecule gp82 by its receptor leads to PTK activation, phosphorylation of p175, activation of PLC and IP_3 generation, culminating with IP_3-mediated Ca^{2+} release from internal stores, probably endoplasmic reticulum. The components of the G isolate signaling cascade, triggered at the cell surface by gp35/50, are mostly unknown; cAMP generated by adenylyl cyclase may be involved, and acidocalcisomes appear to be the source of Ca^{2+} required for parasite internalization.

sults in protein tyrosine kinase (PTK) activation and phosphorylation of p175, a protein that is undetectable in noninfective epimastigotes (Favoreto et al. 1998). Activation of PTK and Ca^{2+} response are associated events, being both inhibited by gp82, as well as by genistein, a specific inhibitor of PTK (Yoshida et al. 2000). The signaling cascade induced by gp82 includes the participation of phospholipase C (PLC), that generates inositol 1,4,5-triphosphate (IP_3), as inferred from experiments in which the parasite infectivity was impaired by PLC inhibitor U73122, as well as by drugs, such as heparin, a competitive IP_3-receptor blocker, and caffein, which affects Ca^{2+} release from IP_3-sensitive stores

(Yoshida et al. 2000). Our hypothesis based on these findings is that in MT of *T. cruzi* isolates that engage gp82 to enter target cells, such as the CL isolate, a signaling cascade is initiated at the parasite cell surface by gp82 and proceeds downstream through a sequence involving PTK, PLC and IP3, leading to Ca^{2+} mobilization, possibly from endoplasmic reticulum (Fig. 5).

Many questions concerning the gp82-mediated signaling cascade remain to be elucidated. One of these is how gp82, which is GPI-anchored and therefore associates only with the outer leaflet of the MT lipid bilayer, activates PTK. In mammalian cells, association of GPI-anchored proteins and PTKs has

been reported. A neuron survival factor neurturin, for instance, signals through multicomplex receptors that consist of receptor tyrosine kinase and a member of a GPI-linked family of receptors that determine ligand specificity (Buj-Bello et al. 1997). In T cells, PTKs can be found in complexes immunoprecipitated with antibodies directed to GPI-anchored proteins (Stefanova et al. 1991, Shenoy-Scaria et al. 1992, Thomas and Samelson 1992). An alternative possibility is the interaction of GPI-anchored proteins and kinases mediated by transmembrane linker proteins, as suggested by Brown (1993). Also awaiting elucidation is the question concerning the connection between PTK or p175 and PLC.

Gp82, a Mediator of Invasion of Gastric Mucosal Epithelium

Recently, the involvement of gp82 in oral *T. cruzi* infection was inferred from experiments in which the infectivity of CL isolate MT was greatly reduced upon treatment with anti-gp82 MAb 3F6 (Neira et al. 2003). In addition to playing a critical role in promoting invasion of gastric mucosal epithelium, the ability of gp82 to bind to gastric mucin may be important for the establishment of infection by oral route. *Shigella dysenteriae*, for instance, whose pathogenic potential is correlated with its ability to invade and multiply within the cells of colonic epithelium, preferentially adheres to colonic mucin (Sudha et al. 2001).

The role of gp82 in establishing efficient *T. cruzi* infection by oral route has been reinforced by studies using gp82-deficient isolates, such as 569 and 588 (Cortez et al. 2003). When administered orally into mice, MT of these gp82-deficient *T. cruzi* isolates produce patent parasitemia, but to greatly reduced levels when compared to isolate CL. Although the in vivo infectivity of isolates 569 and 588 is lower than that of the CL isolate, the ability to enter host cells in vitro is similar. MT of isolates 569 and 588 can efficiently invade cells in vitro because they express gp30, a surface glycoprotein detectable by MAb 3F6 and displaying Ca^{2+}

signal-inducing activity. Gp30-mediated HeLa cell entry of isolates 569 and 588, which is inhibited by MAb 3F6 as well as by native gp30, is dependent on the parasite signal transduction involving PTK activation and Ca^{2+} mobilization from thapsigargin-sensitive stores, similarly to the gp82-mediated signaling. Notwithstanding the functional similarity between gp82 and gp30, MT of gp82-deficient isolates are less infective by oral route than the CL isolate, and this is probably due to the low efficiency of gp30 in binding to gastric mucin. Accordingly, in the presence of high concentration of gastric mucin, to mimic the in vivo condition, the rate of HeLa cell invasion of gp82-deficient MT, but not of CL isolate, was significantly reduced (Cortez et al. 2003). These findings indicate that the host cell invasion by MT can be mediated either by gp82 or gp30, but efficient mucosal infection depends on the expression of gp82, which promotes the adhesion to gastric mucin, the first step towards the penetration into the underlying epithelial cells.

Gp35/50, the Ca^{2+} Signal-inducing Molecule that Promotes Cell Invasion of Poorly Invasive *T. cruzi* Isolates

The involvement of mucin-like gp35/50 molecules in mammalian cell invasion was determined by inhibition of MT internalization using MAb 10D8 or the purified native glycoprotein (Yoshida et al. 1989, Ruiz et al. 1993). Like gp82, gp35/50 bind to target cells in a receptor-mediated manner and triggers bidirectional Ca^{2+} response, but to a lower degree than gp82 (Ruiz et al. 1998).

Gp35/50 mucins are expressed in metacyclic forms and epimastigotes of all *T. cruzi* isolates examined to date. They are encoded by large multigene family (Di Noia et al. 1998) and are detected in SDS-PAGE gels as two-three bands displaying isolate-dependent size polymorphism (Mortara et al. 1992). Gp35/50 molecules are GPI-anchored glycoproteins rich in threonine. Glycans are O-linked to threonine residues in the mucin protein core through N-acetylglucosamine, rather than N-acetylgalactosamines as usually found in vertebrate

mucins and, depending on the *T. cruzi* isolate, may contain galactofuranose residues in addition to galactopyranose (Previato et al. 1994, Acosta-Serrano et al. 1995, Salto et al. 2000). MAb 10D8, that recognize gp35/50 mucins in MT of poorly invasive isolates, such as G and Tulahuen, and partially neutralize their infectivity (Yoshida et al. 1989), reacts with epitopes containing galactofuranose, whereas MAb 2B10 appears to react with galactopyranose-containing epitopes present in all isolates.

