Published in final edited form as:
Parasitol Res. 2009 June ; 104(6): 1251–1260. doi:10.1007/s00436-009-1383-3.

Perspectives on the *Trypanosoma cruzi*–host cell receptor interactions

Fernando Villalta,
Department of Microbial Pathogenesis and Immune Response, Meharry Medical College, Nashville, TN, USA e-mail: fvillalta@mmc.edu

Julio Scharfstein,
Department Immunobiology, Instituto de Biofísica Carlos Chagas Filho, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil e-mail: scharf@biof.ufrj.br

Anthony W. Ashton,
Department of Pathology, Albert Einstein College of Medicine, Bronx, NY 10461, USA

Division of Perinatal Research, Kolling Institute of Medical Research, University of Sydney, Sydney, Australia

Kevin M. Tyler,
BioMedical Research Center, School of Medicine, Health Policy and Practice, University of East Anglia, Norwich, UK

Fangxia Guan,
Bioengineering Department of Zhengzhou University, Zhengzhou, People' Republic of China

Shankar Mukherjee,
Department of Pathology, Albert Einstein College of Medicine, Bronx, NY 10461, USA

Maria F. Lima,
Department of Microbial Pathogenesis and Immune Response, Meharry Medical College, Nashville, TN, USA

Sandra Alvarez,
Department of Pathology, Albert Einstein College of Medicine, Bronx, NY 10461, USA

Louis M. Weiss,
Department of Pathology, Albert Einstein College of Medicine, Bronx, NY 10461, USA

Huan Huang,
Department of Pathology, Albert Einstein College of Medicine, Bronx, NY 10461, USA

Fabiana S. Machado, and
Department of Biochemistry and Immunology, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

Herbert B. Tanowitz
Department of Pathology, Albert Einstein College of Medicine, Bronx, NY 10461, USA e-mail: tanowitz@aecom.yu.edu

Abstract

Correspondence to: Herbert B. Tanowitz.

Chagas disease is caused by the parasite *Trypanosoma cruzi*. The critical initial event is the interaction of the trypomastigote form of the parasite with host receptors. This review highlights recent observations concerning these interactions. Some of the key receptors considered are those for thromboxane, bradykinin, and for the nerve growth factor TrKA. Other important receptors such as galectin-3, thrombospondin, and laminin are also discussed. Investigation into the molecular biology and cell biology of host receptors for *T. cruzi* may provide novel therapeutic targets.

Chagas disease caused by the parasite *Trypanosoma cruzi* remains an important cause of morbidity and mortality in endemic areas of Mexico and Central and South America. Although there are still areas where acute infection remains a public health problem, the greatest human burdens are due to the consequences of chronic infection including cardiomyopathy and the gastrointestinal megasyndromes (Tanowitz et al. 1992). *T. cruzi* infection has gained notoriety because of its association with immunosuppressive states such as HIV/AIDS (Vaidian et al. 2004) and because of the threat of being transmitted by blood transfusion and organ transplantation.

A critical event in infection with *T. cruzi* is the initial interaction of the trypomastigote form of the parasite with the host cell. These interactions result in the activation of signal transduction pathways important in the pathogenesis of Chagas disease. Over the years, there have been many important studies on the interaction of this parasite and receptors on host cells. We have chosen to highlight some recent aspects of this important relationship.

Trypanosoma cruzi–thromboxane receptor interactions

Chagas disease is characterized by intense inflammation and fibrosis (Tanowitz et al. 2005; Petkova et al. 2000) associated with alterations in cardiovascular function, vascular tone (Factor et al. 1985, Tanowitz et al. 1996), hemostasis (Tanowitz et al. 1992; Petkova et al. 2001), and platelet reactivity. An agent that displays some of these pathophysiological properties is the bioactive lipid thromboxane A_2 (TXA_2). Systemic elevation in TXA_2 levels (measured as the stable hydrolytic product TXB_2) is observed in mice infected with *T. cruzi* (Tanowitz et al. 1990; Cardoni and Antunez 2004) suggesting that TXA_2 may be important in Chagas disease. The assumption has been that the host was the source of the elevated TXA_2 observed in the circulation. However, it was recently reported that the parasite is another source of this mediator (Ashton et al. 2007). TXA_2 has a complicated role in the pathophysiology of Chagas disease; however, parasite-derived TXA_2 alone is sufficient to mediate disease progression as deletion of TXA_2 synthase from the host genome does not influence pathogenesis (Ashton et al. 2007). Conversely, appropriate host response to parasite-derived TXA_2 is essential for maintaining host viability and disease pathogenesis. Employing TXA_2 receptor (TP)-null mice, it was determined that a failure of the host to respond to parasite-derived TXA_2 resulted in a higher parasitemia, increased tissue parasitism, and shorter survival time after infection (Ashton et al. 2007).

The TP is a member of the serpentine family of G-protein-coupled receptors. The coupling of this receptor is complicated involving multiple heterotrimeric G-proteins as well as a number of other signaling intermediates. The key signal from TP that appears to regulate the growth phenotype of the amastigote is linked to the activation of Gαq-containing heterotrimeric G-proteins (Ashton et al. 2007) although the specific mediator involved has yet to be confirmed. These may include phospholipase C β and inositol phosphates (Garg et al. 1997) and extracellular signal-regulated kinase (Leal et al. 2007) or protein kinase C (PKC; Einicker-Lamas et al. 2007) activation. Many of these have been previously identified as mediators of experimental Chagas disease. The commonality between these pathways and the host receptors that activate them (such as those for endothelin, TXA_2 and bradykinin) indicates that G-αq signaling from the host may contribute to the pathogenesis of Chagas disease.

In addition to mediating the symptomatic aspects of the disease, there are a number of suggested roles for TP activation in the development of Chagas disease. The first is a means by which the parasite manipulates the responses of the host during infection. The intracellular amastigote produces TXA_2 in substantial quantities (about half as much as platelets). Parasite-derived TXA_2 acts on putative receptors in somatic cells of the host to regulate parasite growth and differentiation. This signaling loop ensures that the parasite does not overwhelm the host too quickly increasing the likelihood of further transmission to a new host. These data may explain differences in the susceptibility to experimental *T. cruzi* infection (Cardoni and Antunez 2004). The higher the TXA_2 production, the earlier this regulatory system would be established which would slow the rate of parasite growth.

Similarly, the response of the host to parasite-derived TXA_2 appears to be largely anti-inflammatory. TP-null mice show significant areas of inflammation while wild-type (WT) mice display minimal pathology (Ashton et al. 2007). Despite being considered a pro-inflammatory mediator, the anti-inflammatory effects of TXA_2 may result from the suppression of NFκB activation by other inflammatory mediators in the more complex setting of *T. cruzi* infection in vivo as previously indicated (Ashton et al. 2003). Moreover, the secretion of TXA_2 also prevents the initiation of an adaptive immune response by the host (Kabashima et al. 2003). Thus, TXA_2 release by the parasite would severely compromise the adaptive and innate immune responses of the host to infection, allowing continued parasite survival and progression to the chronic phase of the disease. Collectively, these events produce some of the diverse pathophysiological changes that result in the complicated phenotype of acute and chronic Chagas disease.

Eicosanoids are produced by several parasitic organisms (Liu and Weller 1990; Belley and Chadee 1995; Kubata et al. 2000). While the contribution of these modulators to disease pathogenesis remains largely unexplored, they represent ideal modulators of infection. If parasite-derived eicosanoids act as immunoregulatory agents for the host, similar to TXA_2, then this mechanism may represent a common mechanism used by intracellular parasites to affect host response. This hypothesis is supported by data indicating enhanced mortality rates in Chagas patients (Celentano et al. 1995; Sterin-Borda et al. 1996) and *T.-cruzi*-infected mice (Celentano et al. 1995; Hideko-Tatakihara et al. 2008) with cyclooxygenase inhibitor use. Thus, TXA_2 appears to be one of a few parasite-derived molecules shown to be essential for host survival and disease progression.

