Cell invasion by *Trypanosoma cruzi* and the type I interferon response

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Chapter Outline

Introduction

The life cycle of *Trypanosoma cruzi* is digenetic, comprising developmental stages in an invertebrate host (vector) as well as a mammalian host. Well over a 100 species of blood feeding triatomines may harbor *T. cruzi*,[1] while a variety of wild and domestic mammals, in addition to humans, can serve as the mammalian hosts.[2] In the triatomine vector, only extracellular developmental forms of the parasite occur. Epimastigotes, which are slender flagellated replicative forms, attach to the lumen

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of the vector digestive tract, multiply in the anterior midgut,[3] and differentiate into metacyclic trypomastigotes in the hindgut.[4,5] Metacyclic trypomastigotes, which are capable of infecting the mammalian host, are released along with the triatomine feces, from where they are involuntarily placed in the bite wound or the mucous membranes of the eyes by the bitten individual.

Intracellular forms of *T. cruzi*, on the other hand, occur exclusively within mammalian cells. Trypomastigotes are capable of invading a wide variety of nonphagocytic cells in vivo. In addition, the parasite invades virtually every nucleated mammalian cell type in culture. Once trypomastigotes have gained access to the host cell, they transform into amastigotes, which multiply in the host cell cytoplasm for several days.[6] Finally, the parasites transform back into trypomastigotes, which egress the host cell[7] and may infect additional cells or reach the circulation from where they can be taken up by triatomines. Fig. 26.1 summarizes the *T. cruzi* life cycle, indicating the different extracellular and intracellular forms of the parasite.

In terms of experimental models, trypomastigotes equivalent to those found in the circulation of infected mammalian hosts (blood forms) are obtained from infected mammalian cells in tissue culture (tissue culture-derived trypomastigotes), while metacyclic trypomastigotes can be differentiated in vitro from epimastigote cultures.[8]

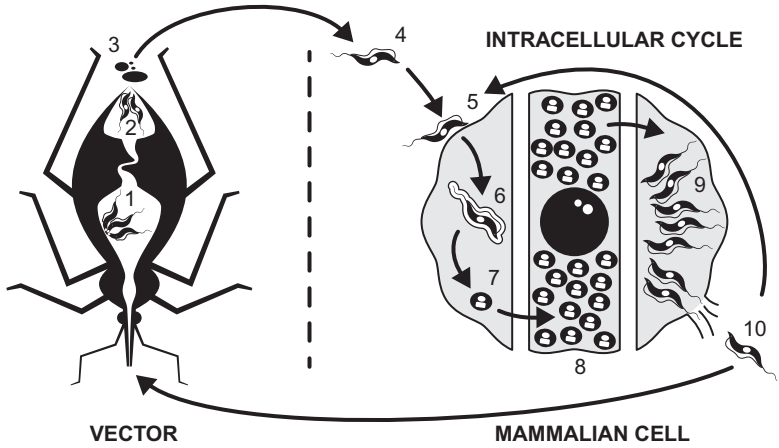

Figure 26.1 The digenetic life cycle of *T. cruzi*. Infected triatomine bugs harbor *T. cruzi* epimastigotes in the midgut. Epimastigotes attach to the lumen of the midgut and multiply (1). In the triatomine hindgut, the parasite transforms to metacyclic trypomastigotes (2), which are expelled along with triatomine feces (3). The metacyclic trypomastigotes contained in the feces contaminate the mammalian host through either the bite wound or the mucosal membranes (4). The parasite attaches to the surface of nonphagocytic host cells (5) and invades them, entering in a vacuole (6), from which it escapes as it transforms into an amastigote, which resides in the host cell cytoplasm (7). The amastigotes multiply by binary fission for approximately 5 days (8), filling the host cell cytoplasm before transforming back into trypomastigotes (9), which egress from the host cell (10). These free trypomastigotes are then capable of infecting new cells or reaching the circulation, from where they can be taken up and infect a new triatomine bug, reinitiating the cycle.

The invasion of nonphagocytic mammalian cells by *T. cruzi* is a complex and elegant biological process, during which the parasite triggers diverse signaling pathways to subvert cellular processes and facilitate its entry into the host cell. The invasion process by trypomastigotes involves continuous parasite motility and repeated probing by the parasite on the surface of host cells,[9] requiring energy expenditure from the parasite.[10] Initially, *T. cruzi* enters the host cell enveloped in a parasitophorous vacuole, from which it later escapes, gaining access to the host cell cytoplasm.

Infection by *T. cruzi* results in marked changes in host cell gene expression, the most conspicuous of which is the activation of the interferon-beta (IFN-β) gene and interferon-stimulated genes (ISGs). This chapter explores the invasion of nonphagocytic mammalian cells by *T. cruzi* and the IFN-β response initiated by the host cell as response to host cell colonization by the parasite.

Attachment to the host cell and parasite homing

Prior to entry, *T. cruzi* attaches to the mammalian host cell. A growing number of molecules have been implicated in mediating *T. cruzi* adhesion to the surface of host cells, including members of the gp85/transialidase, mucin-associated surface proteins, and mucin families (reviewed in Romano et al.[11]). These large polymorphic gene families expressed on the parasite surface encompass around one-half of the *T. cruzi* genome,[12,13] and several variants of their members are known to be coexpressed in trypomastigotes.[14] The best characterized among these is the gp85/transialidase family, which is composed of over 1400 members,[12] some of which have been shown to interact with several extracellular matrix components, including laminin and cytokeratins.[15,16] The gp85/transialidase family is specific to tissue culture-derived/bloodstream trypomastigotes and amastigotes,[17] and its members display a conserved amino acid sequence termed the FLY peptide (VTV × NV × LYNR) near the carboxy-terminus.[15,18] Tonelli et al.[19,20] employed phage display methodology and a panel of cultured vascular endothelial cells from different mouse organs to show that the FLY peptide interacts with high avidity with the vascular endothelium of the heart and bladder, and to a lesser extent with the colon. In contrast, only negligible binding was detected to endothelial cells from bone marrow and lung, suggesting that the FLY peptide binds to endothelium in an organ-specific manner. Additionally, when the FLY-displaying phages were intravenously injected into mice, they were showed to home mainly in the heart vasculature (8800-fold over the control phage), followed by the bladder and esophagus. These results suggest that members of the gp85/transialidase family may not only be important for the interaction with the extracellular matrix during infection, but they may influence the tissue tropism of the parasite as well. Furthermore, a similar phage display approach was used to show that TS9, an in silico-identified peptide shared by gp85/transialidase family members, binds to mammalian cell cytokeratins and vimentin, part of the host cell intermediate filaments.[16] Gp82, another member of the gp85/transialidase family which is expressed specifically by metacyclics,[21] has

been shown to act as an adhesion molecule, binding specifically to gastric mucin, and is believed to be implicated in *T. cruzi* invasion of the gastric mucosal epithelium during oral transmission of Chagas disease.[22]

Nonphagocytic cell invasion by *T. cruzi*

Trypomastigotes are capable of invading a wide variety of mammalian cells, through a process that differs from classical phagocytosis in that no pseudopods occur during entry and it is not prevented by actin filament depolymerization.[23,24] Different research groups have characterized at least three processes involved in *T. cruzi* trypomastigote invasion of nonprofessional phagocytic cells, all of which converge in the lysosomal compartment of the infected host cell (reviewed by Caradonna et al.[9]). While initially presented as mechanistically distinct pathways, an invasion model proposing that the three invasion routes are aspects of the same overall process reconciles historical and emerging information. Below, experimental evidence supporting different *T. cruzi* trypomastigote invasion routes/aspects is introduced in separate sections before discussing a unified model of invasion.

