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Critical Review

Molecular and Cellular Mechanisms Involved in the *Trypanosoma cruzi*/Host Cell Interplay

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Summary

The protozoan parasite *Trypanosoma cruzi* has a complex biological cycle that involves vertebrate and invertebrate hosts. In mammals, the infective trypomastigote form of this parasite can invade several cell types by exploiting phagocytic-like or nonphagocytic mechanisms depending on the class of cell involved. Morphological studies showed that when trypomastigotes contact macrophages, they induce the formation of plasma membrane protrusions that differ from the canonical phagocytosis that occurs in the case of noninfective epimastigotes. In contrast, when trypomastigotes infect epithelial or muscle cells, the cell surface is minimally modified, suggesting the induction of a different class of process. Lysosomal-dependent or -independent *T. cruzi* invasion of host cells are two different models that describe the molecular and cellular events activated during parasite entry into nonphagocytic cells. In this context, we have previously shown that induction of autophagy in host cells before infection favors *T. cruzi* invasion. Furthermore, we demonstrate that autophagosomes and the autophagosomal protein LC3 are recruited to the *T. cruzi* entry sites and that the newly formed *T. cruzi* parasitophorous vacuole has characteristics of an autophagolysosome. This review summarizes the current knowledge of the molecular and cellular mechanisms of *T. cruzi* invasion in nonphagocytic cells. Based on our findings, we propose a new model in which *T. cruzi* takes advantage of the upregulation of autophagy during starvation to increase its successful colonization of host cells.

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INTRODUCTION

Trypanosoma cruzi is the etiological agent of Chagas' disease, a potentially life-threatening illness also known as American trypanosomiasis. Epidemiological data from WHO estimates that 10 million people are infected worldwide, mostly in Latin America where Chagas disease is endemic. However, in recent decades, it has been increasingly detected in the United States of America, Canada, and many European and some Western Pacific countries. Additionally, more than 25 million people are at risk of the disease (http://www.who.int/neglected_diseases/diseases/chagas/en/index.html).

Among the trypanosomatids, *T. cruzi* presents one of the most complex life cycles (*1*), with several developmental stages involving the vertebrate and the invertebrate host species. In host vertebrates, *T. cruzi* behaves as an obligate intracellular pathogen that displays multiple mechanisms to manipulate a variety of host cells processes for invasion. Inside the cell, the infective trypomastigote form (Try) is temporarily contained in a membrane vesicle, the *T. cruzi* parasitophorous vacuole (TcPV), and subsequently, the parasite escapes to the cytosol, differentiates into the amastigote form (Ama), and replicates by binary division (*2, 3*). This replication process culminates after several cycles followed by a new differentiation phase, where the parasites undergo a transition back into the Try stage. Try released from the cell can infect the neighboring cells or reach the bloodstream and circulate before they attach to and enter new cells for another cycle of differentiation and replication. This review is focused on the molecular and cellular events that take place in the host cells during *T. cruzi* infection. To date, different and even contradictory mechanisms of infection have been

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proposed: the phagocytic-like *T. cruzi* invasion and the lysosomal-dependent or -independent *T. cruzi* entry models are the most recognized. These models are even used as paradigms to explain this particular mode of infection. The *T. cruzi*–host cell interaction process is extremely complex, due to: the high number of *T. cruzi* strains; the different parasitic forms (Try or Ama) that can infect cells; the variety of trypomastigote sources (MT: metacyclic trypomastigotes, BT: blood trypomastigotes or TCT: tissue cell derived trypomastigotes); and finally the type of host cell involved. According to this concept, different scenarios could be observed depending on the specific combination of these variables. A couple of years ago, we described the participation of autophagy in *T. cruzi* invasion (*4, 5*). Here, we summarize the main aspects of the different mechanisms involved in *T. cruzi* infection and show our recent findings about the interaction between this parasite and the host cell autophagic pathway.

CELLULAR AND MOLECULAR EVENTS DURING *T. cruzi* INVASION

T. cruzi is a key example of adaptation between lower eukaryotic and mammalian organisms. Studies from diverse laboratories using different host cell types and *T. cruzi* strains have demonstrated that this parasite can invade almost all nucleated cells, phagocytic, or nonphagocytic. Interestingly, it has been shown that parasite uptake, differentiation, and multiplication can also take place in L929 murine aneuploid fibrosarcoma enucleated cells, albeit with a significantly lower parasite load at 48 and 72 h (*6*).

Despite not all of the processes involved in cellular infection are fully understood, many cellular and molecular events during *T. cruzi* invasion have been clarified in recent years. During invasion, it is possible to distinguish two main steps: 1) *T. cruzi* attachment to host plasma membrane and 2) cellular engulfment of parasites to allow *T. cruzi* internalization and TcPV formation. Specific signaling events are triggered after parasite adhesion, which leads to a particular mechanism of parasite internalization. In this work, each cascade will be described in association with the cellular processes that are activated.