T. cruzi mucins are the main acceptors of sialic acid in trans-sialidase (TS)-mediated reaction (Schenkman and Eichinger 1993). TS is an enzyme that specifically transfers (α2-3)-linked sialic acid from extrinsic host-derived macromolecules to O-linked oligosaccharides of *T. cruzi* mucin-like glycoproteins (Schenkman et al. 1991b, Schenkman and Eichinger 1993). The enzyme, that is up to 30 times more active in TCT than in MT (Acosta-Serrano et al. 2001), was first identified in TCT and bloodstream trypomastigotes as a developmentally regulated sialidase that releases sialic acid from human erythrocytes and plasma glycoprotein (Pereira 1983). Using *T. cruzi* epimastigotes, Previato et al. (1985) found that sialic acid from exogenous sialylated glycoconjugates is incorporated into parasite macromolecules. TS preferentially tranfers sialyl residues to available galactose acceptors and acts as a sialidase in the absence of appropriate amounts of suitable acceptors (Schenkman and Eichinger 1993). In MT, sialyl residues are transferred exclusively into gp35/50 mucins (Schenkman et al. 1993a).

Sialyl residues of gp35/50 mucins are not required for MT invasion, they may rather impair the interaction with target cells. Treatment of MT of G isolate with bacterial neuraminidase, for instance, removes sialic acid from gp35/50 and increases the parasite infectivity (Yoshida et al. 1997). Resialylation of gp35/50, by incubation of parasites with *T. cruzi* TS and sialyl lactose, restores the reactivity with lectin or monoclonal antibody specific for sialic acid and, accordingly, reduces the rate of MT entry into target cells to levels similar to those before de-

sialylation. Compatible with this finding, the capacity to bind to host cells and to trigger Ca^{2+} response was found to be higher in desialylated gp35/50 as compared to its sialylated counterpart (Yoshida et al. 1997). Infectivity of CL isolate MT is not affected by neuraminidase treatment because these parasites rely on gp82, rather than on gp35/50, for their internalization.

In MT that preferentially engage gp35/50 to invade host cells, as is the case of G isolate, the signaling cascade triggered in the parasite is distinct from that induced by gp82 (Fig. 5). PTK and PLC are not implicated, instead cyclic AMP (cAMP) may play a role, as deduced from the increased parasite infectivity upon treatment with adenylyl cyclase activator forskolin (Neira et al. 2002). The Ca^{2+} required for cell invasion appears to be released from acidocalcisomes, the vacuoles containing a Ca^{2+}/H^+ exchange system (Docampo et al. 1995), provided that treatment of MT with a combination of ionomycin plus NH_4Cl or nigericin, that releases Ca^{2+} from these acidic compartments, significantly diminishes target cell entry (Neira et al. 2002). How gp35/50, an GPI-anchored molecule, relays the external signal to the parasite interior, and what are the components required for that process are questions that remain unanswered.

GP90, THE MT-SPECIFIC DOWN REGULATOR OF HOST CELL INVASION

The property of gp90 as a negative regulator of target cell invasion was demonstrated by experiments using antisense oligonucleotides targeted to gp90 gene sequences. Treatment of MT of G isolate with antisense oligonucleotides reduced the expression of gp90 and increased the parasite ability to enter host cells, whereas their sense counterparts or the mismatched sequences had no effect (Málaga and Yoshida 2001).

GP90 is a metacyclic stage-specific glycoprotein containing N-linked oligosaccharides (Yoshida et al. 1990). It binds to mammalian cells in a receptor-mediated manner but, unlike gp82 or gp35/50, does not trigger Ca^{2+} signal, and intracellular Ca^{2+}

is not mobilized in the parasite upon binding of anti-gp90 MAb 1G7, in contrast to what happens upon interaction with monoclonal antibodies directed to gp82 or gp35/50 (Ruiz et al. 1998). In MT, the interaction of gp90 with its receptor may trigger an inhibitory pathway, similarly to cells of the immune system where, in addition to activation signals, signaling cascades acting as negative regulators can be induced (Veillette et al. 2002, Vivier et al. 2004). Like NK cell surface inhibitory receptors that antagonize activation pathways using protein tyrosine phosphatases (Vivier et al. 2004), gp90 mediates the activation of MT tyrosine phosphatase that counteracts the action of PTK by dephosphorlylating p175 (Manque et al. 2003).

Like gp82 and gp35/50, gp90 is also anchored to the plasma membrane via GPI (Schenkman et al. 1988b). The type of association of these GPI-anchored molecules with other components of the plasma membrane is unknown. One possibility is the interaction through their extracellular domains. Another possibility is the association through GPI lipid moiety, and in this case the nature of the lipid may influence which plasma membrane molecule is recruited. Of note in this regard is that the lipid portion of gp35/50 GPI from noninfective epimastigotes is composed essentially of 1-O-hexadecyl-2-O-hexadecanoyl-PI and of 1-O-hexadecyl-2-O-octadecanoyl-PI, whereas that of metacyclic stage gp35/50 is mainly ceramide-PI (Acosta-Serrano et al. 1995).

