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Molecular Mechanisms of Host Cell Invasion by *Trypanosoma cruzi*

Conrad L. Epting[a,*], **Bria M. Coatesa**[a], and **David M. Engmanb**[b]

[a] Department of Pediatrics, Northwestern University, Chicago, Illinois, United States of America

[b] Departments of Pathology and Microbiology-Immunology, Northwestern University, Chicago, Illinois, United States of America

Abstract

The protozoan parasite *Trypanosoma cruzi*, the etiologic agent of Chagas disease, is an obligate intracellular protozoan pathogen. Overlapping mechanisms ensure successful infection, yet the relationship between these cellular events and clinical disease remains obscure. This review explores the process of cell invasion from the perspective of cell surface interactions, intracellular signaling, modulation of the host cytoskeleton and endosomal compartment, and the intracellular innate immune response to infection.

Keywords

Trypanosoma cruzi; invasion; lysosome; microtubules; signaling

1. Introduction

The protozoan parasite *Trypanosoma cruzi* is the etiologic agent of Chagas disease, a disorder of poverty endemic to Central and South America. 10-16 million people are chronically infected with *T. cruzi*, with widely variable clinical sequelae, ranging from no disease (the majority) to an inflammatory cardiomyopathy and dilatation of the enteric viscera from denervation injury [1-3]. Chagas disease is emerging in North America in animals and humans, likely from the economic migration of infected individuals and extending range of the insect vector [4, 5]. The early diagnosis and treatment of Chagas disease remains a challenge for resource-poor nations, with the acute phase often passing undetected, and therapy during the chronic phase being largely supportive rather than curative [6, 7].

Typically, *T. cruzi* infection occurs when parasites excreted by the triatomine insect vector contaminate the bite wound or a mucous membrane. In non-endemic areas, transmission may occur congenitally, via blood transfusion or organ transplantation, or as a result of a laboratory accident [8]. Despite a century of scientific study, the relationship between the cell biology of the host-parasite relationship and the pathophysiology of Chagas disease

* Corresponding Author. Address: Conrad L. Epting, Northwestern University, Department of Pediatrics, 320 Superior Street, Searle 4-409, Chicago, Illinois 60611, United States of America, 312-503-4784 tel, 312-503-1181 fax c-epting@northwestern.edu.

remains incompletely understood. This review will explore the process of cell invasion with a focus on known cell surface interactions, review the evidence surrounding tissue tropism, explore the intracellular response to infection, and highlight several experimental unknowns and challenges.

T. cruzi has developed complex and redundant mechanisms to ensure successful cell invasion. We will examine the common features that underlie critical events involved in host cell infection by the parasite, with a focus on the trypomastigote. Considerable heterogeneity exists at each step of this process, and the specifics may vary with each unique combination of parasite strain, stage, and host cell. Therefore, the reader is cautioned not to apply a reductionistic viewpoint broadly. Further, the outcome of *T. cruzi* infection is highly heterogeneous across cell types. Aspects of cell invasion which vary across cell types include surface-surface interactions, enzymatic events, calcium-mediated signaling, trafficking of donor and host membranes, cytoskeletal contributions to parasite uptake and, finally, cytoplasmic entry via escape from the parasitophorous vacuole. Many excellent reviews from leaders in the field have been published on the mechanistic aspects underlying cell invasion [9-14].

2. Tissue Tropism

Infective metacyclic trypomastigotes inoculated into a wound generally infect local macrophages, fibroblasts, and other mesenchymal tissues at the site of primary infection, followed by hematogenous dissemination and stable infection of distant tissues [15] (**Figure 1**). Although the parasite is capable of infecting nearly any nucleated cells *in vitro*, a restricted tissue pool, involving cardiac and skeletal muscle and enteric nerves, develop apparent pathology [16]. It is tempting to conclude that the parasite has intrinsic tissue tropism and, indeed, this idea was initially established in classic work from Melo and Brener [17]. Chagas disease demonstrates geographically-restricted clinical profiles [18], lending support to the notion of strain-dependent tissue-specific tropism, and genetically distinct strains and clones can be isolated from patients with primary cardiac or gastrointestinal disease [19]. Further support for tissue tropism comes from the results of experimental infection employing two isolates of the parasite, in which one strain was found to preferentially localize to the heart, and the other to the gastrointestinal tract [20]. However, the rich genetic variation in parasite population clearly contributes to disease outcome [21], as does the host genetic background [22, 23]. A clear molecular or immunologic explanation for apparent tissue tropism is lacking and, at best, the hallmarks of clinical disease appear to result from a complex interplay between parasite and host genetic variation, inflammation, and immunity.