T. cruzi Adhesion Molecules

A large number of molecules have been involved in the binding of Try to the host cell plasma membrane. These molecules, presented in the glycocalyx of *T. cruzi*, are grouped into several families, including mucins, trans-sialidases (TSs), TS-like molecules, and integral membrane proteins. Mucins are the major glycoproteins of *T. cruzi* surface; mucin-encoded genes represent 1% of the parasite genome. These molecules have a small core comprising 50–200 amino acids with sequences rich in Ser and Thr. These residues are the acceptor sites for *O*-linked oligosaccharides, which confer to mucins that have a strong hydrophilic character (*7*). The expression of a large repertoire of mucins containing variable regions during the mammalian infective stages of *T. cruzi* suggests a possible interaction between Try and the host cell to accomplish cellular adhesion and entry and to escape from host immune response. TSs and TS-like molecules transfer sialic-acid residues from host glycoconjugates to parasite mucins. After cleavage of its glycosylphosphatidylinositol anchor by the action of a phosphatidylinositol (PI)-phospholipase C, TSs and TS-like molecules are shed into the bloodstream to upregulate the early infection (*8*). Although all these *T. cruzi* ligands have been implicated in the attachment and invasion of phagocytic and nonphagocytic cells, the connection after parasite adhesion with a specific cellular response is largely unknown. A recently published work showed that the *T. cruzi* surface molecule gp82, present in MT, mediates parasite adhesion and lysosomal exocytosis that are required for parasite invasion (*9*). Putative receptors for these ligands, including Cytokeratin 18 (*10, 11*), P74 (*12*), and Galectin-3 (*13*), have been found in the host cell plasma membrane. Furthermore, components of extracellular matrix-like laminin gamma-1 (*11*), thrombospondin-1, and fibronectin are upregulated during *T. cruzi* infection and have also been implicated in cellular infection (see ref. *14* for a review). Considering the growing list of *T. cruzi* ligands and host cell targets, a large number of parasite–host cell interactions are possible and could explain the high versatility displayed by *T. cruzi* when invading different cell types.

Molecular and Cellular Mechanisms of T. cruzi Engulfment

According to their primary function (i.e., phagocytic or nonphagocytic), host cells show different behaviors during *T. cruzi* internalization. Earlier studies assumed that Try entered all mammalian cells by a phagocytic mechanism (*15*), like most other intracellular parasites, but further investigations showed an alternative mechanism with active penetration of the host cell by the parasite. A morphological approach supported this view since Schenkman et al. (*16*) showed, using scanning electron microscopy, that Try enter epithelial cells and fibroblasts with no pseudopod formation or any other alteration of plasma membrane (like phagocytic cup formation or zippering). On the other hand, it has been shown that the macrophages' plasma membrane covers the parasite by forming a tubular structure or a structure described as a coiled-coil phagosome. Although this process seems to be similar to phagocytosis, evidences suggest that, in contrast to the noninfective epimastigotes (Epi), Try actively direct their own infection in macrophages (*17*).

THE "PHAGOCYTOSIS-LIKE" MODEL OF *T. cruzi* INVASION

The initial studies of parasite–host cell interactions were performed in macrophages as resident tissue macrophages are the first host cell to be invaded by this pathogen during *in vivo* infection. Both Try and Epi are efficiently internalized by macrophages and are found within phagolysosomes (*18, 19*). How-

ever, only Try can escape from the phagolysosome and multiply in the cytosol, whereas Epi are destroyed (*19, 20*). The escape of Try to the cytosol is a crucial event because in activated macrophages, the nitric oxide (NO) generated in the TcPV is the most effective agent against *T. cruzi* (*21*). Also peroxynitrite generated by combination of NO and superoxide anion (O_2^-) in the phagosomal compartment was recently shown to cause parasite killing (*22*). *T. cruzi* is very susceptible to cell damage induced by these metabolites, because enzymes capable of scavenging free radicals are absent or have very low activities in the parasite. Indeed, the trypanocidal drugs such as nifurtimox and benznidazole act via nitrogen free radicals generation (*23*).

Regardless of their final fate, the mechanism by which Try enter macrophages has been a subject of controversy. Although several authors reported that entry was blocked in the presence of actin polymerization inhibitors such as cytochalasin B (*24–27*), others found no inhibition and concluded that invasion was active for a subset of the parasites (*28*). In addition, it has been shown that parasite entry in macrophages may involve the participation of host cell membrane microdomain-like flat domains (rich in flotillin proteins) and caveolae (*29*). Therefore, it can be concluded that in macrophages at least two mechanisms can proceed in parallel, which would explain the apparently contradictory early observations. Some Try stimulate tyrosin-kinases receptors leading to tyrosine phosphorylation, PI 3-kinase recruitment, and assembly of actin filaments in a phagocytic-like actin-dependent invasion mechanism, whereas others enter the cell in membrane depressions formed for flotilin and caveolin. The extent by which these two mechanisms contribute to parasite entry is not well established, but it is known that different *T. cruzi* strains with different subsets of superficial glycoproteins can induce opposite host responses. For example, in nonphagocytic HeLa cells, it was demonstrated that MT from the CL strain that express gp82 activate actin depolymerization, whereas the G strain that has predominantly gp 35/50 produces microfilaments recruitment to the enter sites (*30*). Amastigotes, the intracellular replicative form of *T. cruzi* can, in some cases, be generated in the extracellular milieu and are able to infect cells. In this case, they enter cells by phagocytosis, by means of the actin microfilament system (*31*).