MECHANISMS OF MT INVASION OF GASTRIC MUCOSAL EPITHELIUM UPON ORAL INFECTION

MT, but not blood trypomastigotes, have uniquely specialized functions for mucosal invasion and efficiently enter gastric mucosal epithelium (Hoft 1996, Hoft et al. 1996). This is in accord with the notion that metacyclic and bloodstream trypomastigotes are morphologically similar but are physiologically distinct (Tyler and Engman 2001). Hoft (1996) suggested that MT express stage-specific surface molecules required for adhesion to mucosal epithe-

lial surface receptors and/or for penetration of mucin coat. This hypothesis has been supported by results from experiments of oral infection in mice and in vitro cell invasion assays mimicking the in vivo conditions (Neira et al. 2003, Cortez et al. 2003). Based on these data, the following sequence of events can be visualized (Fig. 6). When MT reach the stomach, they resist destruction because they are protected by protease-resistant gp35/50 mucins, which are abundant on the parasite surface. Pepsin digestion leaves intact the gp82 domain containing both the target cell and the gastric mucin-binding sites. The parasites bind to gastric mucin through gp82 (Fig. 6A) and traverse the mucus to reach the underlying epithelial cells. It has as yet to be demonstrated, but it is possible that *T. cruzi* has mucinase activity, like *Entamoeba histolytica*, which expresses cyteine proteinases that disrupt the polymeric structure of colonic mucin (Moncada et al. 2003), or *Trichomonas vaginalis*, which invades vaginal mucous layer by secreting mucinase (Lehker and Sweeney 1999). Preliminary experiments indicate that *T. cruzi* secrets an enzyme that acts on gastric mucin.

Once the mucous barrier is overcome, MT of *T. cruzi* isolates such as CL efficiently invade gastric epithelial cells by engaging gp82 (Fig. 6B) and triggering bidirectional Ca^{2+} response. MT of G isolate may reach the epithelial cells as effectively as the CL isolate MT, but their entry may be impaired by gp90. Some parasites manage to be internalized by engaging gp35/50 (Fig. 6B).

On the other hand, MT of *T. cruzi* isolates expressing gp30, but deficient in gp82, such as 569 and 588, would have difficulty in penetrating the thick mucin coat, because gp30 binds poorly to gastric mucin. Binding to mucin may be critical for the effective action of mucinase inasmuch as, without binding, the enzyme would be secreted into the medium, not onto the mucin layer. The gp82-deficient parasites that manage to translocate through the mucin coat, and reach the epithelial cells, are then internalized in gp30-mediated manner, probably as efficiently as the gp82-expressing MT.
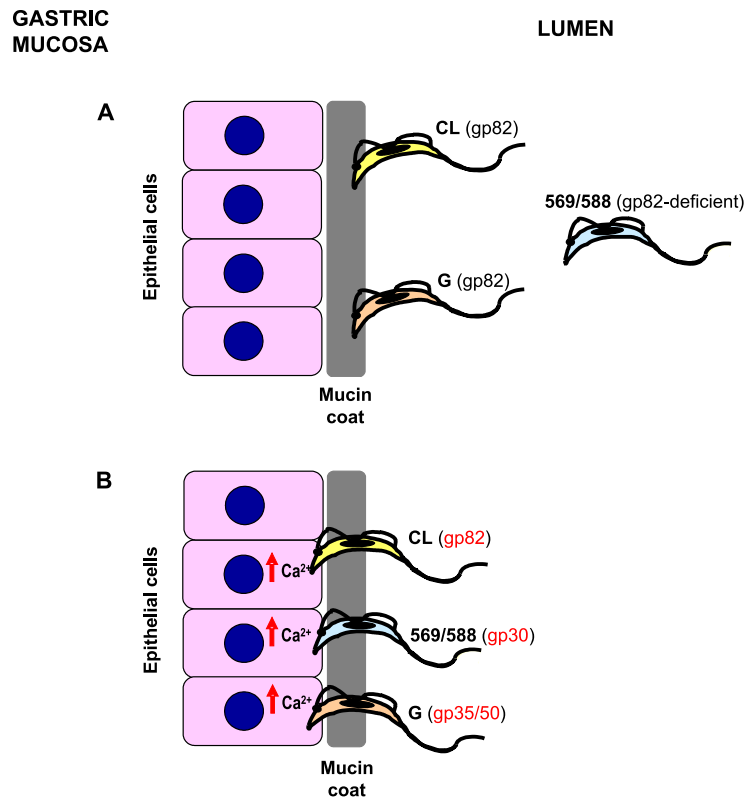
Fig. 6 – Model of molecular interaction of *T. cruzi* metacyclic forms with gastric mucin and gastric mucosal epithelium upon oral infection of mice. In the stomach, MT of different isolates resist destruction by pepsin and acidic pH because they are protected by protease-resistant gp35/50 mucins. A) MT of CL and G isolates bind to gastric mucin via gp82, as the first step to traverse the mucin layer and reach the underlying epithelial cells. Most parasites of gp82-deficient isolate 569 or 588 do not interact with the mucin coat, but a small number of parasites do so, in gp30 mediated manner. B) Once the epithelial cells are reached, MT of CL isolate attach to and efficiently invade them in gp82-dependent manner, whereas MT of gp82-deficient isolate 569 or 588 rely on gp30 for cell invasion. MT of G isolate enter epithelial cells poorly, due to the preferential adhesion through gp35/50, which has lower Ca^{2+} signal-inducing activity than gp82.

TCT MOLECULES IMPLICATED IN HOST CELL INVASION

Diverse TCT molecules have been implicated in host cell invasion. These include surface and/or secreted components such as Tc-85 containing binding sites for laminin and cytokeatin 18 (Giordano et al. 1994, Magdesian et al. 2001), mucins and TS (Schenkman et al. 1991b), cystein proteinases (Meirelles et al. 1992) and members of the prolyl oligopeptidase family of serine proteases (Burleigh et al. 1997, Grellier et al. 2001).

Tc-85 FAMILY AND OTHER TCT COMPONENTS WITH AFFINITY FOR EXTRA CELLULAR MATRIX

Tc-85, the surface glycoprotein expressed in TCT was first implicated in mammalian cell invasion using MAbs that partially block parasite internalization (Alves et al. 1986). Among the members of Tc-85 family, the best characterized is the molecule that contains binding sites for laminin (Giordano et al. 1994, 1999) and host cell cytokeratin 18 (Magdesian et al. 2001). The Tc-85 cell binding site colocalizes to the most conserved mo-

tif (VTVXNVFLYNR) of the gp85/TS superfamily, at the C-terminal domain, and does not contain the laminin-binding site (Magdesian et al. 2001). In the sequence deduced from laminin-binding Tc-85 cDNA, there is a stretch of 122 amino acid residues downstream to this conserved motif (Giordano et al. 1999), whereas in MT gp82 the same motif localizes closer to the C- terminus, being followed by 38 residues, of which 14 correspond to the hydrophobic GPI-anchor sequence (Araya et al. 1994). It is possible that this subterminal localization of conserved motif in MT gp82 precludes its interaction with cytokeratin C18.