Parasite–bradykinin receptor interaction

The term “kinin” refers to a small group of vasoactive metabolites structurally related to the nonapeptide bradykinin (BK), which are released from an internal moiety of high (HK) or low (LK) molecular weight kininogens by the action of plasma or tissue kallikrein (Bhoola et al. 1992). In the settings of infection, kinins can be liberated from the kininogens by the action of microbial cysteine proteases, such as those expressed by *T. cruzi* (Del Nery et al. 1997; Lima et al. 2002; Scharfstein et al. 2000). Once released, the short-lived kinins (half life of <15 s in the plasma) bind to a wide range of cells through distinct subtypes of heterotrimeric G-protein-coupled receptors: bradykinin B2 receptors (B_2R), which are constitutively expressed by cardiovascular cells; B_1R, whose expression is upregulated in injured tissues (Leeb-Lundberg et al. 2005; Marceau and Bachvarov 1998). The effects of BK on the vascular endothelium are prevented by the action of kinin-degrading peptidases, such as the angiotensin converting enzyme (ACE)/kininase II (Skidgel and Erdos 2004). While intact kinins such as BK or lysyl-BK (LBK) are the agonists for B_2R, the proteolytic excision of the C-terminal Arg of BK/LBK by carboxypeptidase N/M (kininase I) generates high-affinity ligands for B_1R(des-Arg-BK or des-Arg-LBK; Marceau and Bachvarov 1998).

Cruzipain (CZ), the major lysosomal-like cysteine protease of *T. cruzi*, was characterized as a potential therapeutic target for the treatment of this infection (McGrath et al. 1995). Initial studies conducted with first-generation CZ inhibitors indicated that trypomastigotes rely on the enzymatic activity of CZ to invade and multiply in cardiac myocytes (Meirelles et al. 1992). The elucidation of the X-ray structure of the catalytic domain of CZ (McGrath et al. 1995) led to development of potent and selective irreversible inhibitors of CZ, some of which were able to protect mice from lethal infection (Engel et al. 1998).

Clues to understanding the functional roles of CZ came from studies demonstrating that this papain-like cysteine protease resembles tissue kallikrein, i.e., both enzymes efficiently cleave HK at the flanking sites of the internal kinin moiety, liberating lysyl-BK (Del Nery et al. 1997). Subsequently, it was demonstrated that HK interactions with heparan sulfate reduced the cysteine inhibitory activity of the cystatin-like domains of HK (Lima et al. 2002). In addition, this sulfated proteoglycan re-directed the substrate specificity of CZ, generating multiple HK breakdown products, including kinin peptides (Lima et al. 2002). These studies suggested that tissue culture trypomastigotes generate kinins through mechanisms that involve cooperative interactions between CZ, HK, and heparan sulfate proteoglycans (Lima et al. 2002).

When Chinese hamster ovary (CHO) cells were transfected with the rat-B_2 receptor (B_2R) gene (Scharfstein et al. 2000; CHO-B_2R) and subsequently infected, there was an induction of IP_3-mediated influx of intracellular calcium in transfected cells. Similarly, activated CZ elicited potent intracellular calcium responses in transfected cells which were blocked by HOE-140, a specific antagonist of the B_2R subtype (Scharfstein et al. 2000). Monoclonal antibodies directed to the BK epitope of kininogens blocked invasion into CHO-B_2R without interfering with the baseline levels of mock infection of CHO cells. Furthermore, parasite uptake by CHO-B_2R was increased upon addition of purified HK or, alternatively, by increasing physiological concentrations of the B_2R peptide agonist, while showing negligible affects on mock-transfected CHO cells. These observations suggest that CZ liberates the B_2R peptide agonist from kininogen molecules docked to cell surfaces of mammalian cells. It is possible that the kinin-releasing reaction occurs within compartmentalized areas of the host cell plasma membrane. This possibility was supported by the observations that membrane-permeable CZ inhibitors efficiently reduced parasite invasion of endothelial cells via the B_2R pathway, while addition of soluble inhibitors such as cystatin C or E-64 did not interfere with cell invasion.

Since trypomastigotes are poorly endocytic and accumulate CZ in the flagellar pocket, the failure of hydrophilic inhibitors in preventing cellular invasion was interpreted as evidence that the kinin-releasing reaction most likely occurs in enclosed areas formed by juxtaposition of host cell and parasite plasma membranes. Thus, Scharfstein et al. (2002) proposed a mechanistic model whereby active CZ molecules diffuse from the flagellar pocket of the parasite into this intercellular space. In this secluded microenvironment, CZ may cleave surface-bound kininogens while being spared from physiological inactivation by soluble forms of plasma protease inhibitors (e.g., cystatins, kininogens, α_2-macroglobulin).

Importantly, there is significant residual infection in cultures (CHO-B_2R), endothelial cells or neonatal cardiac myocytes maintained in the presence of HOE-140 (B_2R antagonist), indicating that the kinin signaling pathway is not the only pathway driving infectivity. However, interaction assays performed with endothelial cells that had been pre-activated by lipopolysaccharide via toll-like receptor (TLR)-4, or with primary murine cardiac myocytes, revealed that the parasites can invade these activated host cells via the upregulated B_1R pathway (Todorov et al. 2003). These effects were canceled after the addition of inhibitors of carboxypeptidase N/M (kininase I) to the cultures, suggesting that the B_1R ligand [des-Arg]-BK/LBK is generated by kininase I. The authors noted that, unlike the effects on B_2R signaling,

addition of ACE inhibitors is not required for parasite invasion via the upregulated B_1R pathway. Additional work is required to determine if the parasites may take advantage of upregulated expression of B_1R in the chronically inflamed myocardium to infect macrophages, fibroblasts, endothelial cells, and/or cardiac myocytes.

After demonstrating that tissue-culture-derived trypomastigotes released kinins while interacting with cultured mammalian cells, additional studies were performed to verify if the parasites were able to activate B_2R at early stages of infection in vivo. Indeed, studies in a mouse subcutaneous infection model demonstrated that tissue-culture-derived trypomastigotes (but not epimastigotes) evoked paw edema through the sequential activation of B_2R and B_1R (Monteiro et al. 2006). Intravital microscopy (Monteiro et al. 2006) demonstrated that signals leading to plasma leakage at early stages of infection allow for the accumulation of kininogens (i.e., substrates for the kinin-releasing cruzipain enzyme) in interstitial spaces. The dissection of the signaling pathways that initiate edematogenic inflammation was performed in the mouse model. These studies demonstrated that tissue-culture-derived trypomastigotes initiate inflammation by triggering Toll-like receptor (TLR) 2-dependent secretion of CXC chemokines by macrophages (Schmitz et al. 2009). Following endothelium/neutrophil activation, there is a discrete extravasation of plasma proteins into interstitial spaces. Acting further downstream, the blood-borne kininogens undergo proteolysis by CZ thus generating high levels of bioactive kinins in the peripheral tissues. The extent of B_2R signaling by the short-lived kinins is tightly regulated by the action of ACE/kininase II, a kinin-degrading metallopeptidase that is highly expressed in subcutaneous tissues (Monteiro et al. 2006). Thus, the intensity of edematogenic inflammation in infected peripheral tissues is controlled by an intricate interplay of TLR2, B_2R, and ACE.