Interestingly, and somewhat surprisingly, given the extensive literature on interactions between host leukocytes and vascular endothelium, the method of parasite egress from the bloodstream into the tissues remains to be established. The parasite is capable of directly infecting the endothelium, and cardiac-specific studies of established Chagas heart disease demonstrate endothelial injury, inflammation, and microcirculatory compromise [24, 25]. The time-course for tissue dissemination is 7-10 days following inoculation [15], yet it is has not been established that parasites must first infect and lyse the vascular endothelium prior to spreading in the surrounding tissues. Alternatively, the parasite could engage in regulated transmigration/diapedesis, stimulate a cytotoxic or inflammatory injury resulting in breach of the permeability barrier, enter and exit the endothelial cell without establishing an infection, or escape via infected inflammatory cells acting as a Trojan horse. The rich complement of surface proteases suggest that enzymatic digestion between the endothelial cell and into the underlying connective tissues is likely a direct, parasite-driven processes. Indeed surface proteases, notably cruzipain [26, 27], play an important role in cellular

infection, and almost certainly are fundamental to permit passage through the intact endothelium as well as the extracellular matrix. It has also been recognized that surface residue modifications through trans-sialidase also contribute to endothelial cell interactions [28]. Further studies are needed to specifically address this fundamental step in parasite dissemination through escape from the vascular compartment.

3. Cell Invasion

Interactions with host cells and the extracellular matrix occur through a large and diverse group of surface glycoproteins and proteases. Since the pioneering work of Dvorak and Hyde [29, 30], researchers have gained tremendous insight into the specific molecules involved during initial cell-cell interactions. Interestingly, many of the glycoproteins share the glycosylphosphatidylinositol (GPI) moiety. GPI-anchored proteins are first synthesized in the ER, conjugated to a GPI-anchor, and attached to the membrane as luminal facing proteins in the endoplasmic reticulum. In the Golgi, they undergo extensive sugar and side-chain modifications, and then fuse with the plasma membrane resulting in extracellular membrane-associated proteins [31, 32]. The structures and functions of these proteins are incredibly diverse, from adhesion, paracrine signaling, surface enzymes, and cell differentiation [33-35]. The GPI anchor confers several additional properties. First, enzymatic cleavage via glycosylphosphatidylinositol-specific phospholipase C (GPI-PLC) can release the head group, and is implicated in lipid and paracrine signaling, as well as signal termination [36, 37]. Additionally, GPI anchored proteins are thought to ubiquitously associate with, and in fact may help define, the lipid raft microdomain compartment [38] in other eukaryotic systems. Trypanosomes were recognized early as cells with abundant expression of GPI-anchored proteins [39], and these proteins form the classic VSG coat critical to immune evasion by *T. brucei* [40, 41]. Many GPI-anchored proteins of *T. cruzi* are involved in both the host response and macrophage infection, as reviewed in [42]. Given the differences in synthesis and side-chain modifications, the responsible enzymes are potential drug targets in the mammalian host.

The mechanisms and route of cell invasion vary greatly with the host cell type, and the reader is cautioned against broad generalizations across cell types. Unlike some infectious agents that rely on uptake and escape from professional phagocytic cells, *T. cruzi* trypomastigotes are capable of directly invading both professional phagocytes and nonphagocytic cells. Among the professional phagocytes, tissue resident macrophages are critical targets for early infection [43], where they initiate both a robust innate immunity and the systemic anti-parasite inflammatory response through epitope processing and presentation. Professional phagocytes have long been recognized both as necessary cellular targets and as a defense mechanisms for the host. Macrophages form the backbone of the infection models exploring the professional phagocytes-parasite interaction. These cells successfully harbor infection [44] yet limit their own infection [45], likely through oxidative burst-dependent killing [46], thus serving as important parasite reservoirs. Trypomastigotes specifically induce their uptake by professional phagocytes by engaging both TLR2 [47] and TLR 9-dependent pathways. The cellular mechanisms of phagocytosis have been well studied, and several reviews are suggested [48-51].