The involvement of fibronectin in target cell invasion by TCT was deduced from experiments in which the peptide RGDS, corresponding to fibronectin cell attachment site, was found to bind to the parasite surface and to inhibit its internalization (Ouaissi et al. 1986b). By using affinity chromatography, the TCT ligand for fibronectin was purified and identified as an 85 kDa protein that interacts with cells bearing fibronectin molecules, such as human monocytes and neutrophils as well as 3T3 fibroblasts (Ouaissi et al. 1986a).

Another TCT surface molecule with affinity for extracellular matrix components is penetrin, a 60 kDa protein that selectively binds to heparin, heparin sulfate and collagen, and promotes fibroblast adhesion and penetration (Ortega-Barria and Pereira 1991). An intriguing observation is that the recombinant penetrin, expressed in *Escherichia coli* and localized on its surface, induced the bacterial attachment to and penetration into Vero cells in a proteoglycan- and collagen-inhibitable manner (Ortega-Barria and Pereira 1991). Assays to probe the role of host cell heparin and heparan sulfate glycosaminoglycans in *T. cruzi* invasion showed that proteoglycan-deficient mutants of Chinese hamster ovary (CHO) cells are poor targets for TCT penetration (Herrera et al. 1994). Penetrin has not been further characterized and its structure remains undefined.

A member of the prolyl oligopeptidase (POP) family of serine proteases, with specificity for human collagen types I and IV, has been identified in cell-free extracts of trypomastigotes, amastigotes and epimastigotes (Santana et al. 1997, Grellier et al. 2001). The 80 kDa enzyme, denominated POP Tc80, also hydrolyses fibronectin and appears to be implicated in host cell invasion. Selective and irreversible inhibitors of POP Tc80 were found to block TCT entry into nonphagocytic mammalian cells (Grellier et al. 2001, Bastos et al. 2005).

In *T. cruzi* infection in vivo, the ability of diverse trypomastigote molecules in binding to laminin, fibronectin, collagen, heparin, heparan sulfate, heparin, in addition to the hydrolytic activity of some of them, may be essential for the parasite transit through the extracellular matrix towards target cells.

TS AND SIALIC ACID ACCEPTOR MOLECULES

The role of TS in mammalian cell invasion has as yet to be fully clarified. On the basis that polyclonal antibodies that block TS activity enhance invasion of host cells by TCT in vitro, Cavallesco and Pereira (1988) proposed that the enzyme negatively modulates *T. cruzi* infection. They also found that, compared to the minor subset of trypomastigotes recognized by anti-TS antibodies (TS[+]), the TS[-] population showed enhanced ability to enter host cells. By using anti-TS monoclonal antibodies and various cell types and parasite isolates, Prioli et al. (1990) confirmed the previous results, reinforcing the hypothesis that TS down regulates *T. cruzi* infection. Subsequently, however, contradictory data were reported by the same group. Pereira et al. (1996) prepared pure TS[+] and TS[-] populations and tested them for host cell invasion. They found that TS[+] trypomastigotes were highly invasive whereas TS[-] parasites were extremely inefficient in invading epithelial cells and fibroblasts. Furthermore, introduction of small amounts of TS into suspensions of nonpenetrating TS[-] trypomastigotes converted them to a highly invasive phenotype (Pereira et al. 1996).

Immunocytochemical studies localized TS on the surface of TCT and in association with the flagellar pockets, suggesting that the enzyme is also se-

creted (Souto-Padrón et al. 1990). The shed acute phase antigen (SAPA), present in *T. cruzi*-infected patients and identified as TS, is the secreted form of enzyme that generates strong immune response (Pollevick et al. 1991). Whether the secreted form of TS and the enzyme expressed on TCT surface act simultaneously during interaction with target cells, or the action of one of them predominates depending on the circumstances, is not clear. According to Ming et al. (1993), TS may function as a counter-receptor for parasite binding to α2,3-sialyl receptors on host cells as a prelude to TCT invasion. Alternatively, after binding of TCT to target cells through another molecule, secreted TS may transfer sialic acid from the mammalian cell membrane to the parasite mucins. These transference reactions could disrupt the binding of sialoadhesins, allowing the parasites to detach and find a new binding site in order to proceed towards their internalization (Schenkman and Eichinger 1993).

In TCT, the sialic acid is incorporated by TS mainly into mucins migrating in SDS-PAGE as a broad band of 70-200 kDa (Schenkman et al. 1991a, b). Mucins and sialyl residues apparently are not primary requirements for TCT invasion (Schenkman et al. 1993b), although monoclonal antibodies to sialic acid-containing epitopes inhibit parasite adhesion to host cells (Schenkman et al. 1991b). On the other hand, the involvement of target cell sialic acid in *T. cruzi* internalization was reported by different groups, using sialic acid-deficient CHO (Lec2) cells. Trypomastigotes entered Lec2 cells to a much lower extent than parental CHO cells, but sialylation by TS restored parasite adhesion and invasion (Ciavaglia et al. 1993, Ming et al. 1993, Schenkman et al. 1993b).