THE LYSOSOMAL-DEPENDENT *T. cruzi* INVASION PROCESS

As mentioned above, *T. cruzi* can enter macrophages either by a phagocytic-like or a nonphagocytic mechanism, whereas nonprofessional phagocytes, like fibroblasts or epithelial cells, are infected by the latter mechanism. The major insights into the mechanism by which nonphagocytic cells are infected were provided by Andrews and colleagues. By using lysosomal markers (e.g., gp120 and Bovin Serum Albumin, BSA gold) in different nonphagocytic cell lines, the authors clearly showed the recruitment of lysosomes to trypomastigote attachment sites (*32*). The lysosome-dependent *T. cruzi* entry model consists in the formation of clusters of lysosomes in proximity to the posterior ends of extracellular parasites, and subsequently with intracellular portions of trypanosomes undergoing internalization. So, it is believed that lysosomal fusion serves as source of membrane to form TcPV.

The initiation of this internalization mechanism requires an early transduction of specific signals across the plasma membrane and the subversion of host cell signaling pathways. A variety of second messengers triggered by host–parasite interaction have been postulated (see ref. *33* for an extensive review). However, transient and repetitive elevations of intracellular calcium concentrations ($[Ca^{2+}]_i$) induced by Try seems to play a major role in the recruitment and fusion of lysosomes, as buffering or depletion of host cell intracellular $[Ca^{2+}]_i$ blocks parasite entry (*34, 35*). In contrast, Epi, the noninfective forms, and the exclusively extracellular trypanosome *T. brucei* do not elicit this response, indicating that only the parasitic infective forms have the competence to induce this signal into the host cell (*34*). In addition to $[Ca^{2+}]_i$ transients, intracellular cyclic adenosine-monophosphate (cAMP) plays a key role in the invasion mechanism, as Try entry is markedly reduced in cells treated with adenylyl cyclase inhibitors (*36*) and also seems to be responsible for the residual invasion capacity of parasites when host cell Ca^{2+} transient is somehow blocked (e.g., thapsigargin) (*25*). The lysosomes recruited to the parasite attachment site require their mobilization from the perinuclear area via microtubules. Significant inhibition of Try entry was observed in cells pretreated with microtubule inhibitors (i.e., colchicine, vinblastine, taxol, and nocodazol) (*37*). As these drugs differentially affect microtubule function (by stabilization or depolymerization), the observed impairment of Try entry is likely due to the inhibition of plus-ended lysosomal trafficking. Two nonmutually excluded links between $[Ca^{2+}]_i$ transients and lysosome recruitment have been proposed. One comes from the possibility that Ca^{2+} signaling modulates the motor activity of kinesin, a plus end-directed microtubule motor protein, through Ca^{2+}–calmodulin interaction (*37*). The other rises from the observation that recombinant Synaptotagmin (Syt) VII C_2A domain and anti-Syt VII C_2A antibodies specifically inhibited host cell invasion by trypomastigotes. Syt VII is a ubiquitously expressed member of Ca^{2+}-binding proteins that modulates Ca^{2+}-dependent exocytosis of lysosomes (*38*).

It has been shown that conversely to a functional microtubule requirement for lysosomal transport to the cell periphery, actin cytoskeleton rearrangements have also a beneficial effect on this process. Disruption of microfilaments by cytochalasin D increased cellular susceptibility to *T. cruzi* infection; the same applies for latrunculin A and phalloidin (*32, 35*). In view of these later findings, it was concluded that a cortical actin rearrangement was a required step that allows contact and fusion between lysosomes and plasma membrane during TcPV formation (*31*).

Further investigations revealed that direct fusion of lysosomes with the plasma membrane represents an extended mechanism present in all mammalian cells, involved in the repair of

plasma membrane lesions (*39*). Therefore, it has been postulated that the parasite takes advantage of this mechanism to gain access to the intracellular environment (*40*). In agreement with this idea, a recently published work showed that acid sphingomyelinase (ASM) is a critical enzyme that, transported in lysosomes, gains access to the outer leaflet of the plasma membrane and promotes a rapid repair of the injury (produced by *T. cruzi* in this case). The mechanism of repair involves ceramide, the product of the action of ASM on sphingomyelin; this lipid favors deformation of the membrane, thereby stimulating the endocytosis of damaged portions of the plasma membrane (*41*). Thus, it has been proposed that *T. cruzi* subverts the ASM-dependent ceramide-enriched endosomes, which normally function in plasma membrane repair to infect host cells.

It is important to mention that *T. cruzi* requires transient residence within acidic organelles for productive infection. The low pH environment of lysosomes facilitates parasite escape from the vacuole and delivery into the host cytosol, a critical step in the *T. cruzi* developmental program. Moreover, early lysosome fusion with invading or recently internalized parasites is critical for cellular retention of parasites. (*42*) The presence of an acidic pH favors the expression and activity of Tc-Tox, a *T. cruzi* pore-forming toxin that permits the escape to the cytosol after vacuole membrane disruption (*20*). The fast interaction of *T. cruzi* with acidic compartments is one of the most important aspects to be considered to maintain *T. cruzi* in the cell as it has been observed that *T. cruzi* can exit back from vacuoles to the extracellular space (*41, 42*).