Analysis of the outcome of kinin system activation in the subcutaneous infection model revealed that kinins released in peripheral tissues activate antigen-loaded dendritic cells (DCs) via B_2R and switch their maturation program. After migrating to the T-cell-rich areas of draining lymph nodes, the mature DCs stimulate adaptive (type-1) immunity. As noted, the extent of DC activation by kinins in vivo is tightly regulated by ACE, a kinin-degrading metallopeptidase expressed in subcutaneous tissues. Consistent with this, mice pretreated with ACE inhibitors developed vigorous innate responses via B_2R, and these effects translated into upregulated Th1 responses (Marceau and Bachvarov 1998). These studies suggest that TLR2 and ACE play opposite roles in the regulation of pathways linking innate immunity (via the kinin/B2R pathway) to adaptive immunity (Monteiro et al. 2006; Aliberti et al. 2003; Scharfstein et al. 2007).

A recent development was the description of the consequences of infection in B_2R-null mice (Monteiro et al. 2007). Infection of these mice results in increased parasitemia, mortality, and myocardial parasitism. The susceptible B_2R-null mice initially developed fairly potent type-1 responses in the spleen, but the number of intracardiac interferon (IFN)-γ-producing $CD4^+$ and $CD8^+$ effector T cells was already reduced at relatively early stages of infection. Furthermore, as the infection progressed, there was a sharp decline in the frequency of type-1 effector cells in B_2R-null mice, both in lymphoid and cardiac tissues. Notably, the decayed T_H1 response of B_2R-deficient mice was accompanied by a rise in the pro-inflammatory T_H17 subset (Monteiro et al. 2007).

The hypothesis that DC signaling via B_2R is required for induction of protective effector T cells was underscored by adoptive cell transfer of WT DCs into B_2R-null mice. This procedure not only rescued the resistant phenotype in the recipient B_2KR-null mice but also restored the development of protective IFN-γ-producing $CD4^+$ $CD44^+$ and $CD8^+$ $CD44^+$ effector T cells in the recipient mutant mice, while dampening the potentially detrimental T_H17 ($CD4^+$ subset) responses (Monteiro et al. 2007). The analysis of $CD11c^+$ DC interaction with tissue-culture-

derived trypomastigotes demonstrated that interleukin (IL)-12 and co-stimulatory molecules (CD86, CD80, CD40) were upregulated in wild-type DCs but not in B_2R-null DCs (Monteiro et al. 2007) and tissue-derived trypomastigotes pre-treated with irreversible inhibitors of CZ failed to induce overt DC maturation. These observations support the notion that *T. cruzi* relies on CZ to enzymatically generate the B_2R agonist while interacting with immature DCs. Although it is not known if conventional DCs are the primary or even unique in vivo targets of *T. cruzi* in the spleen, these results support the idea that kinin-releasing pathogens convert immature DCs into drivers of type-1 adaptive responses through the activation of B_2R, a G-protein-coupled receptor, that acts as a sensor of danger to tissue integrity. Additional studies are required to evaluate if myocardial DCs (Andrade et al. 2000) sense the parasite via the B_2R.

Parasite–host cell interactions with other receptors

Mucins are the major *T. cruzi* surface glycoproteins and their sugar residues interact with invasive trypomastigotes and mammalian cells (Villalta and Kierszenbaum 1984, 1985; Yoshida et al. 1989). The monoclonal antibody B5, recognizing a critical *T. cruzi* 45-kDa mucin epitope, inhibits trypomastigote attachment to heart myoblasts, which prevents parasite entry suggesting that the 45-kDa mucin is used by trypomastigotes to adhere to heart myoblasts (Turner et al. 2002). Others have also implicated mucins in mammalian cell infection (Yoshida et al. 1989;Di Noia et al. 1995; Buscaglia et al. 2006). Mucins function as ligands (Schenkman et al. 1991). For example, mucins bind to galectin-3 (Moody et al. 2000), a β-galactosyl-binding lectin, and the binding has been suggested to mediate parasite attachment and entry (Moody et al. 2000; Kleshchenko et al. 2004). Lipid tail in the GPI-anchor of mucins and other *T. cruzi* ligands interact with the TLRs (Campos and Gazzinelli 2004) and *T. cruzi* infection of cardiac myocytes results in IL-1β-dependent myocyte hypertrophy mediated by TLR-2 (Petersen et al. 2005). These parasite–TLR interactions may promote B_2R signaling by kininogens, which is balanced by ACE signaling as discussed earlier.

Trans-sialidase and enzymatically inactive members of the trans-sialidase superfamily are also present on the parasite surface, but in much lower abundance compared with mucins. Trans-sialidases transfer sialic acid residues from host glycoconjugates to parasite mucins (Previato et al. 1985). Trans-sialidases, independent of its trans-sialidase activity, bind to surface receptors on neurons and glial cells such as Schwann cells, leading to the activation of survival signaling pathways such as mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3 kinase (PI3K)/Akt kinase signaling (Chuenkova and Pereira 2000; Chuenkova et al. 2001). Trans-sialidase binding to neuronal cells leads to survival and differentiation and an enhancement in the synthesis and activity of tyrosine hydroxylase, a rate-limiting enzyme of dopamine and other catecholamine neurotransmitters. A trans-sialidase has also been termed a parasite-derived neurotrophic factor (Chuenkova and PereiraPerrin 2006) and the binding of this trans-sialidase to the nerve growth factor receptor TrkA leads to *T. cruzi* invasion of neuronal and dendritic cells in vitro and in the murine model of this infection (Chuenkova and PereiraPerrin 2004; de Melo-Jorge and PereiraPerrin 2007). Trans-sialidase superfamily members bind to mammalian cell receptors to mediate trypanosome binding and entry (Frasch 2000; Lima and Villalta 1988, 1989; Villalta and Lima 1990; Villalta et al. 1992, 1996, 1998, 1999, 2001; Nde et al. 2006). Although the crystal structure of a *T. cruzi* trans-sialidase has been elucidated (Buschiazzo et al. 2002), its validation as a target for the development of new interventions to block infection appears complex (Agusti et al. 2004). Trans-sialidase and trans-sialidase-like super-family members are shed into the bloodstream and upregulate early infection in phagocytic and nonphagocytic cells (Villalta et al. 1998, 1999, 2001) and exert other biological effects on several cell types.

Gp83 is a ligand expressed in all *T. cruzi* strains and employed by the parasite to attach and enter macrophages as well as nonphagocytic cells (Lima and Villalta 1988; Villalta et al. 1998, 1999, 2001, 2008). Notably, it is expressed only in invasive trypomastigotes (Villalta et al. 1992) and is more highly expressed in highly infective trypomastigote clones (Lima and Villalta 1989). Monovalent Fab fragments of the monoclonal antibody 4A4 specific for gp83 inhibit gp83 binding to myoblasts, fibroblasts, and macrophages, block trypanosomes from attaching to and entering these cells, and neutralize *T. cruzi* infection in vivo (Villalta et al. 2001). A subset of the trans-sialidase super gene family, Tc-85, has been implicated in cell infection since antibodies to Tc-85 partially block parasite internalization (Alves et al. 1986); Tc-85 binds to laminin (Giordano et al. 1999) and cytokeratin 18 (Magdesian et al. 2001). Trypomastigotes release gp83 via parasite glycosylphosphatidylinositol–phospholipase C (PLC) cleavage to activate the host MAPK pathway and PKC in order to promote parasite infection (Villalta et al. 1998, 1999; Nde et al. 2006).