<small>Cruzipain, the Major *T. cruzi* Cysteine Proteinase</small>

Murta et al. (1990) identified *T. cruzi* antigen gp57/ 51 as a cysteine proteinase which is active across pH range 5-7.5. This enzyme, named cruzipain, is expressed in all developmental forms of different *T. cruzi* isolates (Murta et al. 1990, Paiva et al. 1998). By using peptidyl diazomethane derivatives, a class of irreversible inhibitors of cysteine proteinase, the involvement of cruzipain enzyme in host cell invasion and intracellular development was inferred (Meirelles et al. 1992). The role of cruzipain in vivo was also determined. Engel et al. (1998) cured experimental *T. cruzi* infection by treating mice with peptide-fluoromethyl ketones, inhibitors that inactivate cruzipain and arrest intracellular replication as well as intercellular transmission in vitro (Harth et al. 1993).

The participation of cruzipain in host cell invasion by TCT is associated with its ability to generate bradykinin, according to Scharfstein et al. (2000), who investigated the involvement of B_2 type of bradykinin receptor (B_2R) using human umbilical vein endothelial cells (HUVECs) or CHO cells overexpressing B_2R (CHO-B_2R). They found that captopril, an inhibitor of bradykinin degradation by kininase II, potentiated TCT entry into HUVECs and CHO-B_2R, but not into mock-transfected CHO cells, whereas the B_2R antagonist HOE 140 or monoclonal antibody to bradykinin blocked these effects. Purified cruzipain enhanced parasite invasion and triggered Ca^{2+} mobilization in CHO-B_2R in a manner inhibitable by HOE 140 or cruzipain inhibitor E-64, indicating that the enzyme plays a role in generating the kinin agonist from cell-bound kininogen. This kinin-mediated signal transduction route is not ubiquitous, its activation depending on the cell type and the parasite isolate used (Scharfstein et al. 2000).

It has also been shown that MGTA, an inhibitor of kininase I, selectively decreases TCT infectivity for B_1R-expressing cells and that addition of B_1R or B_2R antagonists to host cells coexpressing these receptors inhibit parasite infectivity to a similar extent (Todorov et al. 2002). Because the combined application of both antagonists had no additive effect on both cardiomyocytes and HUVECs, the authors deduced that B_1R and B_2R operate interdependently to meet the intracellular Ca^{2+} concentration threshold required for efficient TCT internalization.

Processing of kininogens presumably takes

place within the secluded spaces formed by juxta-position of parasite and the target cell, inasmuch as membrane-permeable but not soluble cruzipain inhibitors block parasite invasion of cells that naturally overexpress kinin receptors (Scharfstein et al. 2000, Todorov et al. 2002). Cruzipain appears to be modulated by both the host and *T. cruzi* components. On the premise that kininogen molecules may be displayed on cell surfaces by binding to glycosaminoglycans, Lima et al. (2002) examined whether the ability of cruzipain to release kinins from high molecular weight kininogen is modulated by heparin sulfate. In the presence of heparin sulfate, they found an enhancement of 6-fold in cruzipain activity towards synthetic substrates and of up to 35-fold by direct measurement of bradykinin. On the other hand, a tight-binding cysteine proteinase inhibitor, chagasin, was identified in *T. cruzi* (Monteiro et al. 2001). It is localized in the flagellar pocket and cytoplasmic vesicles of TCT, and its expression is inversely correlated with that of cruzipain.

T. cruzi OLIGOPEPTIDASE B

A soluble factor of unknown structure, secreted by TCT, is so far the sole component of this *T. cruzi* developmental form reported to directly trigger Ca^{2+} response in host cells. This soluble factor is generated by the action of a 120 kDa alkaline peptidase on precursors present only in infective trypomastigotes (Burleigh and Andrews 1995). The purified peptidase is devoid of Ca^{2+} signaling activity on its own and is also present in noninfective epimastigotes (Burleigh and Andrews 1995).

By cloning and sequencing of the corresponding cDNA, the TCT peptidase was found to be a cytosolic enzyme closely related to members of the prolyl oligopeptidase family of serine endopeptidases, and was denominated *T. cruzi* oligopeptidase B (Burleigh et al. 1997). The oligopeptidase B null mutant trypomastigotes are defective in mobilizing Ca^{2+} from thapsigargin-sensitive stores in mammalian cells, and in establishing infection in vitro and in vivo (Caler et al. 1998). Based on

experimental evidences, it has been proposed that the Ca^{2+} agonist generated by oligopeptidase B is exported from the parasite, binds to a receptor on the surface of target cells, activating phospholipase C and generating IP_3, which binds to its receptor on the membrane of the endoplasmic reticulum and promotes Ca^{2+} release.

OTHER *T. cruzi* MOLECULES

Several other *T. cruzi* molecules have been implicated in host cell invasion. Surface antigens with metalloprotease activity, which are homologous to *Leishmania* gp63, were identified in MT and TCT and affinity-purified antibodies to these antigens inhibited host cell invasion by ∼50% (Cuevas et al. 2003).

Moro et al. (1995) characterized a secreted *T. cruzi* protein with peptidyl-prolyl cis-trans isomerase activity, susceptible to inhibition by the immunosuppressant FK506 and related drugs, and showed that the addition of the recombinant protein to simian epithelial or HeLa cells enhances parasite invasion. The monomeric protein has a peptidyl-prolyl cis-trans isomerase core, encompassing the characteristic rotamase hydrophobic active site, and its mechanism of action may be the triggering of host cell signal, with or without the contribution of rotamase activity (Pereira et al. 2002).

A 67 kDa lectin-like glycoprotein, which binds to desialylated human erythrocyte membranes in a galactose-dependent way and recognizes receptors in mouse cardiac tissue and human cardiac aortic endothelium, has been described and the cell invasion inhibitory effect of anti-gp67 antibodies reported (Silber et al. 2002).