THE LYSOSOMAL-INDEPENDENT MODEL

A few years ago, a group described a new form of *T. cruzi* entry in nonphagocytic cells based on the generation of a vacuole initially enriched in PtdIns*P*3/PtdIns(3,4)*P*2 and devoid of lysosomal markers (*43*). Using the Akt-PH-GFP probe, these authors showed the acquisition of these phosphoinositides in the nascent *T. cruzi* vacuole at very early times after interaction (between 2 and 5 min). This association is maintained for approximately 15 min; in contrast, the Lamp-1 association is minimal at this time but increases at 60 min, indicating that the lysosomal recruitment to TcPV occurred later. Based on the above observations, the authors postulated a new lysosomal-independent model of *T. cruzi* entry in nonphagocytic cells consisting of an intimate association with host cell plasma membrane markers as an initial step in cell invasion. As the labeling with those markers was around 50% compared to the 20% represented by the lysosomal markers, this pathway seems to be more important at the times analyzed.

Compared with the lysosomal-dependent process, the lysosomal-independent *T. cruzi* entry process appears to be more significant early after internalization favoring the plasma membrane invagination, whereas lysosomal fusion is a late but essential step required for the establishment of a productive infection (*42*) and for progression and completion of *T. cruzi* intracellular cycle (*20, 44*). For this reason, parasites that first enter cells in a plasma membrane-derived vacuole gradually accumulate the lysosomal markers Lamp-1 and endocytosed TR-dextran (Texas Red dextran), like those observed for the maturation of latex bead phagosomes. In agreement with this model, Rab 5, Dynamin, and Rab 7 are acquired on TcPV at different times after infection and can regulate vacuole formation and maturation in nonphagocytic cells (*45*). However, the kinetics of Lamp-1 and lysosomal TR-dextran acquisition by the *T. cruzi* vacuole was found to be insensitive to PI 3-kinase inhibition by wortmannin, in contrast to the effect of this inhibitor on phagosomal maturation and lysosomal exocytosis, indicating that both lysosome-dependent and lysosome-independent *T. cruzi* invasion are mechanistically related but temporally distinct processes. Although both models lead to harbor *T. cruzi* in lysosomes, the initial events that characterize each process are different. Although the lysosomal-dependent model shows that *T. cruzi* can directly enter in lysosomes after fusion of these compartments with the plasma membrane, the lysosomal-independent model demonstrates that the parasite can enter by invagination of the plasma membrane generating a vacuole initially different from lysosomes (i.e., with early endosomal features). Thus, the different early events presented in each model strongly suggest that both mechanisms may exist as separated entities.

THE AUTOPHAGIC MODEL OF *T. cruzi* INVASION

New data from our laboratory have recently revealed a connection between *T. cruzi* and the host cell autophagic pathway. Using epithelial chinese hamster ovary cells (CHO cells) overexpressing green fluorescent protein tagged LC3 (GFP-LC3), the best characterized autophagosome marker, colocalization between the TcPV and LC3 was observed by confocal microscopy (*4*). Quantification studies showed that the maximal recruitment of LC3 occurs between 1 and 6 h after infection, related to the intrinsic capacity of the particular Try strain to escape the vacuole. At earlier times (i.e., less than 1 h) we observed by real time video microscopy, numerous GFP-LC3 decorated autophagosomes concentrated near the plasma membrane in the vicinity of the Try contact site. This observation, associated with the fact that GFP-LC3 was specifically recruited to the cytosolic face of the plasma membrane at *T. cruzi* contact sites, indicates that parasite interaction with LC3 positive compartments occurs at very early times in the TcPV formation and persists during vacuole transport until parasite escape. At later times, 48 or 72 h after infection, when amastigotes were distributed in the cytoplasm, interaction with GFP-LC3 or endogenous LC3 proteins was no longer observed (*4*).

The recruitment of LC3 to the TcPV is modified in relation to the level of autophagic activity of the host cell. Under conditions that induce autophagy, such as starvation or rapamycin treatment, the extent of colocalization significantly increases. In contrast, the LC3 acquisition was impaired under conditions that inhibit autophagy. Furthermore, recent results from our