A surface casein kinase II (CKII) substrate (Tc-1) of trypomastigotes participates in early cell infection (Augustine et al. 2006). Exogenous human CKII phosphorylates serine residues on recombinant and trypomastigote Tc-1 and this phosphorylation is inhibited by CKII inhibitors (Augustine et al. 2006). Antibodies to Tc-1 or CKII inhibitors block the invasion of host cells by trypomastigotes and reduce parasite load in cells. Tc-1 is phosphorylated by human CKII, and whether the latter functions as a Tc-1 receptor is unknown. Since there are no human homologs of Tc-1, the gene and encoded protein could provide targets for drug discovery. Since Tc-1 is highly immunogenic and antibodies directed against Tc-1 neutralize *T. cruzi* infection of mammalian cells, Tc-1 could also be a candidate for vaccine development.

The LYT1 *T. cruzi* protein is required for efficient in vitro infection (Manning-Cela et al. 2001). The LYT1 gene product was characterized and is involved in parasite lysis and therefore affects its infectivity. Other parasite proteins of unknown molecular structure have been implicated in the invasion process since antibodies to these molecules partially inhibit cellular infection. These include penetrin (Ortega-Barria and Pereira 1991), a secreted peptidyl-prolyl cis-trans isomerase (Pereira et al. 2002) and a lectin-like 67-kDa glycoprotein (Silber et al. 2002).

T. cruzi proteases have been implicated in the infection process. Thus, an inhibitor of the *T. cruzi* prolyloligopeptidase Tc80, a member of the serine protease family that hydrolyses fibronectin, reduces trypomastigote entry into nonphagocytic cells (Grellier et al. 2001; Bastos et al. 2005). This suggests that prolyloligopeptidase Tc80 may be important for the parasite's transit through the extracellular matrix (ECM) towards target cells. The parasite secretes oligopeptidase B, a cytosolic serine endopeptidase, which triggers calcium release in host cells (Burleigh and Andrews 1995) required for trypanosome entry and antibodies directed against *T. cruzi* surface metalloproteases partially reduce cell invasion (Cuevas et al. 2003).

Several other candidate host cell receptors mediating the first step of infection have been suggested; however, validation of these candidate receptors at the molecular genetics level is needed in vitro and in vivo. Interestingly, another heterotrimeric G-protein-coupled receptor, the cannabinoid receptor CB1, is present at parasite host cell junction (parasite synapse). Ligation of this receptor induces Gi/o signaling and actually prevents parasite infection of cultured myocytes although use of cannabinoids in mice models did not improve outcome presumably because of their well-characterized immunosuppressive effects (Croxford et al. 2005). Cytokeratin 18, a cytoskeletal protein of Vero cells, was suggested to function as a *T. cruzi* receptor (Magdesian et al. 2001). However, recently, it has been shown that it does not function as a receptor for *T. cruzi* since silencing of cytokeratin 18 expression by RNAi does not affect trypomastigote binding to host cells nor its entry (Claser et al. 2009). Furthermore, a p74 heart myoblast surface protein has also been suggested to function as a *T. cruzi* receptor

mediating attachment leading to entry since it binds to the trypomastigote surface and p74 antibodies block parasite attachment to mammalian cells (Villalta et al. 1993). The ECM, human lectins, and parasite mucins have been shown to play an important role in the early process of *T. cruzi* infection. Accordingly, human galectin-3 binds to a trypomastigote surface mucin (Turner et al. 2002; Moody et al. 2000)and to human coronary artery smooth muscle cells in a lectin-like manner (Kleshchenko et al. 2004) to significantly increase the adhesion of trypomastigotes to human coronary artery smooth muscle cells. Silencing galectin-3 expression in mammalian cells by antisense approach significantly reduces trypomastigote adhesion to cells. Galectin-3 molecules interact with *T. cruzi* 45- (mucin), 32-, and 30-kDa surface proteins on one hand and with laminin on the other, via their carbohydrate recognition domains and are joined together using the R-domains (Moody et al. 2000). In this way, galectin-3 binds to laminin and trypomastigotes to recruit them to the extracellular matrix thus facilitating initial infection. Thus, galectin-3 provides a bridge between parasite and host cell thereby enhancing infection. Some ECM proteins play critical roles in early *T. cruzi* infection and the parasite regulates them to facilitate infection. Interestingly, silencing laminin γ-1 and thrombospondin 1 expression in human cells by stable RNAi significantly reduced *T. cruzi* binding to mammalian cells leading to infection (Nde et al. 2006; Simmons et al. 2006). *T. cruzi* gp83 ligand upregulates the expression of laminin γ-1 to facilitate entry (Nde et al. 2006). Additionally, *T. cruzi* surface antigens bind to laminin (Giordano et al. 1999) and fibronectin (Ouaissi et al. 1986) and have been postulated to participate in the infection process. Thus, the parasite modulates some ECM components and interacts with them to facilitate infection by exploiting these molecules to recruit parasites in the early process of infection.

The completion of the *T. cruzi* genome project highlighted the need to extend the range of techniques available to study gene function of trypanosome attachment and entry. The inability to use RNAi in *T. cruzi* to rapidly study gene function in *T. cruzi* during early infection and pathogenesis poses significant limitations. However, a recent development has been described (Taylor and Kelly 2006) to overcome this problem. The pTcINDEX expression vector for *T. cruzi* (Taylor and Kelly 2006) may facilitate studies of inducible expression of tagged proteins, the generation of conditional knockout trypanosome cell lines, and dominant-negative approaches to validate the role of candidate invasive genes in the process of parasite infection of heart and other cells. Microarray platforms containing the whole human genome and the whole *T. cruzi* genome may facilitate the rapid identification of host and parasite genes involved in the process of early infection and the molecular signature induced by *T. cruzi* in host cells during attachment and entry of cardiac and other cells. Microarray studies have been reported using only partial host and parasite genomes. Silencing cellular host gene expression by RNAi has been an important method to validate candidate host genes implicated in the early process of infection of cardiac and other cells.

T. cruzi induces calcium-triggered recruitment of lysosomes and lysosome fusion with the plasma membrane. This process is dependent on subversion of host cell microtubule dynamics by the parasite (Tyler et al. 2005) as part of as a strategy to form a vacuole, through which they gain entry to the host cells (Burleigh and Andrews 1998; Andrade and Andrews 2005). While lysosome-dependent entry was initially considered to be the primary mechanism by which the parasite gains access to nonprofessional phagocytic cells, it now appears that trypomastigotes can penetrate cells utilizing a PI3K-mediated mechanism which induces invagination of the host cell plasma membrane and is independent of host cell micro-filaments (Woolsey et al. 2003; Burleigh 2005). There are several reviews that deal with host–parasite interaction and cellular response (Burleigh and Andrews 1998; Andrade and Andrews 2005; Woolsey et al. 2003) and molecular analysis of early infection (Villalta et al. 2008).

T. cruzi activates several signal transduction events during entry for its initial establishment in cells (Burleigh and Andrews 1998; Andrade and Andrews 2005; Burleigh 2005). *T. cruzi*

induces calcium mobilization in cardiac myocytes and other cells, mediated by IP3, which is generated upon PLC activation. PI3K, other protein kinases, and phosphatases are also implicated in the mechanisms of *T. cruzi* invasion of phagocytic and nonphagocytic cells (Chuenkova and Pereira 2000; Chuenkova and PereiraPerrin 2004; de Melo-Jorge and PereiraPerrin 2007). The *T. cruzi* gp83 activates the MAPK pathway and PKC to enhance infection of macrophages (Villalta et al. 1998, 1999) and infection of endothelial vascular smooth muscle cells activates the MAPK pathway (Mukherjee et al. 2004, Hassan et al. 2006)

Activation of parasite protein tyrosine phosphorylation is involved in trypomastigote entry into nonphagocytic cells (Favoreto et al. 1998). Trypomastigotes induce tyrosine dephosphorylation of heart myoblast proteins (Favoreto et al. 1998) and the transcription growth factor beta pathway is required for *T. cruzi* invasion of epithelial cells (Ming et al. 1995).