Lysosome exocytosis invasion route

Evidence for lysosome involvement in the process cell of invasion by *T. cruzi* originally arose in the early 1990s. Lysosomes on the host cell periphery were shown to be recruited toward the parasite attachment site and fuse with the plasma membrane during invasion of nonphagocytic cells by *T. cruzi*.[25,26] Experimental conditions which facilitate movement of lysosomes toward the host cell surface lead to increased parasite invasion, whereas those which deplete cells from peripheral lysosomes or prevent their fusion with the plasma membrane reduce invasion.[25] For example, parasite entry can be impaired by lysosome agglutination through microinjection of antibodies against lysosomal proteins or by chemical disruption of the microtubules required for lysosome migration.[26]

Trypomastigotes are able to trigger signaling cascades through host cell trimeric guanine nucleotide-binding protein (G-protein) receptors, activating cyclic adenosine monophosphate (cAMP)-dependent pathways and inducing cytosolic Ca^{2+} concentration fluxes in the host cell cytoplasm.[27,28] Liberation of Ca^{2+} from intracellular stores was suggested to activate the phospholipase C/inositol-3-phosphate signaling pathway and results in transient actin fiber reorganization in the host cells,[29] which is known to facilitate parasite entry.[25] Synaptotagmin VII serves as a Ca^{2+} sensor in lysosome−plasma membrane fusion,[30] and blocking its activity causes a significant reduction ($\sim$50%) of *T. cruzi* invasion.[31]

T. cruzi appears to have evolved redundant mechanisms to elicit Ca^{2+} signaling in host cells. A parasite-derived cytosolic serine endopeptidase, termed oligopeptidase-B, triggers Ca^{2+} transients over a variety of mammalian cells.[32,33] This enzyme has recently been shown to form dimers in solution,[34] and is believed

to act by producing a Ca^{2+} agonist through proteolysis in the cytoplasm of the parasite. The agonist, which has not been identified to date, is proposed to act by initiating signaling cascades resulting in Ca^{2+} transients in mammalian cells.[32,33] Parasites carrying oligopeptidase-B gene deletions show defects in cell invasion in vitro and are less infective in the murine model as well.[35] Furthermore, they are defective in the initiation of Ca^{2+} transients in fibroblasts, myoblasts and HeLa cells, an activity which is restored by addition of recombinant oligopeptidase-B, showing that this enzyme plays a key role in invasion of mammalian cells by the parasite.[35]

However, even in parasites carrying a double KO for oligopeptidase-B, a residual capacity to induce Ca^{2+} transients and to infect cells persists; reinforcing the notion that the parasite possesses redundant mechanism involved in the generation of Ca^{2+} transients.[35] In fact, several other *T. cruzi* proteins have been shown to be able to initiate Ca^{2+} signaling in nonphagocytic cells, including cruzipain,[36] members of the gp85/transialidase family (reviewed by Maeda et al.[8]), a novel family of *T. cruzi* surface membrane proteins (TcSMP),[37] the variant of trypomastigote small surface antigen present in *T. cruzi* lineage VI (TSSA VI),[38,39] and *T. cruzi* serine-, alanine-, and proline-rich proteins (SAP).[40] Among these, the mechanisms of action of cruzipain and that of metacyclic trypomastigotes surface glycoproteins are the best characterized, and will be discussed in further detail below.

The major *T. cruzi* protease, cruzipain, initiates signaling through kinin generation. Kinins are short-lived peptidic hormones involved in circulatory homeostasis which signal through the B2 bradykinin receptor (B2R).[41] Scharfstein et al.[36] showed that purified cruzipain triggers robust Ca^{2+} responses in umbilical vein endothelial cells and B2R-expressing Chinese hamster ovary (CHO) cells. HOE, a specific, and E-64, an irreversible inhibitor of cruzipain, were shown to block such Ca^{2+} transients. Furthermore, live tissue culture-derived trypomastigotes induced Ca^{2+} transients in B2R-expressing CHO but not in mock transfected cells, and addition of purified kininogens or physiological concentrations of bradykinin to the culture medium increased parasite entry into B2R-expressing CHO cells.[36,42] These findings imply that cruzipain is capable of initiating Ca^{2+} transients in the host cell by proteolytically cleaving host cell-bound kininogens to generate kinins in umbilical vein endothelial cells and B2R expressing CHO cells.[36] Since protease inhibitors which block cruzipain activity, such as cystatin-C or E-64, do not block parasite entry into the host cells, it has been proposed that the kininogen lysis by cruzipain requires close contact between the cell membranes of the host cell and the parasite,[36,42] although this has not been demonstrated experimentally.

The work by Yoshida and collaborators regarding the signaling of surface glycoproteins in metacyclic trypomastigotes is also compelling. It has been postulated that tissue culture-derived trypomastigotes (equivalent to blood stream forms) and metacyclic trypomastigotes induce Ca^{2+} transients by different mechanisms (reviewed by Maeda et al.[8]). While tissue culture-derived trypomastigotes would induce Ca^{2+} flux through oligopeptidase B and cruzipain, metacyclics of the CL strain are believed to signal through gp82, a gp85/transialidase family member, activating phospholipase C, the mammalian target of rapamycin (mTOR) and

phosphoinositide 3-kinase (PI3K), and resulting in Ca^{2+} transients in HeLa cells.[8,43] The less infective G-strain is believed to signal through the gp35/50 surface mucin and to generate Ca^{2+} transients in a cyclic AMP-dependent fashion.[43]

The relative expression of gp82, gp35/50, and gp90 (a nonsignaling member of the gp85/trans-sialidase family) has been proposed to govern the infectivity of *T. cruzi* stocks, where immunoprecipitation, immunoblotting, and FACS analysis with monoclonal antibodies revealed that the poorly infective strains (including G strain) express relatively high levels of gp35/50 and gp90, while highly infective strains (including the CL strain) expressed high levels of gp82 and negligible gp90 on its surface.[44] These three metacyclic trypomastigote-specific surface proteins interact with receptors in the surface of mammalian cells, where gp82 induces a robust Ca^{2+} response, while the response induced by gp35/50 is weaker and that of gp90 is negligible.[44] Experimentally induced reduction of gp90 expression through antisense nucleotides was shown to increase parasite infectivity,[45] and it is believed that gp90 negatively regulate *T. cruzi* infectivity.[46] Furthermore, parasite isolates expressing gp90 variants that are sensitive to gastric peptidases are infective through the oral route in vivo despite showing poor infectivity in vitro. High infectivity in vitro can be induced in such isolates by gp90 degradation though exposure to gastric juice.[47]

Plasma membrane invasion route

A second cell invasion route employed by *T. cruzi*, which does not depend on lysosome exocytosis, was discovered by Woolsey et al.[48] These authors noted that after infection of mammalian cells in culture with tissue culture-derived trypomastigotes, only 20−30% of the newly formed parasitophorous vacuoles could be stained for the lysosomal marker LAMP-1 (lysosomal-associated membrane protein) in fluorescence microscopy experiments. The remaining vacuoles are formed by invagination of the host cell plasma membrane, without immediate association with lysosomes. The interaction between the invading parasites and the host cell plasma membrane during the early stages of invasion was evidenced by microscopy experiments involving green fluorescent protein (GFP)-tagged plasma membrane markers. Parasites which enter the cells in plasma membrane-derived vacuoles later fuse with early endosomes and lysosomes, as the vacuole matures. This cell invasion route is not cell type-specific, as it was shown to occur in a variety of mammalian cells (including myoblasts, CHO cells, and primary cardiomyocytes).

Additionally, Woolsey et al.[48] showed that cell invasion by *T. cruzi* is a reversible process, i.e., parasites which have entered the cell through plasma membrane invagination may exit from the host cell. The frequency of reversible invasion increased under conditions which prevent lysosome fusion with the parasitophorous vacuole (disruption of actin filaments with cytochalasin D or inhibiting phosphoinositide-3-kinase with wortmannin).[48,49] These results have generated opposing interpretations, since based on microscopy experiments employing wortmannin, Andrade and Andrews[50] concluded that the plasma membrane invasion route results in reversible, nonproductive cell infection. However, wortmannin affects not only lysosome fusion,

but also endosome fusion with the parasitophorous vacuole (part of the vacuole maturation according to the plasma membrane invasion model) and autophagosome formation (implicated in a third invasion route discussed in the next section). Therefore, the experiments involving treatment of host cells with wortmannin may not be definitive, and the need for time-lapse experiments to clarify the controversy has been pointed out.[9]

The autophagosome route

More recently, a third invasion route employed by *T. cruzi* has been described, which involves elements of the autophagy pathway.[11] Autophagy is a conserved catalytic pathway in mammalian cells, involved in the turnover of old or surplus organelles and macromolecules by their engulfment into phagosomes and subsequent delivery to endosomes and/or lysosomes for their degradation and reuse.[51] The products of several autophagy related genes form part of the core molecular autophagy machinery, and are essential for the formation of phagosomes.[51] Among them, microtubule-associated protein 1A/1B-light chain 3 (LC3), a soluble protein distributed ubiquitously in mammalian cells, constitutes one of the best characterized markers used to monitor autophagy and autophagy-related processes.[52]

Using CHO cells overexpressing a GFP-tagged version of the autophagic marker LC3, Romano et al.[11] evidenced colocalization of GFP-LC3 with *T. cruzi* parasitophorous vacuoles by confocal microscopy, indicating interaction of the parasite with the autophagic compartments during host cell invasion. Abundant phagosomes displaying LC3 on their membrane were recruited to the parasite entry site at early time points (1-h postinfection), but no LC3 was detectable in association with the parasite at later time points, when the parasite is free on the cytoplasm and no longer contained in the parasitophorous vacuole (48−72 h).