For infection of non-phagocytic cells, at least two major pathways have been characterized. The first relies upon a calcium-mediated signaling at the surface for lysosomal trafficking to provide donor membranes for the vacuole in a manner dependent upon actin polymerization and microtubules [52-55], while the second is a plasma membrane-mediated invagination involving PI_3 kinase signaling and independent of actin polymerization [56-59]. While these observations form a core understanding of cell invasion, significant diversity, complexity, and redundancy in the process have emerged over the past two decades.

The capacity for cell invasion is not restricted to metacyclic or cell-derived trypomastigotes. Both the dividing amastigotes [60] and insect stage epimastigotes [61] are fully capable of establishing infections, and amastigotes are increasingly recognized to share comparable infectivity to trypomastigotes. Amastigote cell entry may follow more stereotyped pattern of cell invasion than the relatively diverse patterns noted between trypomastigotes and their targets. Unlike trypomastigotes, amastigotes invade in a Ca^{2+}-dependnet manner insensitive to PI_3 inhibition but involving both cAMP signaling and Ca^{2+}-release from parasite acidocalcisomes. This has been modeled in HeLa cells [62] and MDCK cells, where infection with G-strain amastigotes demonstrate a role for Rac1-mediated cell invasion [63]. While this review will largely focus on pathways established in trypomastigotes, do not discount the important role for infective amastigotes in propagating the local spread of infection within tissues of the parasitized host.

4. Surface Interactions

At the outset, parasites must survive, gain access to the cell surface, and form stable attachments to host cells prior to entry. A cadre of protease-resistant surface glycoproteins either attach to matrix components, bind cell surface receptors, or possess proteolytic activity against matrix components. Many of these surface molecules serve as adhesion anchors, some enabling matrix destruction or ligand cleavage, others help with immune evasion, and others initiate bidirectional signaling events in the parasite and host cell. Nearly 50 percent of the *T. cruzi* genome is dedicated to encoding these surface proteins, broadly divided into several families: the gp63 surface proteases, the gp85/trans-sialidase superfamily (TS), the mucins, and the mucin-associated surface proteins [9, 64, 65]. A few of the prominent surface glycoproteins and, if known, their ligands, together with selected (not comprehensive) references are shown (**Table 1**). A seminal example is the role of sialic acid in parasite virulence [66]. Surface mucins, a subset of the GPI-linked proteins, are modified by sialic acid scavenged from the host through the action of the trans-sialidase [67], as the parasite lacks the ability to produce these modifications directly. These sugar-modified residues, have been demonstrated to have critical roles in cell attachment, invasion, and replication [68]. Other surface sugar residues upon glycoproteins, notably mannose and galactose, also figure prominently in the interaction and infection of host cells [69-71]. Many of the surface glycoproteins impact invasion or serve as virulence factors, since deletion, disruption of their enzymatic activity, or blockade of the receptor-ligand interaction, usually reduces cell invasion *in vitro* and improves outcome of infection *in vivo*. The extent and diversity of these surface protein families cannot be overemphasized. Despite decades of study, a unified single invasion mechanism has not emerged. Rather, a series of redundant and overlapping mechanisms, varying with the parasite-host strain-strain combination, have been reported [9]. This diversity likely contributes to the co-evolutionary success of this parasite in the infection of vertebrate cells and tissues.

The importance of the plasma membrane lipid environment is rapidly gaining attention. Specialized regions, the lipid microdomains/rafts, coordinate and regulate signaling events through temporal-spatial organization of proteins. The kinetoplastids are no exception, and GPI-anchored proteins are known to cluster in lipid rafts in this family [72]. The host-parasite signaling event likely depends upon surface-surface events coordinated through lipid rafts, and indeed, cholesterol scavengers, which impair membrane fluidity and raft lateral reorganization, also impair cell invasion [73, 74].