Global gene expression profiling of host responses to *T. cruzi* and to critical surface molecules involved in the initial cellular infection has not been performed in detail. Only partial genome was used for evaluating gene profiling of host responses to *T. cruzi* (Vaena de Avalos et al. 2002; Garg et al. 2003; Mukherjee et al. 2003, 2008). Is the molecular signature caused by early infection the same, similar, or different across various cell types? What are the molecular signatures induced by trans-sialidases, transsialidase-like molecules, mucins, CKII substrate, and other molecules involved in the process of infection of cardiac myocytes and other cells? Since *T. cruzi* infects all human cells except red blood cells, it is important to investigate if the same types of surface receptors are used in all cells or does the parasite use different classes of receptors depending on the host cell type? Research in this area is in infancy. We know very little about the *T. cruzi* genes that may be important in the molecular pathogenesis of cardiac and other cells, contributing to chagasic cardiovascular disease. The recent completion of the *T. cruzi* genome project and the generation of microchip platforms containing the whole *T. cruzi* genome may facilitate these studies. No global gene networking analysis of the parasite and host cells during the invasion process has been achieved. Functional genomics and systems biology of early infection of cardiac and other cells by *T. cruzi* has not been fully explored. Future progress in this area will facilitate the full understanding of the participation of the parasite and the host during early infection. The structural and atomic analysis of validated *T. cruzi* surface ligands and the co-crystallization of these ligands and their validated host receptors that mediate trypanosome attachment leading to entry are required to understand this interaction at the molecular and atomic levels and to develop small inhibitors to block the first steps of infection. High-throughput drug screening based on specific trypanosome target molecules is not fully developed.

The nature of the host–parasite interaction continues to be an area of great interest not only in Chagas disease but in other types of infection. Understanding these interactions may provide novel targets for therapy.

Acknowledgments

This paper was supported in part by NIH grants AI080580, AI007281, T-32-AI070117 and HL007737 (FV), AI-076248, AI-052739, and R21AI068538 (HBT), AI-05893 (HH), American Heart Association Grant SDG 0735252 N (SM), National Health and Research Council of Australia (Biomed CDA 402847; project grant 512154) (AWA), and CNP_q and FAPERJ (JS).

References

Agusti R, Paris G, Ratier L, Frasch AC, de Lederkremer RM. Lactose derivatives are inhibitors of *Trypanosoma cruzi trans*-sialidase activity toward conventional substrates *in vitro* and *in vivo*. Glycobiology 2004;14:659–670. [PubMed: 15070857]

Aliberti J, Viola JP, Vieira-de-Abreu A, Bozza PT, Sher A, Scharfstein J. Cutting edge: bradykinin induces IL-12 production by dendritic cells: a danger signal that drives Th1 polarization. J Immunol 2003;170:5349–5353. [PubMed: 12759407]

Alves MJ, Abuin G, Kuwajima VY, Colli W. Partial inhibition of trypomastigote entry into cultured mammalian cells by monoclonal antibodies against a surface glycoprotein of *Trypanosoma cruzi*. Mol Biochem Parasitol 1986;21:75–82. [PubMed: 3534565]

Andrade LO, Andrews NW. The *Trypanosoma cruzi*–host–cell interplay: location, invasion, retention. Nat Rev Microbiol 2005;3:819–823. [PubMed: 16175174]

Andrade SG, Pimentel AR, de Souza MM, Andrade ZA. Interstitial dendritic cells of the heart harbor *Trypanosoma cruzi* antigens in experimentally infected dogs: importance for the pathogenesis of chagasic myocarditis. Am J Trop Med Hyg 2000;63:64–70. [PubMed: 11357998]

Ashton AW, Ware GM, Kaul DK, Ware JA. Inhibition of tumor necrosis factor-α-mediated NFκB activation and leukocyte adhesion, with enhanced endothelial apoptosis, by G protein-linked receptor (TP) ligands. J Biol Chem 2003;278:11858–11866. [PubMed: 12517920]

Ashton AW, Mukherjee S, Fnu Nagajyothi, Huang H, Braunstein VL, Desruisseaux MS, Factor SM, Lopez L, Berman JW, Wittner M, Scherer PE, Capra V, Coffman TM, Serhan CN, Gotlinger K, Wu KK, Weiss LM, Tanowitz HB. Thromboxane A_2 is a key regulator of pathogenesis during *Trypanosoma cruzi* infection. J Exp Med 2007;204:929–940. [PubMed: 17420269]

Augustine AJS, Kleshchenko YY, Nde PN, Pratap S, Ager EA, Burns JM Jr, Lima MF, Villalta F. Molecular cloning of a *Trypanosoma cruzi* cell surface casein kinase II substrate, Tc-1, involved in cellular infection. Infect Immun 2006;74:3922–3929. [PubMed: 16790765]

Bastos IM, Grellier P, Martins NF, Cadavid-Restrepo G, de Souza-Ault MR, Augustyns K, Teixeira AR, Schrevel J, Maigret B, da Silveira JF, Santana JM. Molecular, functional and structural properties of the prolyl oligopeptidase of *Trypanosoma cruzi* (POP Tc80) that is required for parasite entry into mammalian cells. Biochem J 2005;388:29–38. [PubMed: 15581422]

Belley A, Chadee K. Eicosanoid production by parasites: from pathogenesis to immunomodulation. Parasitol Today 1995;11:327–334. [PubMed: 15275314]

Bhoola KD, Figueroa CD, Worthy K. Bioregulation of kinins: kallikreins, kininogens, and kininases. Pharmacol Rev 1992;44:1–80. [PubMed: 1313585]

Burleigh BA. Host cell signaling and *Trypanosoma cruzi* invasion: do all roads lead to lysosomes? Sci STKE 2005;293:36–40.

Burleigh BA, Andrews NW. A 120-kDa alkaline peptidase from *Trypanosoma cruzi* is involved in the generation of a novel Ca^{2+}-signaling factor for mammalian cells. J Biol Chem 1995;270:5172–5180. [PubMed: 7890627]

Burleigh BA, Andrews NW. Signaling and host cell invasion by *Trypanosoma cruzi*. Curr Opin Microbiol 1998;1:461–465. [PubMed: 10066513]

Buscaglia CA, Campo VA, Frasch AC, Di Noia JM. *Trypanosoma cruzi* surface mucins: host-dependent coat diversity. Nat Rev Microbiol 2006;4:229–236. [PubMed: 16489349]

Buschiazzo A, Amaya MF, Cremona ML, Frasch AC, Alzari PM. The crystal structure and mode of action of *trans*-sialidase, a key enzyme in *Trypanosoma cruzi* pathogenesis. Mol Cell 2002;10:757–768. [PubMed: 12419220]

Campos MA, Gazzinelli RT. Trypanosoma cruzi and its components as exogenous mediators of inflammation recognized through Toll-like receptors. Mediators Inflamm 2004;13:139–143. [PubMed: 15223603]

Cardoni RL, Antunez MI. Circulating levels of cyclooxygenase metabolites in experimental *Trypanosoma cruzi* infections. Mediators Inflamm 2004;13:235–240. [PubMed: 15545053]

Celentano AM, Gorelik G, Solana ME, Sterin-Borda L, Borda E, González Cappa SM. PGE2 involvement in experimental infection with *Trypanosoma cruzi* subpopulations. Prostaglandins 1995;49:141–153. [PubMed: 7652183]

Chuenkova MV, Pereira MA. A trypanosomal protein synergizes with the cytokines ciliary neurotropic factor and leukemia inhibitory factor to prevent apoptosis of neural cells. Mol Biol Cell 2000;11:1487–1498. [PubMed: 10749944]