Induction of autophagy by cell starvation or pharmacological means (rapamycin) increases host cell invasion by the parasite, along with LC3 colocalization with parasitophorous vacuoles.[11] Subsequent studies have shown that the parasitophorous vacuoles for different strains of *T. cruzi* (Brazil, K98, and CL Brenner) associate with LC3 in various other cell types, including rat myoblasts, mouse cardiomyocytes, and epithelial cells, suggesting that the usage of this invasion route is not limited to a particular parasite strain/host cell combination, and that it constitutes a widespread phenomenon.[53] Additionally, Vanrell et al.[54] showed that blocking the synthesis of polyamines (ubiquitous low molecular weight polycations involved in nucleic acid/protein and protein/protein interactions) through the inhibition of the biosynthetic enzyme ornithine decarboxylase with difluoromethylornithine results in suppression of autophagy in mammalian cells, thus preventing cell invasion by *T. cruzi*.[11]

Toward a unified model for T. cruzi invasion

Despite the differences in their surface molecules and signaling pathways they employ for invasion, trypomastigotes, both metacyclic and tissue culture-derived,

share the ability to enter a wide variety of mammalian host cells through an actin-independent process which retains in common a similar parasitophorous vacuole formation and maturation, suggesting that shared host cell traits must exist which allow for responsiveness to parasite signals and to support parasite entry.[55]

Injuries compromising the plasma membrane integrity of mammalian cells are repaired by a mechanism dependent on intracellular free Ca^{2+} signaling, in which membrane wound resealing occurs by targeted lysosome exocytosis.[56] Interestingly, the evidence demonstrating that bona fide acidic lysosomes could be mobilized to participate in this wound repair process came from studies of the invasion of non-phagocytic mammalian cells by *T. cruzi*, and it is believed that the parasite triggers this repair pathway and subsequently subverts it, hijacking the lysosomes to form the vacuole in which it gains access to the host cell.[57] More recently, the repair process has been further characterized to demonstrate that pore-forming protein-induced lesions as well as mechanical wounds are removed from the plasma membrane by a coupled endocytosis step[58] promoted by lysosomal acid sphingomyelinase, ASM.[59] Therefore, Fernandes et al.[60] studied whether this enzyme also plays a role during *T. cruzi* invasion, and showed that blocking ASM activity through inhibitors (desipramine) or RNA interference (RNAi) hampers trypomastigote invasion. Additionally, they showed that trypomastigotes cause wounds in the host cells plasma membrane during invasion (presumably through their intense motility and secretion of pore-forming molecules) allowing the flow of extracellular Ca^{2+} into the cell through the lesions. The presence of ceramide in the membranes surrounding $>60\%$ of recently invading parasites was shown by immunostaining, and additional lysosomes were shown to progressively fuse with the parasitophorous vacuole.[60] Therefore, the proposed model for invasion of nonphagocytic cells by *T. cruzi* has been recently updated as follows. Lysosome exocytosis toward parasite-induced wounds takes place, and fusion of lysosomes with the host cell plasma membrane occurs as part of the repair process. Upon fusion, lysosome content is released toward the cell surface, liberating ASM, which in turn hydrolyzes sphingomyelin on the external layer of the host cell plasma membrane and generating ceramide microdomains, which invaginate and facilitate the formation of the parasitophorous vacuole and the entrance of the parasite into the cell.[55,60] This invagination of the plasma membrane, which in the lesion repair removes the membrane lesions by endocytosis is likely to represent the "plasma membrane" invasion route described above.[48] Subsequent fusion of lysosomes with the parasitophorous vacuole would ensure parasite retention inside the host cell.[55,60] It has been proposed that the frequency with which striated and cardiac muscle cells undergo plasma membrane injury may be related to the parasite tropism toward these cell types.[55] This unified model thus reconciles the lysosome-dependent and plasma membrane invasion routes as part of the same invasion process. Additionally, in an attempt to include the knowledge about cruzipain-induced kinin signaling within this unified invasion framework, Scharfstein et al.[61] have hypothesized that lipid rafts containing B2R and other G-protein coupled receptors could be internalized during the ASM-mediated invagination of ceramide-rich plasma membrane domains. This hypothesis would predict that during the cell membrane invagination and/or

inside the parasitophoruos vacuole the flagellar pocket and the receptors would be in close proximity allowing for the signaling events triggered by the cruzipain-mediated generation of kinins, although experimental support for this is still lacking.

As the unified model of invasion evolves, it is predicted that newer studies will be interpreted within its framework. In some cases, recent findings regarding the *T. cruzi* invasion process have already been presented by the authors within the framework of this unified invasion model. In one such case, Zhao et al.[62] reported that trypomastigotes invade in with parallel orientation to the host cell microtubules. Microtubule cytoplasmic linker associated protein-1 (CLASP1), a plus end-tracking protein involved in microtubule stabilization at the cell periphery, was shown to play a key role in trypomastigote internalization, lysosome fusion with the parasitophorous vacuole and postentry parasite localization near the host cell nucleus.[62] Furthermore, knocking down CLASP1 impaired all of these events without affecting Ca^{2+}-mediated lysosome exocytosis. Importantly, besides the findings about *T. cruzi* invasion, this study showed evidence that CLASP1 is involved in the dynamics of intracellular localization of lysosomes in mammalian cells. Fig. 26.2 shows a model for *T. cruzi* invasion where the different invasion routes are unified. Some of the findings in the study by Zhao et al.[62] are also included in Fig. 26.2.

In other cases, even very recent studies are still interpreted within a framework which considers different invasion routes for *T. cruzi* exist. For example, Cortez et al.[63] recently reported that lysosome exocytosis and scattering induced by short-term nutrient deprivation of the host cells, promotes host cell invasion by metacyclic trypomastigotes while reducing invasion by tissue culture-derived trypomastigotes. Rapamycin treatment of the host cells prior to infection produced the opposite effect, where metacyclics infected less. The interpretation of these findings by the authors was that metacyclic trypomastigotes and tissue culture-derived trypomastigotes invasion occur by different mechanisms, where the former invade mainly through a lysosome-dependent route, while the later invade through a lysosome-independent route.[63] These results would argue that even if *T. cruzi* invasion occurs though a unified mechanism, different parasite life cycle forms could favor the usage of different components of it. If this were true, it is likely that different genetic lineages and isolates of *T. cruzi* could display such preferences as well.

Escape from the lysosome

Once it has invaded the host cell, *T. cruzi* escapes from the phagolysosome to gain access to the cytoplasm, and this process is dependent on phagolysosome acidification.[64] Lysis of the phagolysosome membrane is believed to be mediated by a parasite-derived secreted pore-forming molecule known as Tc-Tox, which works optimally at low pH.[65,66] Tc-Tox cross-reacts with antibodies against complement C9 protein,[66] and this property was used to screen an expression library and identify a *T. cruzi* gene (LYT1) coding for a hemolytic protein acting at low pH, which cross-reacts with anti-C9 antibodies.[67] In addition, *T. cruzi* neuraminidase

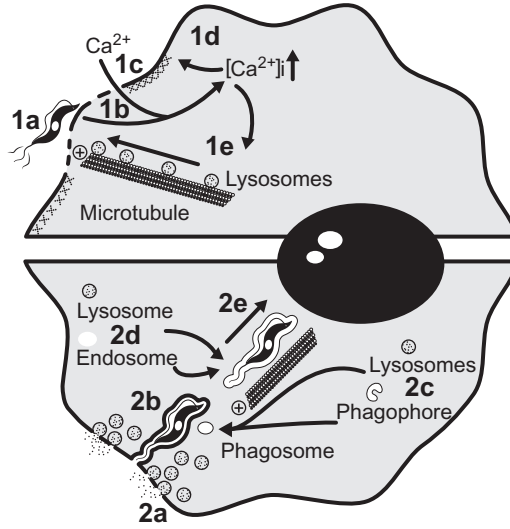

Figure 26.2 Intracellular invasion by *T. cruzi* trypomastigotes. TOP. Trypomastigotes are highly motile and they attach to the surface of nonphagocytic mammalian cells (1a). The parasite causes increased intracellular Ca^{2+} levels by triggering signaling cascades (1b), and by inducing plasma membrane lesions, through which extracellular Ca^{2+} can flow into the cell (1c). Increased intracellular Ca^{2+} concentrations facilitate parasite entry by inducing transient depolymerization of the cortical actin cytoskeleton (1d) and lysosome anterograde (toward the " + " end) translocation on microtubules to reach the parasite entry site in the cell periphery (1e).
BOTTOM. At the parasite entry site, lysosomes exocytose while sealing membrane wounds, and release acid sphingomyelinase, ASM (2a). ASM generates ceramide domains in the outer leaflet of the plasma membrane and favors its invagination, allowing parasites to enter the cell enveloped in a plasma membrane-derived vacuole, in most cases devoid of lysosomal markers (2b). Phagosomes, formed by the fusion of phagophores with lysosomes, may fuse to the nascent parasitophorous vacuole (2c), which may also fuse with endosomes and additional lysosomes as it matures (2d). The parasitophorous vacuole undergoes retrograde (away from the "plus" end) translocation on microtubules toward the perinuclear region (2e).