5. Intracellular Signaling and Calcium

The end result of host-parasite surface interaction is triggering of bidirectional (host and parasite) signaling cascades which initiate the invasion event. After extracellular matrix

proteolysis and surface binding through robust and redundant mechanisms, the parasite initiates a bidirectional calcium signaling cascade. This event can also be trigged in a cell-free system by isolated membrane components or parasite lysate [52, 75]. This calcium signaling is fundamental to the downstream signaling cascade, which ends with the parasite encased in an acidic parasitophorous vacuole [13, 76]. Some of the major signaling events downstream of these surface receptors in the host and parasite trypomastigotes are tabulated (**Table 2**).

The precise molecular mechanisms leading to host and parasite intracellular calcium release remain unknown. In the parasite, at least two pathways have been identified. In metacyclic trypomastigotes, the engagement of gp82 with an unknown ligand triggers a cascade in the parasite involving tyrosine phosphorylation of p175 [77], the serine protein kinase C, and IP_3-medated release of ER calcium stores [78]. An alternative, overlapping pathway occurs upon gp30 activation [79]. Another major pathway, mediated by gp35/50 binding to an unknown ligand, induces calcium release from acidocalcisomes through adenylate cyclase and a rise in cAMP [11, 13], a pathway shared during amastigote invasion. The protein tyrosine phosphatase gp90 is a negative regulator of invasion [14, 80-82]. Additionally, TGF-β and integrin signaling on host cells have been implicated in the invasion process, as have toll-like receptors (TLR2 and 9) [83-85], and the nerve growth factor receptor TrkA has been identified to bind to a trans-sialidase [14]. Signaling in the host cell is even less well characterized. The generation of kinins by cruzipain results in bradykinin receptor (B_2R)-mediated signaling through PLC and IP_3-kinase to release ER-bound calcium, opposed by the actions of the kininases (angiotensin converting enzyme-ACE) [14, 86, 87]. Evidence suggests that the anti-inflammatory properties of ACE inhibition is useful to modulate cardiac inflammation in Chagas [87, 88], as it does in models of experimental autoimmune myocarditis [89]. Surface signaling through other bradykinin receptors (B_1R) by the actions of kininase I, support invasion [90], and the action of oligopeptidase B on its substrate is thought to generate an agonist for host cell calcium release through adenylate cyclase and phospholipase C [91]. Additional receptors are proposed for ligand interactions with TS/Tc85, as well as additional substrates for cruzipain and chagasin, which interface with downstream signaling in both the lysosome-dependent and independent pathways. Scharfstein and Lima recently published a detailed review on the subject of the cysteine proteases, cruzipain, and protease inhibitors [92]. MAPK pathways have also been implicated in macrophages through gp83 signaling [36]. Alternative pathways for amastigote involving calcium release from acidocalcisomes in a PI_3 insensitive manner have already been described above [62].

6. Host Membranes and the Parasite Vacuole

The classic model for parasite entry is based upon the rapid recruitment of lysosomes to the parasite attachment point [93] in a manner dependent upon microtubules and kinesin motors [94]. Host cell plasma membrane and lysosomes have been assumed to be the donor membranes necessary for vacuole formation, and inhibition of membrane fusion, vesicle trafficking, microtubule reorganization, molecular motors, or calcium/cAMP signaling impairs successful invasion. This vesicle-dependent pathway has been shown to be sensitive to wortmannin, a PI_3 kinase inhibitor, known to involve G-protein coupled receptors, and depend upon synaptotagmin-VII [56, 95, 96]. The precise characterization and sources of these donor membranes have become more diverse with further investigation, including early and late endosomes [56, 97], involvement of dynamin and Rab5 [97, 98], and, recently, the autophagocytic pathway [99]. Localized alterations in calcium concentration are known signals for both microtubule-dependent lysosomal trafficking and fusion [100, 101]. More recently, this classic pathway was usurped by a dominant alternative, a direct invagination of the plasma membrane at the site of attachment in a wortmannin-insensitive

and lysosome-independent [102] manner. However, lysosomal fusion is thought to remain fundamental for a productive infection to occur through vacuole acidification [102], and thus these diverse results represent further insight into the components of the maturing parasitophorous vacuole. While the precise molecular events that lead to successful invasion have yet to be elucidated, the overarching theme is one of parasite entry through surface-initiated signaling leading to a bi-directional rise in intracellular calcium, causing reorganization, trafficking, and fusion of selected donor membranes along the host cytoskeleton to the site of membrane attachment and invagination.