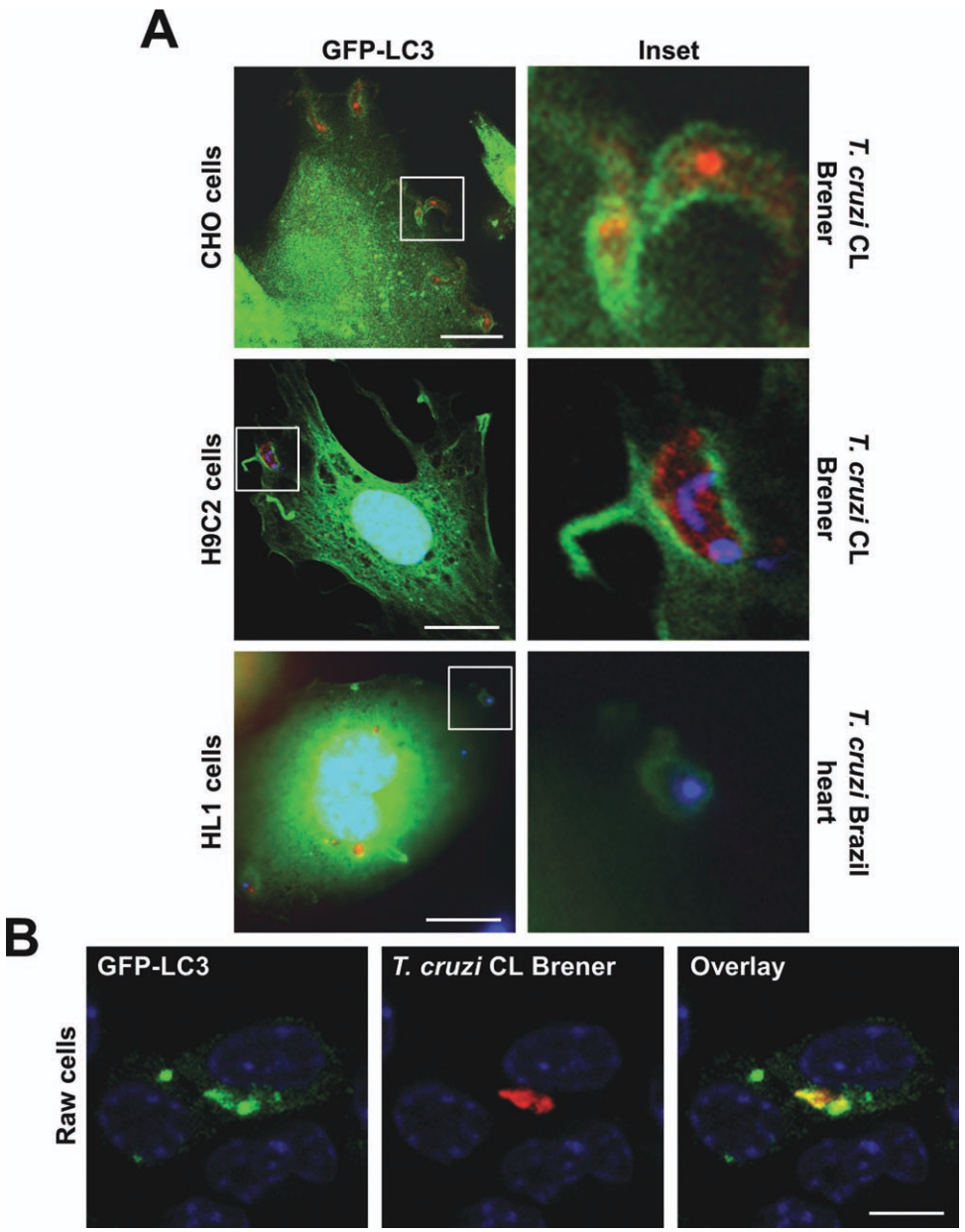

Figure 1. Interaction of *T. cruzi* trypomastigotes and host cell autophagic pathway proceeds in different classes of host cells and *T. cruzi* strains. Stably transfected cells overexpressing GFP-LC3 were infected for 1 h with TCT of *T. cruzi* (Multiplicity of infection, MOI = 50) followed by detection of the parasites by indirect immunofluorescence using specific antibodies or by the DNA marker Hoechst. Panel A: confocal images showing invasion of trypomastigotes of different *T. cruzi* strains enwrapped with GFP-LC3 from the indicated epithelial or cardiac muscle derived cells. Panel B: confocal images depicting the *T. cruzi*/GFP-LC3 interaction in macrophages Raw 264.7, 1 h after infection. Bars: 10 μm.

laboratory have confirmed that this protein is present at the membrane that envelops *T. cruzi* Try during invasion and TcPV formation by different *T. cruzi* strains in distinct classes of host cells. Figure 1A shows parasites surrounded by this autophagic protein at the time that they are entering the cells. Different examples of epithelial and cardiac muscle derived host cells (CHO cells, rat heart myoblast H9C2 cells and mouse cardiomyocyte HL1 cells) overexpressing GFP-LC3 were used and also different *T. cruzi* strains including Brazil heart, K98 (not shown) and CL Brener clone. These observations suggest that *T. cruzi*–host autophagy interaction represents a widespread phenomenon that proceeds in cells relevant for the pathophysiology of the infection and Chagas disease. Moreover, we extended our studies to phagocytic cells and observed in

RAW264.7 macrophages the same interaction events (Fig. 1B) with a percentage of colocalization that increased after starvation induction (data not shown).