(trans-sialidase), which has also been shown to be active at low pH, has also been implicated in the disruption of parasitophorous vacuoles.[68] Interestingly, coinmunoprecipitation experiments suggest that Tc-Tox/LYT1 protein is capable of physically interacting with trans-sialidase.[69] However, direct proof of the proposed functions of these proteins in egress from the parasitophorous vacuole is still lacking.

Host cellular processes required for *T. cruzi* invasion

An RNAi-based genome-wide functional screen designed to identify mammalian cell genes and processes which support establishment of intracellular infection and

intracellular growth by *T. cruzi* was performed by Caradonna et al.[70] The screen employed a multistep process, with an initial library of >25,000 siRNA pools which was assayed on an in vitro culture system using HeLa cells, to determine the effect of silencing individual genes over the *T. cruzi* intracellular infection process. The initial screen was followed by secondary screens with endpoints at 18 h and 72 h postinfection, corresponding to the prereplication phase of infection (i.e., invasion, <24 h after cell infection) and intracellular growth (i.e., amastigote multiplication, >24−90 h after cell infection). Although functional confirmation experiments performed in this study concentrated on host pathways which support the parasites intracellular growth (host metabolic networks and cellular signaling pathways were identified as important for this phase of the parasite life cycle), the screen did identify several dozen candidate host genes involved in the establishment of intracellular residence by *T. cruzi*. Genes that were found to affect parasite invasion included host cell signaling molecules, cytoskeletal proteins (CLASP1, cofilin-1), extracellular matrix proteins (laminin, collagen 1α), and genes involved in protein trafficking and organelle biosynthesis, among others. These findings are in line with what would be expected based on the knowledge regarding mammalian cell invasion described in previous sections of this chapter. The identification of CLASP1 as a protein required for parasite invasion in the RNAi knockdown screen was immediately followed by functional confirmation in subsequent studies,[62] highlighting the validity of the use of unbiased screens to identify functional leads that can be subsequently confirmed and characterized in the pursuit of unveiling the details of the infection process by *T. cruzi*, and intracellular pathogens in general.

Global transcriptional responses to *T. cruzi* infection: type I IFN response

Interaction with intracellular pathogens produces characteristic transcriptional signatures in host cells.[71] Vaena de Avalos et al.[72] dissected the transcriptional response of human fibroblast to *T. cruzi* infection using microarray technology and showed that the parasite elicits very little transcriptional changes early in the intracellular infection. However, at 24 h postinfection, the transcriptional signature shows very clear upregulation of ISGs due to infected IFN-β production by the infected fibroblasts. Importantly, this transcriptional signature characterized by ISGs was not present in similar studies with other intracellular pathogens, including *T. gondii*[73] and *Salmonella*.[74] Additional studies characterized transcriptional responses to *T. cruzi* infection in three different primary human cell types relevant to the infection in vivo: fibroblasts, smooth muscle cells, and endothelial cells, reporting a shared ISGs induction signature that was driven by soluble/secreted cytokines as demonstrated using transwell plates.[75] When analyzing the gene-ontology functions to which the commonly upregulated genes belonged to, "IFN signaling" and "antigen presentation" emerged as the top signaling pathways induced in all three cell types, consistent with strong increase in IFNβ gene

transcription. In conjunction, these studies demonstrate that type I IFN drives the early transcriptional response to *T. cruzi* infection.

Type I IFNs

The IFNs are classified into type I (> 20 members), type II (IFN-ɣ), and type III (three types of IFN-ʎ). IFN-ɣ is produced by natural killer (NK) and natural killer T (NKT) cells stimulated by IL-12, while type I and III IFNs are generally produced in response to viral infections.[76] Type I IFNs form part of the most diverse family cytokines, and upon binding to the interferon-α/β receptor (IFNAR), induce transcriptional modulation of over 1000 genes with diverse biological properties as part of the innate immune response.[76]

The IFNAR receptor consists of two transmembrane protein chains (IFNAR1 and 2), associated to the cytoplasmic Janus Kinase 1 (JAK1) and tyrosine kinase 2 (TYK2). In the canonical signal transduction pathway, STAT1 (signal transducer and activator of transcription 1) and STAT2 proteins are phosphorylated, bind interferon regulatory factor 9 (IRF9), and this complex translocates to the nucleus to activate ISGs.[77] However, this signal transduction pathway is not isolated, and it is extensively interconnected with signaling pathways from the innate immune system involved in pattern recognition, including the Toll-like receptors (TLRs), RIG-I like receptors (RLGs), NOD-like receptors, and C-type lectin receptors.[78]

There are different mechanisms by which pathogens can activate type I IFN production, namely interaction with TLRs and cytosolic surveillance molecules. TLRs are transmembrane receptors specialized in the recognition of pathogen associated molecular patterns which are expressed in immune and some nonimmune cells. Upon binding of a ligand, TLRs dimerize and signal through the adaptor molecules myeloid differentiation factor 88 (MyD88) and TIR (Toll/interleukin-1 receptor) domain-containing adaptor protein inducing interferon beta (TRIF) (reviewed in Ref. [79]).

The signal transduction networks activated downstream of the innate immunity receptors is vast and complex and a detailed description of it is beyond the scope of this chapter. We refer the reader to the excellent reviews on the subject in Refs. [76,78,79]. However, in the following paragraphs of this section we will mention some of the molecules in the signaling networks downstream of TLRs and other cytoplasmic receptors, which are relevant to the discussion of studies about type I IFN induction during *T. cruzi* infection.

Among the molecules known to act downstream of MyD88 and TRIF are the interferon regulatory transcription factor 3 (IRF3) and TANK-binding kinase 1 (TBK1). IRF3 is sufficient to induce the expression of IFN-β, and upon phosphorylation by TBK1 dimerizes and translocates to the nucleus, binding the IFN-β promoter[80,81] and driving the expression of IFN-β itself (autocrine loop) and of ISGs.[79]

Production of type I IFNs can also occur in a TLR-independent manner. Cytoplasmic pathogens are detected by a different set of receptors, among which the retinoic acid-inducible protein I (RIG-I) and melanoma differentiation-associated

gene 5 (MDA5) are cytoplasmic receptors which recognize dsRNA of viral origin, and are required for type I IFN responses against several types of viruses. The signal transduction pathway downstream of RIG-I and MDA5 converges with that of TLRs in the activation of TBK-1, which phosphorylates IRF3.[79] A variety of other DNA cytoplasmic sensors capable of inducing IFN production are known (reviewed by Gurtler and Bowie[82]).

Signaling pathways involved in type I IFN production in *T. cruzi* infected cells

The signaling pathways responsible for induction of type I IFN production by host cells during *T. cruzi* infection have not been fully characterized. The process has been shown to depend on IRF3/TBK1 (as all known pathways leading to IFN-α and -β production), and to follow the dynamics of an autocrine induction loop.[83] Additionally, type I IFN production by host cells is known to be elicited before *T. cruzi* escapes the parasitophorous vacuole, and can take place in the absence of active infection (since heat-killed trypomastigotes also triggered it).[83]

Despite earlier indications of TLR3 involvement,[84] Chessler et al.[83] found no evidence for TLR-signaling in the process by exploiting mouse fibroblasts (MEFs) and mouse bone marrow-derived macrophages (BMDM) defective for TLR3, TLR4, and their downstream mediators Myd88 and TRIF. Moreover, the cytosolic sensors known at the time (RIG-I and MDA5) were also not required during *T. cruzi*-induced type I IFN production. A possible role for more recently discovered cytoplasmic sensing mechanisms[82,85] remains to be studied. Additionally, it is tempting to speculate that Ca^{2+} signaling associated to perturbations of host cell membrane could activate IRF3, causing type I IFN production during *T. cruzi* cell invasion, as it has been shown to occur during viral infection.[86]