7. Host Cytoskeleton

The host cytoskeleton is critical for successful invasion. Host cells are encased in an actin corset parallel to the inner membrane. Calcium-mediated actin de-polymerization likely facilitates initial parasite entry and negatively impacts parasite retention [53, 56, 103]. The specific role of actin polymerization appears to depend on the specific cell type and parasite stage examined, with cytochalasin D treatment enhancing trypomastigote, yet impairing amastigote, invasion [104]. A host of actin-associated elements has been identified, including intermediate filaments, myosin-associated components, integrins, and extracellular matrix components, as noted in a recent review [105]. The Rho/Rac family of small GTPases is known to be a critical link between surface signaling and changes in the underlying cytoskeleton. However, evidence suggests that trypomastigotes do not rely on this family for productive infection. In contrast, the invasion mechanism employed by amastigotes does depend upon Rac1 signaling, again highlighting the diversity of cell invasion [63, 106]. Members of the Rab family of GTPases, necessary for endosomal compartment trafficking, are essential for infection [97]. The many components of the endosomal compartment (early, late, lysosomal, and autophagocytic) traffic along microtubules, which are necessary for infection. Evidence suggests that parasite entry itself may serve as a nucleation point for microtubule radiation from the parasitophorous vacuole membrane, further facilitating endosomal attraction initiated by calcium flux [107]. The relationship between apparent parasite microtubule nucleation and lysosomal attraction is unknown, nor is it understood if the parasite stimulates this organization, or if this represents part of the host response to invasion. It remains possible that the forming vacuole is somehow attractive for γ-tubulin but that the vacuole simply “sticks” to microtubules in the vicinity and that lysosome fusion is a relatively passive rather than an active process.

8. Cytoplasmic Entry and Parasite Differentiation

Now encased in the acidic parasitophorous vacuole (**Figure 2**), parasite protection is offered by surface trans-sialidases [108], which also serve to facilitate parasite maturation and release [109]. There is clear evidence that the parasite breaks down the vacuolar membrane to facilitate cytoplasmic entry [110]. The mechanism for vacuolar escape is known to be lysosome and pH dependent [111]. Early reports indicated that secretion of a porin-like/complement 9-related factor TcTOX [112], and later a lytic factor LYT1 [113, 114] were critical for this final step in cell invasion. While TcTOX has defined further molecular characterization, LYT1 null mutants [113] demonstrate markedly attenuated infectivity [115]. At the acidic pH of the vacuole achieved through lysosomal fusion, LYT1 and/or TcTOX, are expressed and assume conformations capable of promoting membrane lysis to permit cytoplasmic entry. The invasive trypomastigote thus functions as a loaded weapon, and, teleologically, has completed its task to achieve successful invasion.

As a digenetic organism, *T. cruzi* follows a differentiation continuum from insect vector to mammalian host and back again. The epimastigote differentiates into the metacyclic trypomastigote in the insect hindgut and, once introduced into the host, differentiates into the replicative intracellular amastigote. These ultimately differentiate again into bloodform

trypomastigotes, which lyse out of the cell. Both the amastigote and trypomastigote forms are capable of propagating the local and metastatic infection. Transient intermediate forms are thought to develop during the differentiation process from amastigotes to trypomastigotes and appear in the mammalian host with the general morphology of epimastigotes. [54, 116]. Of specific interest here are the factors regulating intracellular differentiation. Most notably is the acidic pH achieved in the vacuole. This environment initiates the differentiation program into the amastigote over a period of several (2-8) hours. *In vitro* the parasite spontaneously undergoes differentiation if placed in an acidic environment [117]. The replication of amastigotes also demonstrates an absolute requirement for L-proline [116], and the activity of phosphatases may be required for differentiation as well [118]. After a period of quiescence, the amastigotes reenter the cell cycle and undergo nine rounds of replication prior to further differentiation (or perhaps considered de-differentiation) into motile trypomastigotes [10, 59]. Interestingly, this process of invasion, infection, and replication will occur even in cells stripped of their nuclei, indicating that new host gene transcription is not necessary [119]. The trypomastigotes destroy the host cell by unclear mechanisms, although evidence does not support apoptotic cell death [120]. After cytolysis, the infection cycle begins again for new host cell targets or uptake by a naïve triatomine taking a bloodmeal.