The crucial role of the *T. cruzi*–host autophagy interaction was demonstrated when quantification studies showed that autophagy induction significantly increased the percentage of infected cells at 1–3 h after infection. Interestingly, this percentage was markedly reduced in the presence of the PI3K inhibitors wortmannin or 3-methyladenine or in the absence of the specific autophagy genes Beclin-1 or Atg5, necessary for the first steps of the autophagic pathway (*4*). In addition, in the new host cell systems analyzed, increased host cell colonization by *T. cruzi* was always observed under autophagy induction (data not shown). Nevertheless, different parasitic forms could engage different host cell responses during invasion as shown for MT, which activate signaling cascades involving mammalian target of rapamycin and/or PI3K/PKC (*9*), which are opposite to previously demonstrated for *T. cruzi* TCT (*4*).

We have also shown that when autophagy is induced, the localization of lysosomal markers like Lamp-1 and Cathepsin D increase on TcPV, whereas in Atg 5 KO cells the localization of these markers are significantly reduced at 15 min or 1 h after infection. Taking into account that *T. cruzi* needs the lysosomal environment to effectively infect host cells (*20, 42, 46*), this result provides an explanation for why these autophagy impaired cells (or cells pretreated with autophagy inhibitors) have a reduced capacity to become infected. Although Lamp-1 and Cathepsin D are typically used as lysosomal markers, a strong body of evidences shows that these proteins are located in other cellular compartments like the trans-Golgi network, late endosomes, and even in the plasma membrane in the case of Lamp-1 or Lamp-2. For this reason, to further analyze the specific localization of lysosomes and autophagosomes during *T. cruzi* invasion, we preloaded CHO GFP-LC3 cells with a self-quenched bovine albumin (DQ-BSA, Molecular Probes, Eugene, Oregon) and performed time-lapse live images of the infection process. The advantage of this probe is that it only emits fluorescence when albumin is degraded to small peptides; this reaction occurs in the presence of acidic proteases restricted to lysosomes. Thus, under starvation, compartments labeled with GFP-LC3 and DQ-BSA, representing autophagolysosomes (or autolysosomes), are transported and fuse to plasma membrane domains that contain invading Try (Fig. 2, Copyright Landes Bioscience). Originally published in Ref. *4*, PMID: 19115481; http://dx.doi.org/10.4161/auto.5.1.7160. Furthermore, it is possible to see the parasite body inside the cell wrapped by a membrane containing GFP-LC3 and autolysosomes in close proximity to this large structure that is forming. GFP-LC3 could have been left behind in this locale after autolysosomes have fused with the plasma membrane. Nevertheless, we cannot exclude the possibility that the GFP-LC3 protein may be recruited directly from the cytosol before the arrival of autolysosomes. Both situations support the crucial role of this pathway in the *T. cruzi* invasion process.

Considering the aforementioned results, we propose a general model in which under autophagic induction host cells produce a new set of autolysosomal vesicles that are available to migrate to the cell surface and to shelter the parasites after fusion with the plasma membrane (Fig. 3). To invade host cells, *T. cruzi* exploits the lysosomal exocytosis machinery to attract these compartments to the cell surface, but under normal conditions when cells are maintained in full nutrient media, the number of lysosomes available for *T. cruzi* is more limited, compared to the numerous acidic vesicles present under starvation conditions (*47*). *T. cruzi* has also the capacity to recruit the autophagolysosomes specifically to the invasion sites. The final result is an enhanced infection rate in starved compared to control cells. According to our working model, all conditions that activate the autophagic flux (and the autophagolysosome generation) would benefit *T. cruzi* colonization of cells. Interestingly, cells cotransfected to overexpress both LC3 and VAMP-7 proteins present an elevated degree of infection in both full nutrient and starvation conditions (data not shown). As VAMP-7 is a SNARE protein that favors fusion between autophagosomes and late endosomes/lysosomes, activating the autophagic flux (*48*), these results support the proposed model.

The autophagolysosomal nature of the recently formed TcPV could have other benefits for the parasites within, compared with the canonical lysosomal compartments. In principle, autophagolysosomes are compartments enriched in a high variety of simple nutrients originated from complex intracellular components previously entrapped by autophagic transport and degraded by lysosomal hydrolases. Furthermore, the membrane of autophagosomes has particular physical properties that permit the interaction (and concentration) of specific compounds, like monodansyl cadaverine (MDC), a fluorescent polyamine used to label late autophagosomes/autolysosomes (*49*). In a previous work, we have demonstrated that polyamines are required for *T. cruzi* differentiation (*50*); thus, it is possible that other physiological polyamines (e.g., putrescine, spermidine, and spermine) could be concentrated in these compartments in the same way as MDC generating a more favorable environment to initiate Try differentiation.

Another interesting point to consider is that invading trypomastigotes cause a substantial mechanical lesion of the plasma membrane that should be rapidly repaired to maintain the internal electrolyte equilibrium and cell viability. Considering that autophagy is the main process involved in the clearance of damaged organelles, the rapid recruitment and accumulation of LC3 at the specific *T. cruzi* entry sites and the subsequent fusion with autolysosomes could be interpreted as a mechanism to repair the recently produced injury. In this sense, as mentioned above the work from the group of Andrews (*41*) revealed that Try wound the host cell membrane and trigger a Ca-dependent plasma membrane repair mechanism mediated by lysosomal exocytosis. Although the majority of the processes described in this report reinforce the lysosomal-dependent *T. cruzi* entry model, none of them discard the participation of the autophagic pathway.