Chuenkova MV, PereiraPerrin M. Chagas' disease parasite promotes survival and differentiation through TrkA nerve growth factor receptor. J Neurochem 2004;91:385–394. [PubMed: 15447671]

Chuenkova MV, PereiraPerrin M. Enhancement of tyrosine hydroxylase expression and activity by *Trypanosoma cruzi* parasite-derived neurotrophic factor. Brain Res 2006;1099:167–175. [PubMed: 16806115]

Chuenkova MV, Furnari FB, Cavenee WK, Pereira MA. *Trypanosoma cruzi* trans-sialidase: a potent and specific survival factor for human Schwann cells by means of phosphatidylinositol 3-kinase/Akt signaling. Proc Natl Acad Sci U S A 2001;98:9936–9941. [PubMed: 11481434]

Claser C, Curcio M, Mello SM, Silveira EV, Monteiro HP, Rodrigues MM. Silencing cytokeratin 18 gene inhibits intracellular replication of *Trypanosoma cruzi* in Hela cells but not binding and invasion of trypanosomes. BMC Cell Biol 2009;9:68. [PubMed: 19087356]

Croxford JL, Wang K, Miller SD, Engman DM, Tyler KM. Effects of cannabinoid treatment on Chagas disease pathogenesis: balancing inhibition of parasite invasion and immunosuppression. Cell Microbiol 2005;7:1592–1602. [PubMed: 16207246]

Cuevas IC, Cazzulo JJ, Sanchez DO. gp63 homologues in *Trypanosoma cruzi*: surface antigens with metalloprotease activity and a possible role in host cell infection. Infect Immun 2003;71:5739–5749. [PubMed: 14500495]

de Melo-Jorge M, PereiraPerrin M. The Chagas' disease parasite *Trypanosoma cruzi* exploits nerve growth factor receptor TrkA to infect mammalian hosts. Cell Host Microbe 2007;1:251–261. [PubMed: 18005706]

Del Nery E, Juliano MA, Lima AP, Scharfstein J, Juliano L. Kininogenase activity by the major cysteinyl proteinase (cruzipain) from *Trypanosoma cruzi*. J Biol Chem 1997;272:25713–25718. [PubMed: 9325296]

Di Noia JM, Sanchez DO, Frasch AC. The protozoan *Trypanosoma cruzi* has a family of genes resembling the mucin genes of mammalian cells. J Biol Chem 1995;270:24146–24149. [PubMed: 7592617]

Einicker-Lamas M, Nascimento MT, Masuda CA, Oliveira MM, Caruso-Neves C. *Trypanosoma cruzi* epimastigotes: regulation of myo-inositol transport by effectors of protein kinases A and C. Exp Parasitol 2007;117:171–177. [PubMed: 17586497]

Engel JC, Doyle PS, Hsieh I, McKerrow JH. Cysteine protease inhibitors cure an experimental *Trypanosoma cruzi* infection. J Exp Med 1998;188:725–734. [PubMed: 9705954]

Factor SM, Cho S, Wittner M, Tanowitz HB. Abnormalities of the coronary microcirculation in acute murine Chagas' disease. Am J Trop Med Hyg 1985;34:246–253. [PubMed: 3985268]

Favoreto S Jr, Dorta ML, Yoshida N. *Trypanosoma cruzi* 175 kDa protein tyrosine phosphorylation is associated with host cell invasion. Exp Parasitol 1998;89:188–194. [PubMed: 9635442]

Frasch AC. Functional diversity in the *trans*-sialidase and mucin families in *Trypanosoma cruzi*. Parasitol Today 2000;16:282–286. [PubMed: 10858646]

Garg N, Postan M, Mensa-Wilmot K, Tarleton RL. Glycosylphosphatidylinositols are required for the development of *Trypanosoma cruzi* amastigotes. Infect Immun 1997;65:4055–4066. [PubMed: 9317007]

Garg N, Popov VL, Papaconstantinou J. Profiling gene transcription reveals a deficiency of mitochondrial oxidative phosphorylation in *Trypanosoma cruzi*-infected murine hearts: implications in chagasic myocarditis development. Biochim Biophys Acta 2003;1638:106–120. [PubMed: 12853116]

Giordano R, Fouts DL, Tewari D, Colli W, Manning JE, Alves MJ. Cloning of a surface membrane glycoprotein specific for the infective form of *Trypanosoma cruzi* having adhesive properties to laminin. J Biol Chem 1999;274:3461–3468. [PubMed: 9920891]

Grellier P, Vendeville S, Joyeau R, Bastos IM, Drobecq H, Frappier F, Teixeira AR, Schrevel J, Davioud-Charvet E, Sergheraert C, Santana JM. *Trypanosoma cruzi* prolyl oligopeptidase Tc80 is involved in nonphagocytic mammalian cell invasion by trypomastigotes. J Biol Chem 2001;276:47078–47080. [PubMed: 11598112]

Hassan GS, Mukherjee S, Nagajyothi F, Weiss LM, Petkova SB, de Almeida CJ, Huang H, Desruisseaux MS, Bouzahzah B, Pestell RG, Albanese C, Christ GJ, Lisanti MP, Tanowitz HB. *Trypanosoma*

cruzi infection induces proliferation of vascular smooth muscle cells. Infect Immun 2006;74:152–159. [PubMed: 16368968]

Hideko Tatakihara VL, Cecchini R, Borges CL, Malvezi AD, Graçade Souza VK, Yamada-Ogatta SF, Rizzo LV, Pinge-Filho P. Effects of cyclooxygenase inhibitors on parasite burden, anemia and oxidative stress in murine *Trypanosoma cruzi* infection. FEMS Immunol Med Microbiol 2008;52:47–58. [PubMed: 18031539]

Kabashima K, Murata T, Tanaka H, Matsuoka T, Sakata D, Yoshida N, Katagiri K, Kinashi T, Tanaka T, Miyasaka M, Nagai H, Ushikubi F, Narumiya S. Thromboxane A_2 modulates interaction of dendritic cells and T cells and regulates acquired immunity. Nat Immunol 2003;4:694–701. [PubMed: 12778172]

Kleshchenko YY, Moody TN, Furtak FA, Ochieng J, Lima MF, Villalta F. Human galectin-3 promotes *Trypanosoma cruzi* adhesion to human coronary artery smooth muscle cells. Infect Immun 2004;72:6717–6721. [PubMed: 15501810]

Kubata BK, Duszenko M, Kabututu Z, Rawer M, Szallies A, Fujimori K, Inui T, Nozaki T, Yamashita K, Horii T, Urade Y, Hayaishi O. Identification of a novel prostaglandin $F_{2\alpha}$ synthase in *Trypanosoma brucei*. J Exp Med 2000;192:1327–1338. [PubMed: 11067881]

Leal AL, Pantaleao TU, et al. Hypothyroidism and hyperthyroidism modulates Ras-MAPK intracellular pathway in rat thyroids. Endocrine 2007;31:174–178. [PubMed: 17873330]

Leeb-Lundberg F, Marceau F, Muller-Esterl W, Pettibone DJ, Zuraw BL. Classification of the kinin receptor family: structure, function and regulation. Pharmacol Rev 2005;2005(57):27–77. [PubMed: 15734727]

Lima MF, Villalta F. Host-cell attachment by *Trypanosoma cruzi*: identification of an adhesion molecule. Biochem Biophys Res Commun 1988;155:256–262. [PubMed: 3046614]

Lima MF, Villalta F. *Trypanosoma cruzi* trypomastigote clones differentially express a cell adhesion molecule. Mol Biochem Parasitol 1989;33:159–170. [PubMed: 2657421]