SIGNAL TRANSDUCTION IN HOST CELL DURING *T. cruzi* INVASION

Ca^{2+} SIGNALING AND LYSOSOME RECRUITMENT IN TARGET CELLS

In 1994, Tardieux et al. (1994) reported that TCT, or their isolated membranes, but not the non-infective epimastigotes, induce repetitive cytosolic-

free Ca^{2+} transients in normal rat kidney (NRK) fibroblasts, and that parasite entry is inhibited by depletion of host cell cytosolic-free Ca^{2+} or pretreatment with Ca^{2+} channel blockers. In addition to NRK cells, soluble fraction of TCT induced Ca^{2+} response in a variety of cell types, such as hamster CHO and Dede, dog MDCK, monkey CV-1, human A7 (Burleigh and Andrews 1995), L_6E_9 myoblasts (Moreno et al. 1994) and isolated primary canine cardiac myocytes (Barr et al. 1996). MT were also found to trigger Ca^{2+} signaling in diverse cell types which included human epithelial HeLa cells and macrophages (Dorta et al. 1995, Wilkowsky et al. 1996). IP3, generated upon PLC activation, mediated intracellular Ca^{2+} mobilization triggered by TCT soluble factor (Rodriguez et al. 1995).

According to Andrews (1995), host cell Ca^{2+} response induces the recruitment of lysosomes to the site of *T. cruzi* penetration. At that site, lysosomal markers are immediately incorporated into parasitophorous vacuole without accumulation of polymerized actin around the recently internalized parasites, and invasion is facilitated by disruption of microfilaments (Tardieux et al. 1992). Lysosome redistribution and TCT invasion of NRK or L_6E_9 cells is inhibited upon treatment with microtubule-binding drugs nocodazole, colchicine, vinblastine and taxol, or after microinjection with antibodies to kinesin, indicating that lysosome transport is mediated by microtubule/kinesin (Rodriguez et al. 1996). Recently, Jaiswal et al. (2002) reported that lysosomes that fused were predominantly pre-docked at the plasma membrane, Ca^{2+} being primarily responsible for fusion and not recruitment of lysosomes to the cell surface. By fusing with the plasma membrane, lysosomes would contribute to formation of the parasitophorous vacuole (Andrews 1995).

Elevation in intracellular free Ca^{2+} concentration triggered lysosome fusion and exocytosis, as deduced from the appearance on the plasma membrane of the lysosomal glycoprotein lgp120, and the release of the lysosomal enzyme beta-hexosaminidase or the lysosomally processed form of cathepsin D (Rodriguez et al. 1997). Ca^{2+}-dependent exocytosis of lysosomes is cAMP-regulated and is enhanced by isopreternol, a β-adrenergic agonist that activates adenylyl cyclase through heterotrimeric G protein G_s (Rodriguez et al. 1999). Sinaptotagmin VII, a ubiquitously expressed sinaptotagmin isoform that is localized on the membrane of lysosomes in different cell types and regulates exocytosis of these organelles, appears to mediate *T. cruzi* invasion. TCT entry was impaired in CHO cells loaded with antibodies that recognize the Ca^{2+}-binding domain of sinaptotagmin VII and inhibit the Ca^{2+}-triggered exocytosis of lysosomes (Caler et al. 2001).

Targeted lysosome exocytosis may not be the predominant mechanism by which TCT gain access to non-professional phagocytic cells. It has been found that only a minimal fraction of invading TCT associate with host cell lysosomes whereas the majority of parasites induce plasma membrane invagination and the TCT-containing vacuoles gradually acquire lysosomal markers (Woolsey et al. 2003). The newly forming *T. cruzi* compartments first interact with an early endosome compartment and subsequently with other late endosomes, before interaction with lysosomes (Wilkowsky et al. 2002).

HOST CELL ACTIN CYTOSKELETON

As a rapid and transient reorganization of host cell microfilaments is induced by TCT soluble factor and live trypomastigotes, probably as a direct consequence of increased intracellular Ca^{2+} concentration, it has been proposed that this disassembly of the cortical actin cytoskeleton plays a role in *T. cruzi* invasion (Rodriguez et al. 1995). In accord with this hypothesis is the observation that treatment of NRK cells with cytochalasin D, a drug that disrupts microfilaments, enhances TCT internalization (Tardieux et al. 1992, Caler et al. 2000). However, such an effect has not been detected by other groups. Cytochalasin D had little or no effect on TCT entry into MDCK or HeLa cells (Schenkman et al. 1991c) whereas marked inhibiton was detected in diverse cell types, including Vero, LLCMK 2, HFSF fibrob-

last, L-6 skeletal muscle myoblast and resident peritoneal macrophages (Rosestolato et al. 2002), in addition to heart muscle cells (Barbosa and Meirelles 1995). As regards MT invasion, it was significantly inhibited by treatment of HeLa cells with cytochalasin B or latrunculin B (Osuna et al. 1993), but unaffected by cytochalasin D (Schenkman and Mortara 1992). It is not clear why the results from different groups differ so widely, provided that apparently the experimental conditions and the drug concentration used are similar.

Recent data implicate the actin cytoskeleton in the intracellular retention of parasites. Woolsey and Burleigh (2004) demonstrated that cytochalasin D treatment of host cells inhibits early lysosome association with invading TCT by uncoupling the cell penetration step from lysosome recruitment and/or fusion, and prolonged disruption of actin microfilaments results in significant loss of internalized parasites from infected cells. That a significant fraction of the internalized parasite is not retained inside host cells for a productive infection was confirmed by Andrade and Andrews (2004), by blocking lysosome-mediated TCT invasion through phosphoinositide 3-kinase inhibition.

An interesting observation, that reinforces the role of the cortical actin cytoskeleton disassembly in *T. cruzi* invasion, has been made in human placenta syncytiotrophoblasts. Using immunohistochemical techniques, Sartori et al. (2003a) observed the presence of actin in the syncytiotrophoblasts throughout the brush border in placentae from non-chagasic women but, after culture with trypomastigotes, this labeling disappeared, indicating that the parasite induced disassembly of the cortical actin cytoskeleton.