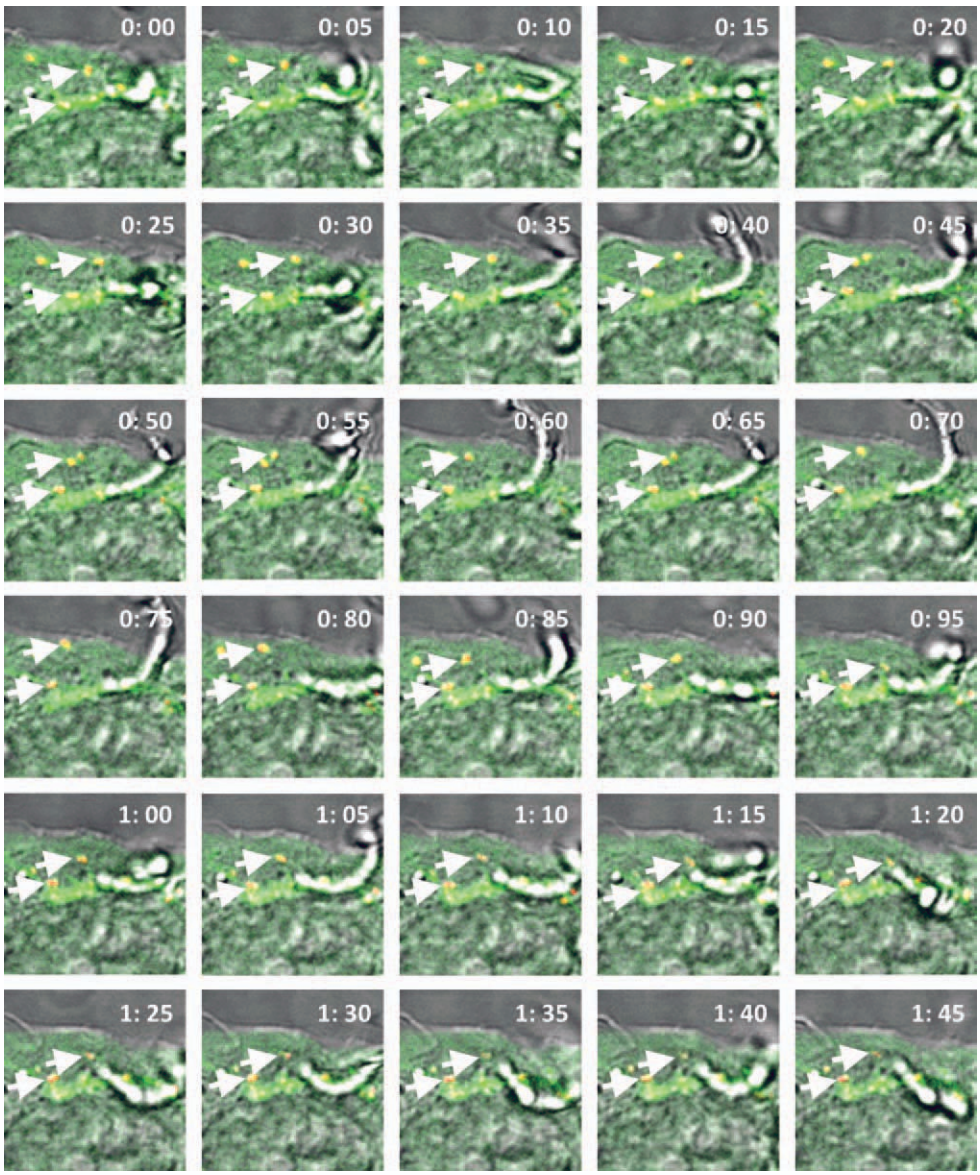

Figure 2. Autolysosomes associate to parasites during *T. cruzi* infection. Stably transfected CHO cells overexpressing GFP-LC3 were labeled with DQ-BSA (10 μg/mL) for 1 h and incubated for 2 h in starvation medium before infection with TCT of the CL Brener strain (MOI = 50). Cells were mounted on a biosafety chamber maintained at 37 °C and immediately analyzed by time-lapse confocal microscopy. Parasites were visualized by phase contrast and autolysosomes labeled with DQ-BSA (red) and decorated by GFP-LC3 (green) moving toward the parasite are indicated by arrows. A total of 30 slides every 5 sec were taken. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Another interesting consideration is that some pathogens enter phagocytic cells using mechanisms to avoid the activation of the respiratory burst. The lipophosphoglycan, the major surface glycoconjugate of *Leishmania* promastigotes, has been reported to play an active role in protecting parasites within phagolysosomes via the impairment of killing mechanisms (*51*). It could be possible that the generation of a vacuole with autophagosomal characteristics in macrophages (Fig. 1B) can help *T. cruzi* Try to prevent the generation of NO and peroxynitrites that can kill them.