Type I IFN responses to *T. cruzi* infection in the mouse model

Chagas disease transmission occurs primarily by the vectorial route.[6] Therefore, infection on a dermal or mucosal site would be expected to constitute the first parasite−host contact. Chessler et al.[87] studied the transcriptional responses at the site of intradermal infection with *T. cruzi* in a murine model for dermal infection. As evidenced by transcriptomic analysis of mouse dermal tissue in the inoculation site with Affymetrix microarray chips, strong activation of ISGs occurs at the primary infection site. Similar to what occurs in cultured cells,[72,75] the major pathway activated by *T. cruzi* infection in mouse skin is IFN signaling.[87]

Among the genes activated during dermal infection, several were related to the recruitment or activity of natural killer (NK) cells, macrophages, lymphocytes,

neutrophils, and dendritic cells. The presence of these cells in the infection site was confirmed by histologic analysis of dermal tissue, suggesting that neutrophil and NK cells recruited to the infection site could be responsible for IFN-γ production, leading to the strong activation of transcription of ISGs which was observed. Therefore, mice were depleted of NK cells or neutrophils through treatment-specific antibodies. Surprisingly, however, NK cell or neutrophil depletion had little effect over the expression of ISGs, indicating that although they are recruited to the infection site, these cells are not required for the IFN response.[87]

Additionally, microarray analysis showed a robust ISG activation at the *T. cruzi* inoculation site in IFN-γ-deficient mice. On the other hand, when mice lacking the type I IFN receptor were infected with *T. cruzi*, the transcriptional activation of ISGs at the inoculation site is abrogated, clearly showing that type I IFNs are responsible for activation of this transcriptional signature.[87]

Subsequently, the authors measured the IFN-β and ISGs induction by three parasite strains displaying different degrees of virulence. Parasitemia, parasite tissue load, and pathologic manifestations in the infected dermal tissue displayed by the strains correlated directly with the intensity of the IFN-β induction at the dermal inoculation site (Brazil > Y > G). However, the intensity of the overall induction of ISGs did not (Y > Brazil > G).[87]

It has been shown that CD8 T cells play a key role for the control of *T. cruzi* infection, and mice rapidly succumb when infected by the parasite if they are unable to mount CD8 responses.[88,89] Martin et al.[90] studied the impact of IFN-β over the CD8 T cell response in a murine model. Over 30% of the CD8 T cell population of B6 mice infected with the Brazilian strain of *T. cruzi* specifically responded to epitopes of the transialidase peptides TSKB20 (ANYKFTLV) and TSKB18 (ANYDFTL).[90] When mice lacking the type I IFN receptor ($IFNAR^{-/-}$) were infected with *T. cruzi*, the numbers of CD8 T cells, their expansion dynamics, as well as their capacity for IFN-γ production specific for TSKB20 and TSKB18 peptides did not change as compared to wild-type infected mice. This shows that CD8 T cell responses, known to be crucial for *T. cruzi* infection, are independent from type I IFN signaling.[90]

In an additional study, infections with infections in $IFNAR^{-/-}$ mice with Brazil and Y strain were characterized.[91] When sublethal inoculation doses were employed, no differences in parasitemia or mortality between $IFNAR^{-/-}$ and control mice were detected. However, when lethal parasite doses were used, wild-type mice succumbed to the infection while $IFNAR^{-/-}$ mice had significantly better survival rates. No differences were found among the mice in terms of inflammation, parasite burden in tissues, cellular infiltrate, or apoptotic cells in the infected tissues. Additionally, the splenocytes from $IFNAR^{-/-}$ mice produced significantly higher amounts of IFN-γ in response to *T. cruzi* antigen, although both types of mice had a similar number of effector cells present. In summary, according to Chessler et al.,[91] type I IFN appears not to provide protection against *T. cruzi* infection, and to even be harmful for the host when the parasite burden is high (Fig. 26.3).

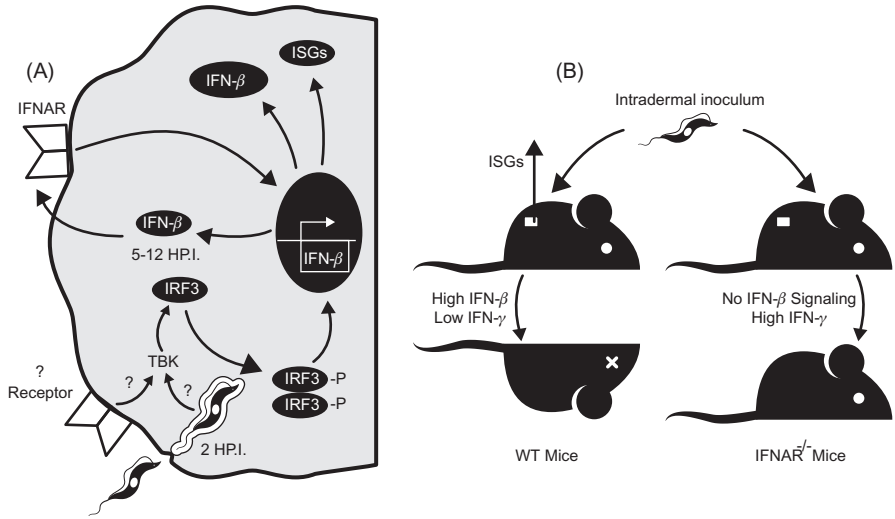

Figure 26.3 The type I IFN response during *T. cruzi* infection. (A) IFN-β production in a nonphagocytic cell infected by *T. cruzi*. The identity and intracellular/extracellular nature of the receptors and signaling molecules initially triggered by *T. cruzi* remains unknown (indicated by question marks). However, as early as 2-h postinfection, while the parasite is still contained in the parasitophorous vacuole, IRF3 is phosphorylated by TBK. IRF3 dimers activate IFN-β transcription. Through a transcription activation loop involving signaling through the IFNAR receptor, IFN-β intensifies its own production as well as that of ISGs. (B) In vivo intradermal *T. cruzi* infection in a murine model under high parasite burden conditions (lethal dose inoculum). Upon dermal infection with *T. cruzi*, wild-type mice produce high amounts of IFN-β, which in turn activates ISGs. However, low levels of IFN-γ are produced, and the wild-type mice die. On the other hand, mice lacking the type I IFN receptor ($IFNAR^{-/-}$) produce high levels of IFN-γ and survive the infection.

The balance between type I and type II IFN responses during infection with intracellular pathogens

The fact that type I IFNs do not protect against *T. cruzi* infection, and that they might be harmful to the host under specific circumstances[91] fit a broader trend in the literature regarding the innate responses to intracellular pathogens like *Mycobacterium* spp. and *Leishmania* spp. Studies in human leprosy[92] have demonstrated that polarized clinical manifestations of infection with *M. leprae* correlate with IFN-β and IFN-γ expression profiles. IFN-β and the genes it activates (including IL-10) are preferentially expressed in lepromatous lesions, a disseminated and progressive clinical manifestation of leprosy where abundant bacilli and a Th2 type immune response profile are present. Meanwhile, in tuberculoid leprosy, characterized by self-healing localized lesions, an IFN-γ transcriptional signature, including a

vitamin D-dependent antimicrobial peptide response, is present along with a Th1 profile. The responses in tuberculoid leprosy can be inhibited by IFN-β and IL-10, which suggests that the type I versus type II IFN balance in the site of infection might affect protective and pathogenic responses during *M. leprae* infection.[92] Additionally, an IFN-β transcriptional signature was detected in peripheral blood mononuclear cells from symptomatic tuberculosis patients, while an IFN-γ transcriptional profile is present in latent tuberculosis, suggesting that type I versus type II IFN balance can affect the progression of *M. tuberculosis* infection as well.[92]

Several recent reports[93–96] highlight a link between destructive disseminative mucocutaneous leishmaniasis and type I IFN-induced hyperinflammatory Th1 type responses. Interestingly, type I IFN responses have been associated to *Leishmania* isolates carrying the *Leishmania*-specific totivirus LRV1.[93] Infection of macrophages functionally deficient for TLR3, 7, or 9, or for the adaptors MyD88 and TRIF, indicate that the double-stranded genome of LRV1 carried by *L. guayanensis* induces IFN-β production by engaging the TLR3−TRIF-dependent pathway in macrophages. Heavier infection with LRV1 induces more robust IFN-β expression which exacerbates the clinical manifestations of leishmaniasis.[93]

Collectively, these reports support the view that the double-stranded RNA genome of the LRV1 virus within *Leishmania* parasites acts as in innate immunogen which promotes parasite persistence and alters the clinical course of leishmaniasis. This probably occurs when LRV1 particles are liberated from dead *Leishmania* parasites. This knowledge may allow for better prognosis of the risk of developing mucocutaneous leishmaniasis as well as for better treatment options. However, other factors, such as the genetic variability of the host and parasite, may play a role in the clinical outcome as well.