PHOSPHOINOSITIDE (PI)-3 KINASES,
PROTEIN KINASES AND PHOSPHATASES

Among the mechanisms of *T. cruzi* invasion are those dependent on lipid as well as protein kinases. Infection of macrophages with trypomastigotes stimulates the formation of the lipid products of PI 3-kinases and treatment with wortmannin, an inhibitor of PI 3-kinases, impaires parasite internalization (Todorov et al. 2000). Immunofluorescence microscopy, using antibodies against p85, the regulatory subunit of PI 3-kinase, localized the enzyme at the site of parasite interaction with macrophages, which was rich in F-actin (Vieira et al. 2002). In addition to phagocytic human macrophages and J774 murine cells, nonphagocytic Vero, L_6E_9 and 3T3 cells become less susceptible to *T. cruzi* infection upon treatment with wortmannin (Wilkowsky et al. 2001). According to Woolsey et al. (2003), host cell PI 3-kinases activated by TCT early in the cell invasion process regulate lysosome-dependent parasite entry. Treatment of *T. cruzi* with wortmannin also inhibited parasite internalization (Wilkowsky et al. 2001), indicating that both parasite and target cell PI 3-kinase activities are implicated in cell invasion.

The involvement of protein tyrosine kinase (PTK) in *T. cruzi* invasion of macrophages has been reported by Vieira et al. (1994). By treating either TCT or macrophages with genistein, a specific PTK inhibitor, these authors found a significant decrease in parasite endocytosis. Monoclonal anti-phosphotyrosine antibodies revealed an accumulation of tyrosine-phosphorylated residues at the site of parasite association with the macrophage surface, colocalizing with host cell F-actin-rich domains (Vieira et al. 2002). Activation of parasite PTK is required for MT and TCT entry into nonphagocytic cells (Favoreto et al. 1998) but, in contrast to what is seen in macrophages, host cell PTK activity is not involved, as inferred from the lack of inhibition of TCT or MT internalization upon treatment of RNK or HeLa cells with genistein (Rodriguez et al. 1995, Favoreto et al. 1998).

Other protein kinases also participate in *T. cruzi* entry into host cells. Wilkowsky et al. (2001) found that infection of transiently transfected 3T3 cells containing an inactive mutant protein kinase B are less susceptible to invasion, as compared to the active mutant-transfected cells. Activation of protein kinase C and enhancement of parasite uptake by macrophages have been detected upon incubation with recombinant gp83, a TCT surface ligand

(Villalta et al. 1999).

Activation of the host cell PTK is not required for *T. cruzi* invasion of nonphagocytic cells. On the other hand, protein tyrosine phosphatases appear to be involved. Invasion of TCT induced tyrosine dephosphorylation of several proteins in L_6E_9 myoblasts, and the parasite internalization was greatly reduced in the presence of protein tyrosine phosphatase inhibitors and in the presence of excess phosphotyrosine, but not of phosphoserine or phosphothreonine (Zhong et al. 1998). In human HEp2 tumor cells, infection with *T. cruzi* resulted in the alterations of their placental alkaline phosphatase activity as well as in a different pattern of actin organization, compared to control cells, and the interference in the enzyme activity before infection decreased the invasion rate (Sartori et al. 2003b).

TARGET CELL SURFACE MOLECULES IMPLICATED IN SIGNAL TRANSDUCTION AND TCT INVASION

In addition to Ca^{2+} signaling through bradykinin receptor, the signaling route mediated by transforming growth factor-β (TGFβ) receptor may be activated during TCT invasion of target cells. In a series of experiments, Ming et al. (1995) showed the requirement of TGFβ pathway for *T. cruzi* invasion of epithelial cells. They found that TCT attached to TGFβ receptor-deficient epithelial cell lines, but were unable to penetrate. Susceptibility to *T. cruzi* infection was restored by transfection with TGFβ receptor genes, and treatment with TGFβ greatly enhanced parasite internalization. As a TGFβ-responsive reporter gene is induced in TGFβ-sensitive cell lines by TCT, but not by noninvasive epimastigotes, Ming et al. (1995) postulated that *T. cruzi* may directly trigger activation of the TGFβ-signaling pathway required for invasion. The putative TGFβ-like factor from TCT has never been characterized.

In macrophages, the heterodimeric $\beta1$ integrins, which belong to a ubiquitous family of integral membrane proteins that link the extracellular matrix to the cortical cytoskeleton, may be involved in signal transduction and *T. cruzi* internaliza-

tion. Fernandez et al. (1993) observed that, when added to human macrophages, monoclonal antibodies to $\beta1$ subunit of VLA integrin family specifically blocked *T. cruzi* uptake, without interfering with the uptake of *Leishmania pinfanoi* or *Escherichia coli*. As that inhibition correlated with the ability to block fibronectin binding to macrophages, it is uncertain whether the parasite interacts with VLA directly or through the binding to fibronectin.

Galectin 3, which increases K-Ras activation and triggers a Ras signal (Elad-Sfadia et al. 2004), is another host cell component that may participate in *T. cruzi* invasion. In experiments with human coronary artery smooth muscle cells, that express galectin-3 on the surface and also secret it, Kleshchenko et al. (2004) found that exogenously added galectin-3 increases trypomastigote binding. *T. cruzi* adhered poorly to cells with reduced expression of galectin-3, but the adhesion property was restored by exogenous galectin-3.

MECHANISMS OF TARGET CELL INVASION BY BLOOD TRYPOMASTIGOTES

From the data of in vitro studies with TCT, the possible mechanisms that the bloodstream trypomastigotes may use to invade host cells can be envisaged (Fig. 7). Before reaching the target cells, in many instances the parasites have to overcome the barrier of extracellular matrix, in the same manner as MT encounter the mucin coat in the stomach. Through the surface molecules of gp85/TS superfamily, the parasites bind to fibronectin/laminin (Fig. 7A) and pave the way for the action of enzymes, such as the serine protease POP Tc80 that hydrolyses collagen/fibronectin (Fig. 7B).