The transcriptional events in the trypanosome that regulate these critical differentiation steps are poorly understood, and current dogma dictates that virtually all regulation occurs after transcription and trans-splicing. Thus message stability, transcription initiation, and post-transcriptional processing are critical events for the kinetoplastids, which generate polycistronic transcripts at a relatively constant rate, and control gene dosage largely through genomic copy amplification [121-123]. Not surprising, proteosome activity is known to be essential for degrading stage-specific proteins during the cytoskeletal remodeling that occurs during the transformation from trypomastigotes to amastigotes [124]. Several *T. cruzi*-specific proteases and other enzymes have been identified in the differentiation event [125, 126], but the upstream signals remain largely unknown. Notably, at the transcriptional level, evidence suggests down-regulation of RNA polymerases I and II occurs upon differentiation from proliferative to nonproliferative forms [127]. Additionally, stage-specific regulation of histone and ubiquitin genes has been reported [128, 129]. Overall, identifying and understanding changes in gene transcription, splicing, mRNA stability, and translational events governing differentiation and replication are incompletely understood and will benefit from additional study to develop specific therapies targeting against *T. cruzi*.

9. Host Response

Successful intracellular pathogens often co-opt the very cellular self-preservation mechanisms designed to thwart parasitism. *T. cruzi* has developed mechanisms of evading the immune responses and suppressing host apoptosis by modulating the expression of host cell surface receptors, secreted factors, and signaling molecules. The pathogenesis of Chagas disease, and the relative contributions of the parasite, inflammation, and autoimmunity remains a matter of much investigation and debate, and is beyond the scope of this review.

In addition to protective, anti-parasite immunity, individuals with Chagas disease develop aberrant, potentially deleterious immune responses. Notably, invasion with the parasite triggers a type I interferon response, known to be critical in during intracellular invasion from bacteria and virus (a foreign protein/DNA/RNA response), which may drive a local immune and autoimmune response [130]. An ancient cellular response to infection, termed the IFN-stimulatory DNA response (ISD), may be a key player in the local and adaptive immune response [131]. Both TLR2 and TLR9 mediated innate immune response from

presentation of *T. cruzi* methylated CpG antigens [132] are involved (reviewed in [133]. Detailed reviews of the TLR-dependent and independent pathways are noteworthy [134], as well as the role for innate immunity clinical disease persistence [135]. In experimental models, dysregulation of several components of the type I interferon response, notably the members of the IFN regulatory factors (1-4) and their cognate binding proteins, drive spontaneous autoimmunity [136]. Stetson et al. found that deletion of the negative regulator Trex1, which serves to downregulate the ISD response, resulting in spontaneous cardiac autoimmunity [137]. It is intriguing to surmise that the cardiac autoimmunity observed in Chagas was the product of an imbalanced IFN response or failure to reset the inflammatory response even after parasite clearance. Further study will be necessary to understand the involvement of the ISD response as a driver of both adaptive immunity and the resultant cardiac autoimmunity.

10. Perspective

Understanding the cellular interaction between parasite and host and the host immune response are fundamental to treating parasitosis and preventing progression to Chagas disease. Decades of research have revealed an incredibly rich surface proteome that confers some degree of tissue specificity while retaining broad plasticity for cellular invasion. Pathways characterizing the upstream and downstream signaling events mediating cell invasion are partially understood at best, and many critical steps lack ligand-receptor pairing. With the developments in advanced proteomic and lipidomic analysis, the time is right to dissect the surface interactions between host and parasite. Our understanding of parasite differentiation, from trypomastigote to amastigote and back, remains incomplete, and detailed investigation into parasite transcriptional and transcriptional regulation underlying cell differentiation is likely to yield important insights. Finally, the emerging role of innate cellular immunity both in facilitating effective cell invasion and differentiation, and perhaps in affording resistance to overwhelming infection, is just now beginning to be investigated.