Concluding remarks

Invasion of nonphagocytic host cells and the concomitant activation of the innate immune response are among the earliest interactions between *T. cruzi* and its mammalian host. An integrated model of nonphagocytic cell invasion by *T. cruzi* is currently favored, which reconciles previously described invasion routes originally considered to be mechanistically independent. Importantly, although it is well established that parasite-triggered signaling cascades initiated by interaction with host cell surface molecules are required for invasion, attaining integrated understanding of the relationship between these signaling events and the different components of the emerging invasion model constitutes a remaining challenge to be tackled in future research efforts.

T. cruzi invasion of nonphagocytic host cells results in the activation of the innate immune system, specifically type I IFN production, which in turn drives the global transcriptional profile of infected cells and influences the subsequent adaptative immune response. The signaling mechanisms leading to type I IFN production during *T. cruzi* infection remain poorly understood. Furthermore, the degree to

which these early events determine the outcome of the infection with *T. cruzi* is currently unknown, although under conditions of high parasite burden, type I IFN production is detrimental for the host.[83] A negative effect of type I IFN responses is in line with a trend found for infection with other intracellular pathogens, such as mycobacteria and *Leishmania*, and gaining a better understanding of these phenomena may provide clues to new therapeutic options and strategies for prognosis of the clinical manifestations of Chagas disease. Finally, further studies are warranted in order to gain broader understanding of the impact that the full breath of genetic variability of *T. cruzi* might have over individual aspects of infection such as cell invasion and type I IFN responses, and how this might in turn impact the overall immune responses and the clinical outcomes of chagasic patients.

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Glossary

AMP	adenosine monophosphate
ASM	lysosomal acid sphingomyelinase
BMDM	mouse bone marrow-derived macrophages
B2R	B2 bradykinin receptor
Ca^{2+}	calcium ion
CHO cells	Chinese hamster ovary cells
CLASP1	microtubule cytoplasmic linker associated protein-1
E-64	an irreversible inhibitor of cysteine proteases. Chemical name (1*S*,2*S*)-2-(((*S*)-1-((4-guanidinobutyl)amino)-4-methyl-1-oxopentan-2-yl)carbamoyl) cyclopropanecarboxylic acid
FACS	fluorescence-activated cell sorting
G protein	guanine nucleotide-binding protein
GFP	green fluorescent protein
Gp82	*Trypanosoma cruzi* metacyclic trypomastigote-specific surface glycoprotein
Gp85	*Trypanosoma cruzi* Gp85 glycoproteins, members of the Gp85/Trans-sialidase superfamily
HOE	a potent antagonist of the bradkynin-2 receptor. Chemical name Arg-(3-hyp-5-thi-7-tic-9-oic)-9-desarg-bradykinin
IFN	interferon
IFNAR receptor	interferon-α/β receptor
IFN-β	interferon β
IFN-γ	interferon γ
IRF3	interferon regulatory transcription factor 3
IRF9	interferon regulatory factor 9
ISGs	interferon-stimulated genes

JAK1	Janus Kinase 1
LAMP-1	lysosomal-associated membrane protein
LC3	microtubule-associated protein 1A/1B-light chain 3
LRV1	*Leishmania* RNA virus
MDA5	melanoma differentiation associated gene 5
mTOR	mammalian target of rapamycin
MyD88	myeloid differentiation factor 88
NK cell	natural killer cell
NKT cell	natural killer T cell
NOD receptor	nucleotide-binding oligomerization domain receptor
PI3K	phosphoinositide 3-kinase
RIG-I	retinoic-acid-inducible protein I
RLGs	RIG-I like receptors
RNAi	RNA interference
SAP	*T. cruzi* serine-, alanine-, and proline-rich proteins
STAT1	signal transducer and activator of transcription 1
STAT2	signal transducer and activator of transcription 2
TBK1	TANK-binding kinase 1
TcSMP	*T. cruzi* surface membrane proteins
TIR	toll/interleukin-1 receptor
TLR3	Toll-like receptor 3
TLR4	Toll-like receptor 4
TLRs	Toll-like receptors
TRIF	domain-containing adaptor protein inducing interferon beta
TSSA VI	trypomastigote small surface antigen present in *T. cruzi* lineage VI
TYK2	tyrosine kinase 2

References

1. Schofield CJ, Galvao C. Classification, evolution, and species groups within the triatominae. *Acta Trop* 2009;**110**:88−100.
2. Noireau F, Diosque P, Jansen AM. *Trypanosoma cruzi*: adaptation to its vectors and its hosts. *Vet Res* 2009;**40**:26.
3. Kollien AH, Schaub GA. The development of *Trypanosoma cruzi* in triatominae. *Parasitol Today* 2000;**16**:381−7.
4. Schmidt J, Kleffmann T, Schaub GA. Hydrophobic attachment of *Trypanosoma cruzi* to a superficial layer of the rectal cuticle in the bug *Triatoma infestans*. *Parasitol Res* 1998;**84**:527−36.
5. Zeledon R, Bolanos R, Espejo Navarro MR, Rojas M. Morphological evidence by scanning electron microscopy of excretion of metacyclic forms of *Trypanosoma cruzi* in vector's urine. *Mem Inst Oswaldo Cruz* 1988;**83**:361−5.
6. Bern C. Chagas' disease. *N Engl J Med* 2015;**373**:1882.
7. Costales J, Rowland EC. A role for protease activity and host-cell permeability during the process of *Trypanosoma cruzi* egress from infected cells. *J Parasitol* 2007;**93**:1350−9.
8. Maeda FY, Cortez C, Yoshida N. Cell signaling during *Trypanosoma cruzi* invasion. *Front Immunol* 2012;**3**:361.

9. Caradonna KL, Burleigh BA. Mechanisms of host cell invasion by *Trypanosoma cruzi*. *Adv Parasitol* 2011;**76**:33–61.
10. Martins RM, Covarrubias C, Rojas RG, Silber AM, Yoshida N. Use of L-proline and ATP production by *Trypanosoma cruzi* metacyclic forms as requirements for host cell invasion. *Infect Immun* 2009;**77**:3023–32.
11. Romano PS, Arboit MA, Vazquez CL, Colombo MI. The autophagic pathway is a key component in the lysosomal dependent entry of *Trypanosoma cruzi* into the host cell. *Autophagy* 2009;**5**:6–18.
12. El-Sayed NM, Myler PJ, Bartholomeu DC, Nilsson D, Aggarwal G, Tran AN, et al. The genome sequence of *Trypanosoma cruzi*, etiologic agent of Chagas disease. *Science* 2005;**309**:409–15.
13. Alves MJ, Colli W. Role of the gp85/trans-sialidase superfamily of glycoproteins in the interaction of *Trypanosoma cruzi* with host structures. *Subcell Biochem* 2008;**47**:58–69.
14. Atwood III JA, Weatherly DB, Minning TA, Bundy B, Cavola C, Opperdoes FR, et al. The *Trypanosoma cruzi* proteome. *Science* 2005;**309**:473–6.
15. Mattos EC, Tonelli RR, Colli W, Alves MJ. The gp85 surface glycoproteins from *Trypanosoma cruzi*. *Subcell Biochem* 2014;**74**:151–80.
16. Teixeira AA, de Vasconcelos Vde C, Colli W, Alves MJ, Giordano RJ. *Trypanosoma cruzi* binds to cytokeratin through conserved peptide motifs found in the laminin-g-like domain of the gp85/trans-sialidase proteins. *PLoS NTD* 2015;**9**:e0004099.
17. Alves MJ, Colli W. *Trypanosoma cruzi*: adhesion to the host cell and intracellular survival. *IUBMB Life* 2007;**59**:274–9.
18. do Carmo MS, dos Santos MR, Cano MI, Araya JE, Yoshida N, da Silveira JF. Expression and genome-wide distribution of the gene family encoding a 90 kDa surface glycoprotein of metacyclic trypomastigotes of *Trypanosoma cruzi*. *Mol Biochem Parasitol* 2002;**125**:201–6.
19. Tonelli RR, Giordano RJ, Barbu EM, Torrecilhas AC, Kobayashi GS, Langley RR, et al. Role of the gp85/trans-sialidases in *Trypanosoma cruzi* tissue tropism: preferential binding of a conserved peptide motif to the vasculature in vivo. *PLoS NTD* 2010;**4**:e864.
20. Tonelli RR, Colli W, Alves MJ. Selection of binding targets in parasites using phage-display and aptamer libraries in vivo and in vitro. *Front Immunol* 2012;**3**:419.
21. Correa PR, Cordero EM, Gentil LG, Bayer-Santos E, da Silveira JF. Genetic structure and expression of the surface glycoprotein gp82, the main adhesin of *Trypanosoma cruzi* metacyclic trypomastigotes. *Sci World J* 2013;**2013**:156734.
22. Staquicini DI, Martins RM, Macedo S, Sasso GR, Atayde VD, Juliano MA, et al. Role of gp82 in the selective binding to gastric mucin during oral infection with *Trypanosoma cruzi*. *PLoS NTD* 2010;**4**:e613.
23. Schenkman S, Andrews NW, Nussenzweig V, Robbins ES. *Trypanosoma cruzi* invade a mammalian epithelial cell in a polarized manner. *Cell* 1988;**55**:157–65.
24. Schenkman S, Robbins ES, Nussenzweig V. Attachment of *Trypanosoma cruzi* to mammalian cells requires parasite energy, and invasion can be independent of the target cell cytoskeleton. *Infect Immun* 1991;**59**:645–54.
25. Tardieux I, Webster P, Ravesloot J, Boron W, Lunn JA, Heuser JE, et al. Lysosome recruitment and fusion are early events required for trypanosome invasion of mammalian cells. *Cell* 1992;**71**:1117–30.
26. Rodriguez A, Samoff E, Rioult MG, Chung A, Andrews NW. Host cell invasion by trypanosomes requires lysosomes and microtubule/kinesin-mediated transport. *J Cell Biol* 1996;**134**:349–62.