The new model presented here proposes that *T. cruzi* exploits a previously induced autophagic pathway to efficiently colonize the host cell. Although *T. cruzi* has the capacity to infect the majority of the cells, it was demonstrated that cardiac and smooth muscle myocytes are the more susceptible cells to this parasitic infection (*52, 53*). Indeed, heart or digestive lesions characterize the chronic stage of Chagas disease (*54*). On the other hand, using mice models it was demonstrated that tissues like cardiac or smooth muscles activate autophagy 24–48 h after starvation and that contrary to

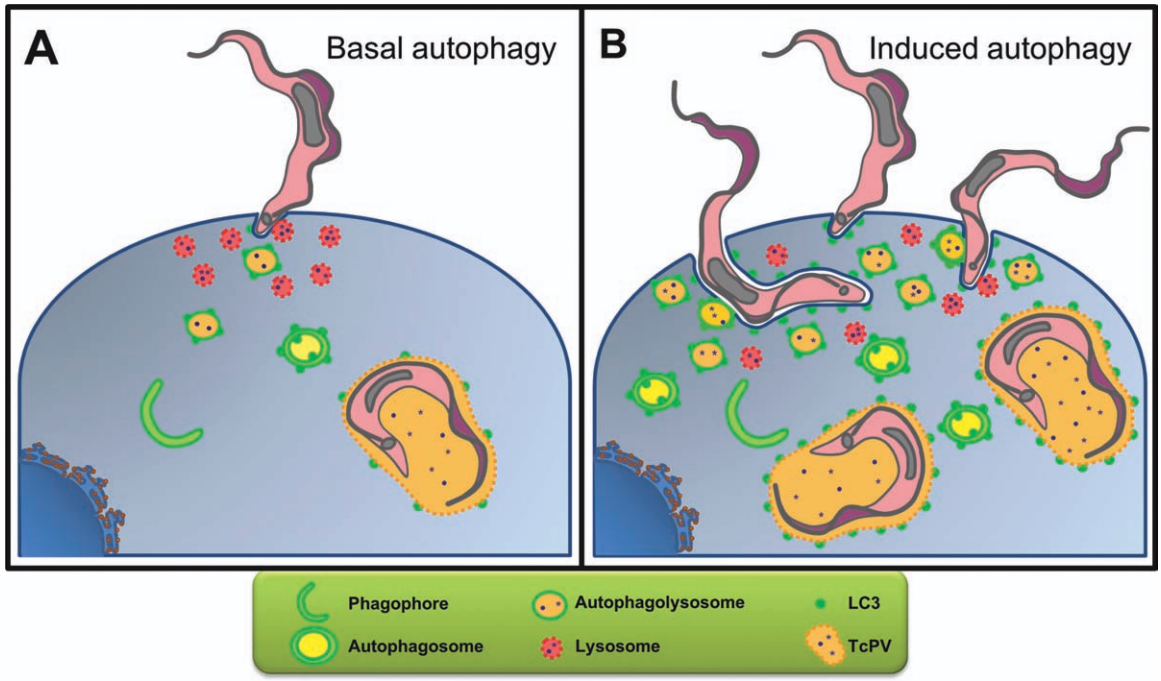

Figure 3. A proposed model of *T. cruzi* infection involving the host cell autophagic pathway. Panel A: the parasite subverts the lysosomal exocytic process to invade host cells in basal conditions. Note that basal autophagy also would provide acidic vesicles (i.e., autophagolysosomes). Panel B: when the autophagic pathway is induced by means of starvation or rapamycin, the number of autophagolysosomes augments compared with basal conditions. The protein LC3 is recruited to the cytoplasmic surface of the plasma membrane at the *T. cruzi* invasion sites leading the parasitophorous vacuole formation. Trypomastigote forms of *T. cruzi* temporally reside in a vacuole with autophagolysosomal characteristics before escaping to cytosol. The final result is an augmented level of infection with *T. cruzi* in cells with increased autophagic response.

organs like liver that quickly returns to the basal level, this response is sustained (*55*). In these conditions, it could be possible that contact with *T. cruzi* favors the rapid colonization of these target tissues increasing the probability to develop heart disease. We do not exactly know which is the specific role of autophagy in the natural human infection. However, epidemiological data show that only 30% of infected people present the chronic clinical manifestations. Although many factors could be participating in the development of chronic disease, one of the most important is the life conditions including the nutritional status of the infected people. It is tempting to speculate that a nutritional deficit produces in humans a strong induction of autophagy that could benefit parasite infection as well as the persistence in target organs and the generation of chronic disease. Although the connection between a low nutrition and the presence of infections may result obvious, to date the molecular aspect of this relationship has not been addressed.

Considering all the concepts and experimental data mentioned above and taking into account the remarkable versatility displayed by *T. cruzi* to infect cells, it is highly probable that the real paradigm will be a hybrid situation where some features of the proposed models of *T. cruzi* entry in nonphagocytic cells occur simultaneously. Moreover, if we consider that host cells have a different subset of molecular components depending of the cell type and the specific environment in which they are immersed, it is likely that *T. cruzi* may induce a specific mechanism by engaging the components available at that particular moment, and that in a different cell type or situation, the parasite will activate another set of cellular responses. Further experiments using powerful technologies available nowadays will be necessary to elucidate all the intrinsic aspects of *T. cruzi*–host cell interplay displayed during the establishment of the chagasic infection.

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