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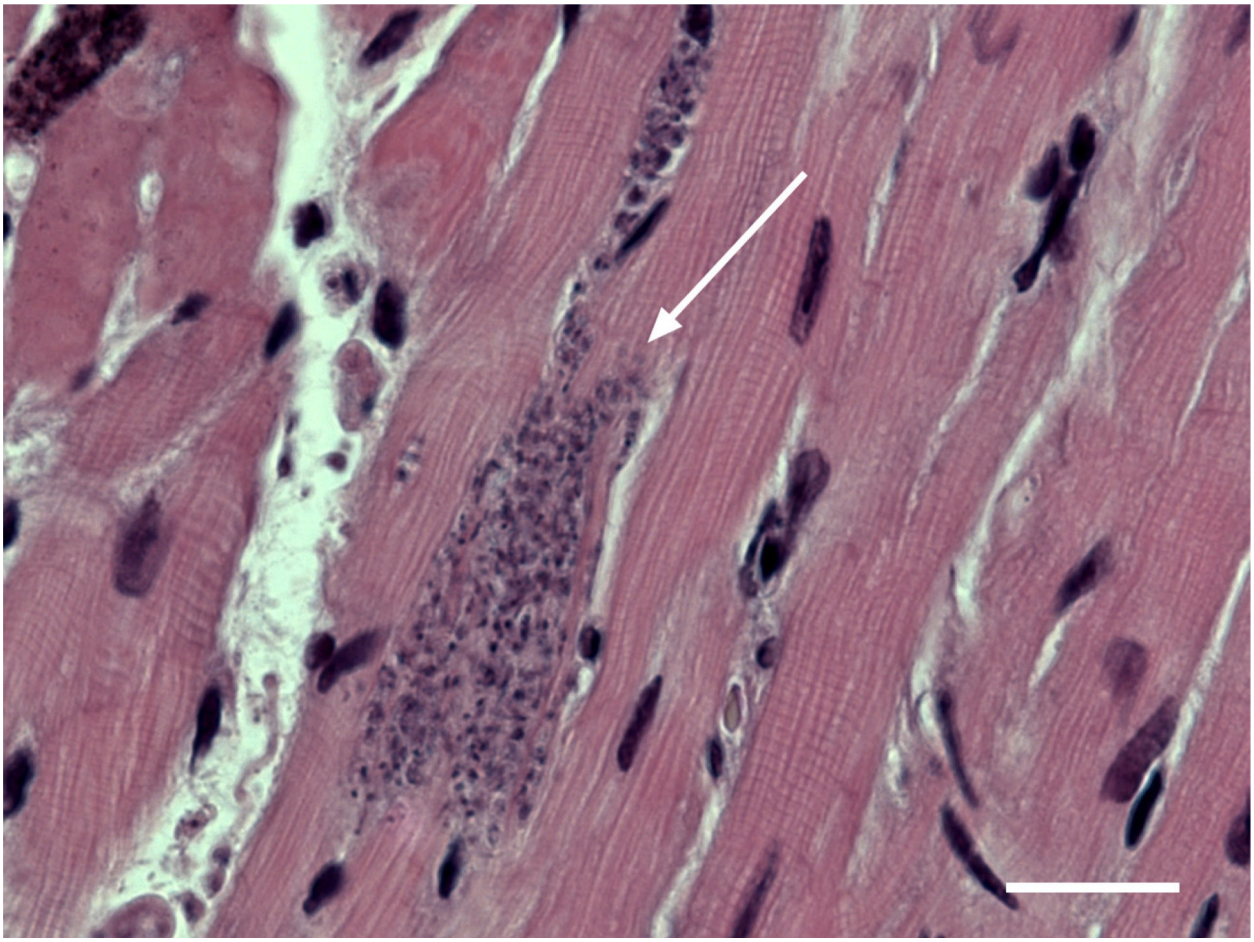

Figure 1.
Trypanosoma cruzi forms nests of intracellular parasites (white arrow) when it infects mammalian cells, especially cardiac and skeletal muscle. Shown here is an H&E stained section of a mouse heart demonstrating parasitosis of adjacent cardiac myocytes. Bar = 20 micrometers.