27. Tardieux I, Nathanson MH, Andrews NW. Role in host cell invasion of *Trypanosoma cruzi*-induced cytosolic-free Ca^{2+} transients. *J Exp Med* 1994;**179**:1017–22.
28. Rodriguez A, Martinez I, Chung A, Berlot CH, Andrews NW. Camp regulates Ca^{2+}-dependent exocytosis of lysosomes and lysosome-mediated cell invasion by trypanosomes. *J Biol Chem* 1999;**274**:16754–9.
29. Rodriguez A, Rioult MG, Ora A, Andrews NW. A trypanosome-soluble factor induces ip3 formation, intracellular Ca^{2+} mobilization and microfilament rearrangement in host cells. *J Cell Biol* 1995;**129**:1263–73.
30. Martinez I, Chakrabarti S, Hellevik T, Morehead J, Fowler K, Andrews NW. Synaptotagmin vii regulates ca(2 +)-dependent exocytosis of lysosomes in fibroblasts. *J Cell Biol* 2000;**148**:1141–9.
31. Caler EV, Chakrabarti S, Fowler KT, Rao S, Andrews NW. The exocytosis-regulatory protein synaptotagmin vii mediates cell invasion by *Trypanosoma cruzi*. *J Exp Med* 2001;**193**:1097–104.
32. 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**:5172–80.
33. Burleigh BA, Andrews NW. Signaling and host cell invasion by *Trypanosoma cruzi*. *Curr Opin Microbiol* 1998;**1**:461–5.
34. Motta FN, Bastos IM, Faudry E, Ebel C, Lima MM, Neves D, et al. The *Trypanosoma cruzi* virulence factor oligopeptidase B (OPBTc) assembles into an active and stable dimer. *PLoS ONE* 2012;**7**:e30431.
35. Caler EV, Vaena de Avalos S, Haynes PA, Andrews NW, Burleigh BA. Oligopeptidase B-dependent signaling mediates host cell invasion by *Trypanosoma cruzi*. *EMBO J* 1998;**17**:4975–86.
36. Scharfstein J, Schmitz V, Morandi V, Capella MM, Lima AP, Morrot A, et al. Host cell invasion by *Trypanosoma cruzi* is potentiated by activation of bradykinin B(2) receptors. *J Exp Med* 2000;**192**:1289–300.
37. Martins NO, Souza RT, Cordero EM, Maldonado DC, Cortez C, Marini MM, et al. Molecular characterization of a novel family of *Trypanosoma cruzi* surface membrane proteins (TcSMP) involved in mammalian host cell invasion. *PLoS NTD* 2015;**9**:e0004216.
38. Di Noia JM, Buscaglia CA, De Marchi CR, Almeida IC, Frasch ACA. *Trypanosoma cruzi* small surface molecule provides the first immunological evidence that Chagas' disease is due to a single parasite lineage. *J Exp Med* 2002;**195**:401–13.
39. Canepa GE, Degese MS, Budu A, Garcia CR, Buscaglia CA. Involvement of TSSA (trypomastigote small surface antigen) in *Trypanosoma cruzi* invasion of mammalian cells. *Biochem J* 2012;**444**:211–18.
40. Zanforlin T, Bayer-Santos E, Cortez C, Almeida IC, Yoshida N, da Silveira JF. Molecular characterization of *Trypanosoma cruzi* sap proteins with host-cell lysosome exocytosis-inducing activity required for parasite invasion. *PLoS ONE* 2013;**8**:e83864.
41. Couture R, Blaes N, Girolami JP. Kinin receptors in vascular biology and pathology. *Curr Vasc Pharmacol* 2014;**12**:223–48.
42. Scharfstein J, Lima AP. Roles of naturally occurring protease inhibitors in the modulation of host cell signaling and cellular invasion by *Trypanosoma cruzi*. *Subcell Biochem* 2008;**47**:140–54.
43. Ferreira D, Cortez M, Atayde VD, Yoshida N. Actin cytoskeleton-dependent and -independent host cell invasion by *Trypanosoma cruzi* is mediated by distinct parasite surface molecules. *Infect Immun* 2006;**74**:5522–8.

44. Ruiz RC, Favoreto Jr. S, Dorta ML, Oshiro ME, Ferreira AT, Manque PM, et al. Infectivity of *Trypanosoma cruzi* strains is associated with differential expression of surface glycoproteins with differential Ca^{2+} signalling activity. *Biochem J* 1998;**330**(Pt 1):505–11.
45. Malaga S, Yoshida N. Targeted reduction in expression of *Trypanosoma cruzi* surface glycoprotein gp90 increases parasite infectivity. *Infect Immun* 2001;**69**:353–9.
46. Yoshida N. Molecular mechanisms of *Trypanosoma cruzi* infection by oral route. *Mem Inst Oswaldo Cruz* 2009;**104**(Suppl 1):101–7.
47. Covarrubias C, Cortez M, Ferreira D, Yoshida N. Interaction with host factors exacerbates *Trypanosoma cruzi* cell invasion capacity upon oral infection. *Int J Parasitol* 2007;**37**:1609–16.
48. 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**:3611–22.
49. Woolsey AM, Burleigh BA. Host cell actin polymerization is required for cellular retention of *Trypanosoma cruzi* and early association with endosomal/lysosomal compartments. *Cell Microbiol* 2004;**6**:829–38.
50. Andrade LO, Andrews NW. Lysosomal fusion is essential for the retention of *Trypanosoma cruzi* inside host cells. *J Exp Med* 2004;**200**:1135–43.
51. Yang Z, Klionsky DJ. Mammalian autophagy: core molecular machinery and signaling regulation. *Curr Opin Cell Biol* 2010;**22**:124–31.
52. Tanida I, Ueno T, Kominami E. Lc3 and autophagy. *Methods Mol Biol* 2008;**445**:77–88.
53. Romano PS, Cueto JA, Casassa AF, Vanrell MC, Gottlieb RA, Colombo MI. Molecular and cellular mechanisms involved in the *Trypanosoma cruzi*/host cell interplay. *IUBMB Life* 2012;**64**:387–96.
54. Vanrell MC, Cueto JA, Barclay JJ, Carrillo C, Colombo MI, Gottlieb RA, et al. Polyamine depletion inhibits the autophagic response modulating *Trypanosoma cruzi* infectivity. *Autophagy* 2013;**9**:1080–93.
55. Fernandes MC, Andrews NW. Host cell invasion by *Trypanosoma cruzi*: a unique strategy that promotes persistence. *FEMS Microbiol Rev* 2012;**36**:734–47.
56. Reddy A, Caler EV, Andrews NW. Plasma membrane repair is mediated by ca(2+)-regulated exocytosis of lysosomes. *Cell* 2001;**106**:157–69.
57. Tan H, Andrews NW. Don't bother to knock—the cell invasion strategy of *Trypanosoma cruzi*. *Trends Parasitol* 2002;**18**:427–8.
58. Idone V, Tam C, Goss JW, Toomre D, Pypaert M, Andrews NW. Repair of injured plasma membrane by rapid Ca^{2+}-dependent endocytosis. *J Cell Biol* 2008;**180**:905–14.
59. Tam C, Idone V, Devlin C, Fernandes MC, Flannery A, He X, et al. Exocytosis of acid sphingomyelinase by wounded cells promotes endocytosis and plasma membrane repair. *J Cell Biol* 2010;**189**:1027–38.
60. Fernandes MC, Cortez M, Flannery AR, Tam C, Mortara RA, Andrews NW. *Trypanosoma cruzi* subverts the sphingomyelinase-mediated plasma membrane repair pathway for cell invasion. *J Exp Med* 2011;**208**:909–21.
61. Scharfstein J, Andrade D, Svensjo E, Oliveira AC, Nascimento CR. The kallikrein–kinin system in experimental Chagas disease: a paradigm to investigate the impact of inflammatory edema on GPCR-mediated pathways of host cell invasion by *Trypanosoma cruzi*. *Front Immunol* 2012;**3**:396.
62. Zhao X, Kumar P, Shah-Simpson S, Caradonna KL, Galjart N, Teygong C, et al. Host microtubule plus-end binding protein CLASP1 influences sequential steps in the *Trypanosoma cruzi* infection process. *Cell Microbiol* 2013;**15**:571–84.