Lima AP, Almeida PC, Tersariol IL, Schmitz V, Schmaier AH, Juliano L, Hirata IY, Müller-Esterl W, Chagas JR, Scharfstein J. Heparan sulfate modulates kinin release by *Trypanosoma cruzi* through the activity of cruzipain. J Bio Chem 2002;277:5875–5881. [PubMed: 11726662]

Liu LX, Weller PF. Arachidonic acid metabolism in filarial parasites. Exp Parasitol 1990;71:496–501. [PubMed: 2226710]

Magdesian MH, Giordano R, Ulrich H, Juliano MA, Juliano L, Schumacher RI, Colli W, Alves MJ. Infection by *Trypanosoma cruzi*: identification of a parasite ligand and its host-cell receptor. J Biol Chem 2001;276:19382–19389. [PubMed: 11278913]

Manning-Cela R, Cortes A, Gonzales-Rey E, Van Voorhis WC, Swindle J, Gonzales A. LYT1 protein is required for efficient in vitro infection by *Trypanosoma cruzi*. Infect Immun 2001;69:3916–23. [PubMed: 11349059]

Marceau F, Bachvarov DR. Kinin receptors. Clin Rev Allergy Immunol 1998;6:385–401. [PubMed: 9926287]

McGrath ME, Eakin AE, Engel JC, McKerrow JH, Craik CS, Fletterick RJ. The crystal structure of cruzain: a therapeutic target for Chagas' disease. J Mol Biol 1995;247:251–259. [PubMed: 7707373]

Meirelles MN, Juliano L, Carmona E, Silva SG, Costa EM, Murta AC, Scharfstein J. Inhibitors of the major cysteinyl proteinase (GP57/51) impair host cell invasion and arrest the intracellular development of *Trypanosoma cruzi* in vitro. Mol Biochem Parasitol 1992;52:175–184. [PubMed: 1620157]

Ming M, Ewen ME, Pereira ME. Trypanosome invasion of mammalian cells requires activation of the TGF-β signaling pathway. Cell 1995;82:287–296. [PubMed: 7628017]

Monteiro AC, Schmitz V, Svensjo E, Gazzinelli RT, Almeida IC, Todorov A, de Arruda LB, Torrecilhas AC, Pesquero JB, Morrot A, Bouskela E, Bonomo A, Lima AP, Müller-Esterl W, Scharfstein J. Cooperative activation of TLR2 and bradykinin B_2 receptor is required for induction of type 1 immunity in a mouse model of subcutaneous infection by *Trypanosoma cruzi*. J Immunol 2006;177:6325–6335. [PubMed: 17056563]

Monteiro AC, Schmitz V, Morrot A, de Arruda LB, Nagajyothi F, Granato A, Pesquero JB, Müller-Esterl W, Tanowitz HB, Scharfstein J. Bradykinin B2 Receptors of dendritic cells, acting as sensors of

kinins proteolytically released by *Trypanosoma cruzi*, are critical for the development of protective type-1 responses. PLoS Pathog 2007;11:1730–17344.

Moody T, Ochieng J, Villalta F. Novel mechanism that *Trypanosoma cruzi* uses to adhere to the extracellular matrix-mediated by human galectin-3. FEBS Lett 2000;470:305–308. [PubMed: 10745086]

Mukherjee S, Belbin TJ, Spray DC, Iacobas DA, Weiss LM, Kitsis RN, Wittner M, Jelicks LA, Scherer PE, Ding A, Tanowitz HB. Microarray analysis of changes in gene expression in a murine model of chronic chagasic cardiomyopathy. Parasitol Res 2003;91:187–196. [PubMed: 12910413]

Mukherjee S, Huang H, Petkova SB, Albanese C, Pestell RG, Braunstein VL, Christ GJ, Wittner M, Lisanti MP, Berman JW, Weiss LM, Tanowitz HB. *Trypanosoma cruzi* infection activates extracellular signal-regulated kinase in cultured endothelial and smooth muscle cells. Infect Immun 2004;72:5274–5282. [PubMed: 15322023]

Mukherjee S, Nagajyothi F, Mukhopadhyay A, Machado FS, Belbin TJ, Campos de Carvalho A, Guan F, Albanese C, Jelicks LA, Lisanti MP, Silva JS, Spray DC, Weiss LM, Tanowitz HB. Alterations in myocardial gene expression associated with experimental *Trypanosoma cruzi* infection. Genomics 2008;91:423–432. [PubMed: 18343633]

Nde PN, Simmons K, Kleschchenko YY, Pratap S, Lima MF, Villalta F. Silencing of laminin γ-1 blocks *Trypanosoma cruzi* infection. Infect Immun 2006;74:1643–1648. [PubMed: 16495535]

Ortega-Barria E, Pereira ME. A novel *Trypanosoma cruzi* heparin binding protein promotes fibroblast adhesion and penetration on engineered bacteria and trypanosomes into mammalian cells. Cell 1991;67:411–421. [PubMed: 1655283]

Ouaissi MA, Cornette J, Capron A. Identification and isolation of *Trypanosoma cruzi* trypomastigote cell surface protein with properties expected of a fibronectin receptor. Mol Biochem Parasitol 1986;19:201–211. [PubMed: 3526144]

Pereira PJ, Vega MC, Gonzales-Rey E, Fernandez-Carazo R, Macedo-Ribeiro S, Gomis-Ruth FX, Gonzales A, Coll M. *Trypanosoma cruzi* macrophage infectivity potentiator has a rotamase core and a highly exposed α-helix. EMBO Reports 2002;3:88–94. [PubMed: 11751578]

Petersen CA, Krumholz KA, Burleigh BA. Toll-like receptor 2 regulates interleukin-1 beta-dependent cardiomyocyte hypertrophy triggered by *Trypanosoma cruzi*. Infect Immun 2005;73:6974–6980. [PubMed: 16177377]

Petkova SB, Tanowitz HB, Magazine HI, Factor SM, Chan J, Pestell RG, Bouzahzah B, Douglas SA, Shtutin V, Morris SA, Tsang E, Weiss LM, Christ GJ, Wittner M, Huang H. Myocardial expression of endothelin-1 in murine *Trypanosoma cruzi* infection. Cardiovasc Pathol 2000;9:257–265. [PubMed: 11064272]

Petkova SB, Huang H, Factor SM, Pestell RG, Bouzahzah B, Jelicks LA, Douglas Weiss LM, SA Wittner M, Tanowitz HB. The role of endothelin in the pathogenesis of Chagas' disease. Int J Parasitol 2001;31:499–511. [PubMed: 11334935]

Previato JO, Andrade AF, Pessolani MC, Mendoca-Previato L. Incorporation of sialic acid into *Trypanosoma cruzi* macromolecules. A proposal for a new metabolic route. Mol Biochem Parasitol 1985;16:85–96. [PubMed: 2412116]

Scharfstein J, Schmitz V, Morandi V, Capella MM, Lima AP, Morrot A, Juliano L, Müller-Esterl W. Host cell invasion by *Trypanosoma cruzi* is potentiated by activation of bradykinin B2 receptors. J Exp Med 2000;192:1289–1299. [PubMed: 11067878]

Scharfstein J, Schmitz V, Svensjö E, Granato A, Monteiro AC. Kininogens coordinate adaptive immunity through the proteolytic release of bradykinin, an endogenous danger signal driving dendritic cell maturation. Scand J Immunol 2007;66:128–136. [PubMed: 17635790]

Schenkman S, Jiang MS, Hart GW, Nussenzweig V. A novel cell surface trans-sialidase of *Trypanosoma cruzi* generates a stage-specific epitope required for invasion of mammalian cells. Cell 1991;65:1117–1125. [PubMed: 1712251]