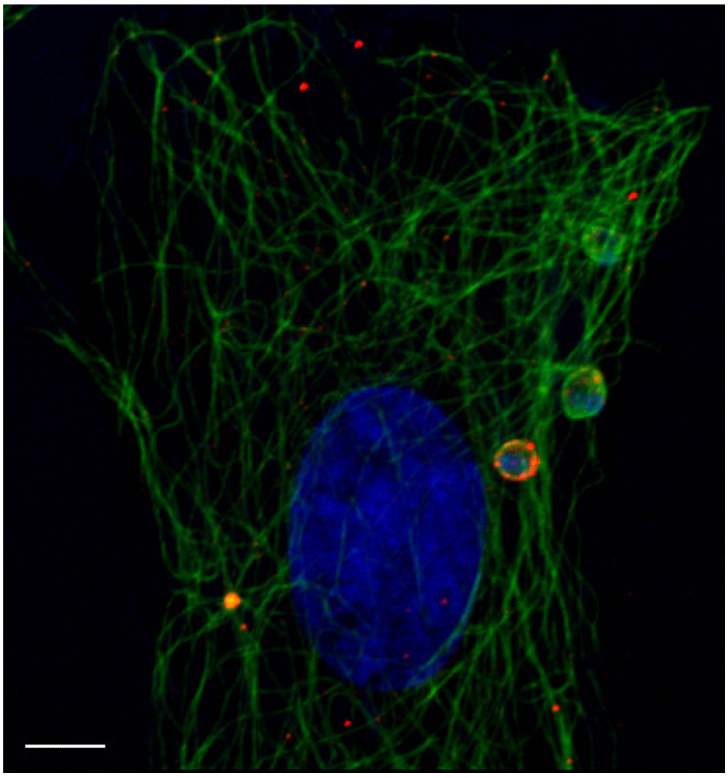

Figure 2.

T. cruzi interacts with the host cytoskeleton during cell invasion. Shown here are H9C2 rat cardiomyoblasts during invasion with *Trypanosoma cruzi*. Host and parasite DNA in blue (DAPI) host lysosomes in red, host α-tubulin in green. Bar = 5 micrometers

Table 1

Surface glycoproteins of *T. cruzi* with extracellular matrix binding or proteolytic activity

Protein	Host Target or Ligand	Biology	Reference(s)
gp82	mucin, unknown surface ligand	binding, signaling	[138]
gp63	fibronectin, laminin	ECM protease, binding	[139]
Penetrin (gp60)	heparan, heparan sulfate, collagen	binding	[139]
Tc-85/ gp85/TS	fibronectin, laminin, cytokeratin 18	binding, retention	[140, 141]
gp35/50	mucins	binding, signaling	[142]
gp90	unknown	inhibitor of invasion, signaling	[143]
gp30	unknown	binding	[79]
Mucins/Trans-sialidase	2,3-sialyl containing host surface glycoproteins (galectin-3)	sialidase, secreted (SAPA) immunogen	[144, 145]
Mucin p45	unknown	cardiac myocyte binding	[146]
gp83	unknown	sialidase, Ca signaling	[147]
Cruzipain	bradykinin	cysteine proteases	[148, 149]
POP Tc80 serine protease	collagen I, IV, fibronectin	ECM protease	[150, 151]

Table 2

Signaling properties of selected *T. cruzi* surface glycoproteins

Protein	Biology	Reference
gp82	parasite: Ca increase, PLC dependent tyrosine phosphorylation of Tc-p175	[144, 152]
gp83	parasite: Ca increase Host: MAPK signaling	[36, 153]
gp30	parasite: Ca increase	[79]
gp35/50	host and parasite cAMP and calcium increase	[154-156]
gp90	phosphatase, ? downregulates gp82 signaling	[157]
cruzipain	bradykinin signaling, calcium increase, kinin generation	[86]
oligopeptidase B	cytosolic, cleaves a 120 kDa substrate, secreted, direct calcium release	[91, 158]