63. Cortez C, Real F, Yoshida N. Lysosome biogenesis/scattering increases host cell susceptibility to invasion by *Trypanosoma cruzi* metacyclic forms and resistance to tissue culture trypomastigotes. *Cell Microbiol* 2015.
64. Ley V, Robbins ES, Nussenzweig V, Andrews NW. The exit of *Trypanosoma cruzi* from the phagosome is inhibited by raising the ph of acidic compartments. *J Exp Med* 1990;**171**:401–13.
65. Andrews NW, Whitlow MB. Secretion by *Trypanosoma cruzi* of a hemolysin active at low pH. *Mol Biochem Parasitol* 1989;**33**:249–56.
66. Andrews NW, Abrams CK, Slatin SL, Griffiths G. A *T. cruzi*-secreted protein immunologically related to the complement component C9: evidence for membrane pore-forming activity at low pH. *Cell* 1990;**61**:1277–87.
67. Manning-Cela R, Cortes A, Gonzalez-Rey E, Van Voorhis WC, Swindle J, Gonzalez A. LYT1 protein is required for efficient in vitro infection by *Trypanosoma cruzi*. *Infect Immun* 2001;**69**:3916–23.
68. Hall BF, Webster P, Ma AK, Joiner KA, Andrews NW. Desialylation of lysosomal membrane glycoproteins by *Trypanosoma cruzi*: a role for the surface neuraminidase in facilitating parasite entry into the host cell cytoplasm. *J Exp Med* 1992;**176**:313–25.
69. Lugo-Caballero C, Ballesteros-Rodea G, Martinez-Calvillo S, Manning-Cela R. Identification of protein complex associated with LYT1 of *Trypanosoma cruzi*. *Biomed Res Int* 2013;493525.
70. Caradonna KL, Engel JC, Jacobi D, Lee CH, Burleigh BA. Host metabolism regulates intracellular growth of *Trypanosoma cruzi*. *Cell Host Microbe* 2013;**13**:108–17.
71. Diehn M, Relman DA. Comparing functional genomic datasets: lessons from DNA microarray analyses of host–pathogen interactions. *Curr Opin Microbiol* 2001; **4**:95–101.
72. 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**:639–44.
73. Blader IJ, Manger ID, Boothroyd JC. Microarray analysis reveals previously unknown changes in *Toxoplasma gondii*-infected human cells. *J Biol Chem* 2001; **276**:24223–31.
74. Eckmann L, Smith JR, Housley MP, Dwinell MB, Kagnoff MF. Analysis by high density CDNA arrays of altered gene expression in human intestinal epithelial cells in response to infection with the invasive enteric bacteria salmonella. *J Biol Chem* 2000;**275**:14084–94.
75. Costales JA, Daily JP, Burleigh BA. Cytokine-dependent and -independent gene expression changes and cell cycle block revealed in *Trypanosoma cruzi*-infected host cells by comparative mrna profiling. *BMC Genomics* 2009;**10**:252.
76. Noppert SJ, Fitzgerald KA, Hertzog PJ. The role of type I interferons in TLR responses. *Immunol Cell Biol* 2007;**85**:446–57.
77. Lopez de Padilla CM, Niewold TB. The type I interferons: basic concepts and clinical relevance in immune-mediated inflammatory diseases. *Gene* 2016;**576**:14–21.
78. Takeuchi O, Akira S. Pattern recognition receptors and inflammation. *Cell* 2010;**140**:805–20.
79. Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. *Cell* 2006;**124**:783–801.
80. Lin R, Mamane Y, Hiscott J. Structural and functional analysis of interferon regulatory factor 3: localization of the transactivation and autoinhibitory domains. *Mol Cell Biol* 1999;**19**:2465–74.

81. Sharma S, tenOever BR, Grandvaux N, Zhou GP, Lin R, Hiscott J. Triggering the interferon antiviral response through an IKK-related pathway. *Science* 2003; **300**:1148–51.
82. Gurtler C, Bowie AG. Innate immune detection of microbial nucleic acids. *Trends Microbiol* 2013;**21**:413–20.
83. Chessler AD, Ferreira LR, Chang TH, Fitzgerald KA, Burleigh BA. A novel IFN regulatory factor 3-dependent pathway activated by trypanosomes triggers IFN-beta in macrophages and fibroblasts. *J Immunol* 2008;**181**:7917–24.
84. Koga R, Hamano S, Kuwata H, Atarashi K, Ogawa M, Hisaeda H, et al. TLR-dependent induction of IFN-beta mediates host defense against *Trypanosoma cruzi*. *J Immunol* 2006;**177**:7059–66.
85. Bhat N, Fitzgerald KA. Recognition of cytosolic DNA by CGAS and other sting-dependent sensors. *Eur J Immunol* 2014;**44**:634–40.
86. Hare DN, Collins SE, Mukherjee S, Loo YM, Gale Jr. M, Janssen LJ, et al. Membrane perturbation-associated Ca^{2+} signaling and incoming genome sensing are required for the host response to low-level enveloped virus particle entry. *J Virol* 2015;**90**:3018–27.
87. Chessler AD, Unnikrishnan M, Bei AK, Daily JP, Burleigh BA. *Trypanosoma cruzi* triggers an early type I IFN response in vivo at the site of intradermal infection. *J Immunol* 2009;**182**:2288–96.
88. Tarleton RL, Koller BH, Latour A, Postan M. Susceptibility of beta 2-microglobulin-deficient mice to *Trypanosoma cruzi* infection. *Nature* 1992;**356**:338–40.
89. Tarleton RL, Grusby MJ, Postan M, Glimcher LH. *Trypanosoma cruzi* infection in MHC-deficient mice: further evidence for the role of both class I- and class II-restricted T cells in immune resistance and disease. *Int Immunol* 1996;**8**:13–22.
90. Martin DL, Murali-Krishna K, Tarleton RL. Generation of *Trypanosoma cruzi*-specific CD8 + T-cell immunity is unaffected by the absence of type I interferon signaling. *Infect Immun* 2010;**78**:3154–9.
91. Chessler AD, Caradonna KL, Da'dara A, Burleigh BA. Type I interferons increase host susceptibility to *Trypanosoma cruzi* infection. *Infect Immun* 2011;**79**:2112–19.
92. Teles RM, Graeber TG, Krutzik SR, Montoya D, Schenk M, Lee DJ, et al. Type I interferon suppresses type II interferon-triggered human anti-mycobacterial responses. *Science* 2013;**339**:1448–53.
93. Ives A, Ronet C, Prevel F, Ruzzante G, Fuertes-Marraco S, Schutz F, et al. *Leishmania* RNA virus controls the severity of mucocutaneous leishmaniasis. *Science* 2011;**331**:775–8.
94. Ronet C, Beverley SM, Fasel N. Muco-cutaneous leishmaniasis in the New World: the ultimate subversion. *Virulence* 2011;**2**:547–52.
95. Hartley MA, Ronet C, Zangger H, Beverley SM, Fasel N. *Leishmania* RNA virus: when the host pays the toll. *Front Cell Infect Microbiol* 2012;**2**:99.
96. Hartley MA, Drexler S, Ronet C, Beverley SM, Fasel N. The immunological, environmental, and phylogenetic perpetrators of metastatic leishmaniasis. *Trends Parasitol* 2014;**30**:412–22.