Upon encountering the target cells, trypomastigotes attach to them in a manner mediated by Tc-85, TS or mucin (Fig. 7C). This interaction induces the activation of *T. cruzi* oligopeptidase B that generates a Ca^{2+} signaling factor from a precursor molecule. Triggering of host cell Ca^{2+} mobilization by this secreted parasite factor promotes invasion. Alternatively, or simultaneously, cruzipain is secreted by attached trypomastigotes within the con-
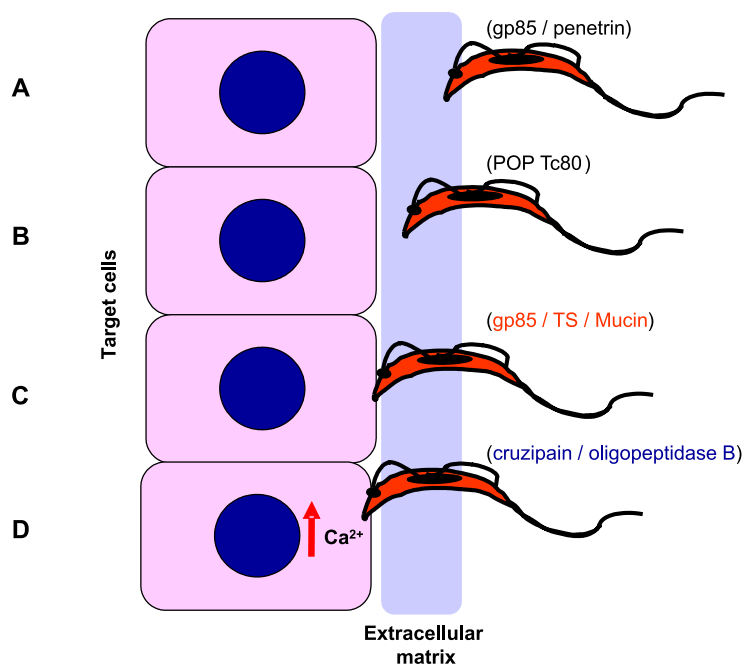
Fig. 7 – Model of molecular interaction of *T. cruzi* bloodstream trypomastigotes with target cells. The extracellular matrix may constitute in many instances a barrier trypomastigotes have to overcome to reach the host cells. A) TCT attach to the extracellular matrix using molecules such as gp85 and penetrin, which have affinity for laminin, fibronectin, collagen, heparin. B) To translocate through the matrix, the parasite may use POP Tc80, which has collagen/fibronectin hydrolyzing activity. C) Adhesion to and invasion of target cells can be mediated by gp85, TS and/or mucins. D) Binding of TCT to target cells induce the activation of oligopeptidase B that generates a Ca^{2+} agonist, as well as the secretion of cruzipain that indirectly triggers Ca^{2+} response in host cells.

fines of parasite-target cell juxtaposition, and its action on the kininogen generates bradykinin, which also induces Ca^{2+} response by interacting with its receptor (Fig. 7D). Trypomastigotes of *T. cruzi* isolates that trigger both signaling pathways may be more invasive. By testing different *T. cruzi* isolates, Aparicio et al. (2004) have found a correlation between the level of secreted cruzipain and the capacity of trypomastigotes to invade host cells. The low invasive capacity of G isolate, with low levels of cruzipain, may result from the lack of activation of cruzipain-mediated signaling pathway. In addition to the referred signaling routes, parasite-target cell interactions mediated by TGFβ receptors and β1 integrins may take place, further contributing to the process of parasite internalization.

CONCLUDING REMARKS

Activation of signal transduction pathways triggered in the parasite and the host cell, leading to intracellular Ca^{2+} mobilization, Ca^{2+}-induced reorganization of the host cell actin cytoskeleton and lysosome recruitment, constitutes the general mechanism by which *T. cruzi* trypomastigotes invade mammalian cells. With a plethora of *T. cruzi* molecules that have been identified and characterized structural and functionally, plus the identification of target cell components involved, the whole process is beginning to be understood at the molecular level. The picture is complex. Not only MT and TCT engage different molecules to interact with host cells, but different *T. cruzi* isolates may use distinct sets of molecules, activating distinct sig-

naling pathways. In addition, some molecular interactions may trigger inhibitory signals that down regulate trypomastigote invasion. Furthermore, as the in vivo infection is concerned, the interaction of parasites with host components before reaching the target cells has also to be considered. Great progress has been made towards understanding the mammalian cell invasion by *T. cruzi*, but a lot more work has to be done before we can draw a more complete and detailed picture of that process.

RESUMO

O estabelecimento da infecção por *Trypanosoma cruzi*, o agente da doença de Chagas, depende de uma série de eventos envolvendo interações de diversas moléculas do parasita com componentes do hospedeiro. Focalizamos aqui os mecanismos de invasão celular por tripomastigotas metacíclicos (TM) e por tripomastigotas de cultura de tecido (TCT). Durante a internalização de TM ou TCT, vias de transdução de sinal são ativadas tanto no parasita como na célula alvo, acarretando a mobilização de Ca^{2+}. Para adesão, TM utiliza as glicoproteínas de superfície como a gp82 e gp35/50, que são moléculas indutoras de sinal de Ca^{2+}. Em isolados de *T. cruzi* que entram na célula hospedeira de maneira dependente de gp82, a proteína tirosina quinase assim como a fosfolipase C do parasita são ativadas, e Ca^{2+} é liberado de reservatórios sensíveis a IP3, enquanto em isolados de *T. cruzi* que se ligam às células alvo através de gp35/50, a via de sinalização envolvendo adenilil ciclase parece ser estimulada, com liberação de Ca^{2+} de acidocalciossomos. Além disso, dependendo do isolado de *T. cruzi*, sinais inibitórios mediados por gp90 específica de TM podem ser desencadeados tanto na célula hospedeira como no parasita. O repertório de moléculas de TCT implicadas na invasão celular inclui glicoproteínas de superfície da família gp85, com membros contendo sitos de ligação à laminina e citoqueratina 18, enzimas como a cruzipaína, trans-sialidase, e uma oligopeptidase B que gera um agonista de Ca^{2+} a partir de uma molécula precursora.

Palavras-chave: *Trypanosoma cruzi*, tripomastigotas, invasão celular, transdução de sinal, mobilização de Ca^{2+}.

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