Schmitz V, Svenso E, Serra RR, Teixeira MM, Scharfstein J. Proteolytic generation of kinns in tissues infected by *Trypanosoma cruzi* depends on CXC-chemokine secretion by macrophages activated via Toll-like 2 receptors. J Leuk Bio. 2009(in press)

Silber AM, Marcipar IS, Roodveldt C, Cabeza Meckert P, Laguens R, Marcipar AJ. *Trypanosoma cruzi*: identification of a galactose-binding protein that binds to cell surface of human erythrocytes

and is involved in cell invasion by the parasite. Exp Parasitol 2002;100:217–225. [PubMed: 12128048]

Simmons KJ, Nde PN, Kleshchenko YY, Lima MF, Villalta F. Stable RNA interference of host thrombospondin-1 blocks *Trypanosoma cruzi* infection. FEBS Lett 2006;580:2365–2370. [PubMed: 16616140]

Skidgel RA, Erdos EG. Angiotensin converting enzyme (ACE) and neprilysin hydrolyze neuropeptides: a brief history, the beginning and follow-ups to early studies. Peptides 2004;25:521–525. [PubMed: 15134871]

Sterin-Borda L, Gorelik G, Goren N, Cappa SG, Celentano AM, Borda E. Lymphocyte muscarinic cholinergic activity and PGE2 involvement in experimental *Trypanosoma cruzi* infection. Clin Immunol Immunopathol 1996;81:122–128. [PubMed: 8906742]

Tanowitz HB, Burns ER, Sinha AK, Kahn NN, Morris SA, Factor SM, Hatcher VB, Bilezikian JP, Wittner M. Enhanced platelet adherence and aggregation in Chagas' disease: a potential pathogenic mechanism for cardiomyopathy. Am J Trop Med Hyg 1990;43:274–281. [PubMed: 2121055]

Tanowitz HB, Kirchhoff LV, Simon D, Morris SA, Weiss LM, Wittner M. Chagas' disease. Clin Microbiol Rev 1992;4:400–419. [PubMed: 1423218]

Tanowitz HB, Kaul DK, Chen B, Morris SA, Factor SM, Weiss LM, Wittner M. Compromised microcirculation in acute *Trypanosoma cruzi* infection. J Parasit 1996;82:124–130. [PubMed: 8627481]

Tanowitz HB, Huang H, Jelicks LA, Chandra M, Loredo ML, Weiss LM, Factor SM, Shtutin V, Mukherjee S, Kitsis RN, Christ GJ, Wittner M, Shirani J, Kisanuki YY, Yanagisawa M. Role of endothelin 1 in the pathogenesis of chronic chagasic heart disease. Infect Immun 2005;73:2496–503. [PubMed: 15784596]

Taylor MC, Kelly JM. pTcINDEX: a stable tetracycline-regulated expression vector for *Trypanosoma cruzi*. BMC Biotechnol 2006;6:32. [PubMed: 16824206]

Todorov AG, Andrade D, Pesquero JB, Araujo RC, Bader M, Stewart J, Gera L, Müller-Esterl W, Morandi V, Goldenberg RC, Neto HC, Scharfstein J. *Trypanosoma cruzi* induces edematogenic responses in mice and invades cardiomyocytes and endothelial cells in vitro by activating distinct kinin receptor (B1/B2) subtypes. FASEB J 2003;17:73–75. [PubMed: 12424228]

Turner CW, Lima MF, Villalta F. *Trypanosoma cruzi* uses a 45 kDa mucin for adhesion to mammalian cells. Biochem Biophys Res Commun 2002;290:29–34. [PubMed: 11779128]

Tyler KM, Murphy SC, Engman DM. Recruitment of host tubulins to the parasite synapse and parasitophorous vacuole during *Trypanosoma cruzi* invasion. Cell Microbiol 2005;7:1579–1591. [PubMed: 16207245]

Vaena de Avalos S, Blader IJ, Fisher M, Boothroyd JC, Burleigh BA. Immediate/early response to *Trypanosoma cruzi* infection involves minimal modulation of host cell transcription. J Biol Chem 2002;277:639–644. [PubMed: 11668183]

Vaidian AK, Weiss LM, Tanowitz HB. Chagas' disease and AIDS. Kinetoplastid Biol Dis 2004;3(1):2. [PubMed: 15142278]

Villalta F, Kierszenbaum F. Host cell invasion by *Trypanosoma cruzi*: role of cell surface galactose residues. Biochem Biophys Res Commun 1984;119:228–235. [PubMed: 6322778]

Villalta F, Kierszenbaum F. Role of surface N-acetyl-glucosamine residues on host cell infection by *Trypanosoma cruzi*. Biochim Biophys Acta 1985;845:216–222. [PubMed: 3888284]

Villalta F, Lima MF. Purification of *Trypanosoma cruzi* surface proteins involved in adhesion to host cells. Biochem Biophys Res Commun 1990;172:925–931. [PubMed: 2241980]

Villalta F, Lima MF, Zhou L, Ruiz-Ruano A. Attachment of *Trypanosoma cruzi* to host cells: a monoclonal antibody recognizes a trypomastigote stage-specific epitope on the gp 83 required for parasite attachment. Biochem Biophys Res Commun 1992;182:6–13. [PubMed: 1370617]

Villalta F, Ruiz-Ruano A, Valentine AA, Lima MF. Purification of a surface 74 kDa glycoprotein from heart myoblasts that inhibits the binding and entry of *Trypanosoma cruzi* into heart cells. Mol Biochem Parasitol 1993;61:217–230. [PubMed: 8264726]

Villalta F, Smith CM, Burns JM Jr, Chaudhuri G, Lima MF. Fab fragments of a mAb to a member of the family 2 of trans-sialidases of *Trypanosoma cruzi* block trypanosome invasion of host cells and

neutralize infection by passive immunization. Ann N Y Acad Sc 1996;97:242–245. [PubMed: 8993367]

Villalta F, Zhang Y, Bibb KE, Burns JM Jr, Lima MF. Signal transduction in human macrophages by gp83 ligand of *Trypanosoma cruzi*: trypomastigote gp83 ligand up regulates trypanosome entry through the MAP kinase pathway. Biochem Biophys Res Commun 1998;249:247–252. [PubMed: 9705866]

Villalta F, Zhang Y, Bibb KE, Pratap S, Burns JM Jr, Lima MF. Signal transduction in human macrophages by gp83 ligand of *Trypanosoma cruzi*: trypomastigote gp83 ligand up-regulates trypanosome entry through protein kinase C activation. Mol Cell Biol Res Commun 1999;2:64–70. [PubMed: 10527894]

Villalta F, Smith CM, Ruiz-Ruano A, Lima MF. A ligand that *Trypanosoma cruzi* uses to bind to mammalian cells to initiate infection. FEBS Lett 2001;21:384–388.

Villalta F, Madison NM, Kleshchenko Y, Nde PN, Lima MF. Molecular analysis of early host cell infection by *Trypanosoma cruzi*. Front Biosci 2008;13:3714–3734. [PubMed: 18508467]

Woolsey AM, Sunwoo L, Petersen CA, Brachmann SM, Cantley LC, Burleigh BA. Novel PI 3-kinase-dependent mechanisms of trypanosome invasion and vacuole maturation. J Cell Sci 2003;116:3611–3622. [PubMed: 12876217]

Yoshida N, Mortata RA, Araguth MF, Gonzales JC, Russo M. Metacyclic neutralizing effect of monoclonal antibody 10D8 directed to the 35- and 50-kilodalton surface glycoconjugates of *Trypanosoma cruzi*. Infect Immun 1989;57:1663–1667. [PubMed: 2656520]