A century of research: what have we learned about the interaction of *Trypanosoma cruzi* with host cells?

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Since the discovery of Trypanosoma cruzi *and the brilliant description of the then-referred to "new tripanosomiasis" by Carlos Chagas 100 years ago, a great deal of scientific effort and curiosity has been devoted to understanding how this parasite invades and colonises mammalian host cells. This is a key step in the survival of the parasite within the vertebrate host, and although much has been learned over this century, differences in strains or isolates used by different laboratories may have led to conclusions that are not as universal as originally interpreted. Molecular genotyping of the CL-Brener clone confirmed a genetic heterogeneity in the parasite that had been detected previously by other techniques, including zymodeme or schizodeme (kDNA) analysis.* T. cruzi *can be grouped into at least two major phylogenetic lineages:* T. cruzi *I, mostly associated with the sylvatic cycle and* T. cruzi *II, linked to human disease; however, a third lineage,* T. cruzi *III, has also been proposed. Hybrid isolates, such as the CL-Brener clone, which was chosen for sequencing the genome of the parasite (Elias et al. 2005, El Sayed et al. 2005a), have also been identified. The parasite must be able to invade cells in the mammalian host, and many studies have implicated the flagellated trypomastigotes as the main actor in this process. Several surface components of parasites and some of the host cell receptors with which they interact have been described. Herein, we have attempted to identify milestones in the history of understanding* T. cruzi*- host cell interactions. Different infective forms of* T. cruzi *have displayed unexpected requirements for the parasite to attach to the host cell, enter it, and translocate between the parasitophorous vacuole to its final cytoplasmic destination. It is noteworthy that some of the mechanisms originally proposed to be broad in function turned out not to be universal, and multiple interactions involving different repertoires of molecules seem to act in concert to give rise to a rather complex interplay of signalling cascades involving both parasite and cellular components.*

Key words: *Trypanosoma cruzi* - cellular invasion - trypomastigotes - amastigotes - parasitophorous vacuole escape - phylogenetic lineages

Outlook

Since the pioneering studies by Hertha Meyer and co-workers (Meyer 1942, Meyer & Xavier 1948), which initiated in vitro studies of *Trypanosoma cruzi* development in cultured cells, and the subsequent detailed descriptions provided by James Dvorak and co-workers on how cells become infected by *T. cruzi* trypomastigotes (Dvorak & Hyde 1973, Dvorak & Howe 1976), numerous studies have been undertaken to elucidate the molecular mechanisms that underlie the complex process of parasite entry into mammalian host cells. In order to accomplish these studies, a great deal of effort has been devoted to isolating parasites and determining the optimal conditions for their growth and differentiation (Camargo 1964, Baker & Price 1973, Pan 1978a, Engel et al. 1982, Villalta & Kierszenbaum 1982, Petry et al. 1987, De Souza 2000). A number of significant contributions have provided insights into both the ultrastructural organisation of the parasite (De Souza 2008) and the participation of both parasite and cellular in infection (Zingales & Colli 1985, De Souza 2000, Alves & Colli 2008). In Fig. 1, we have selected milestones that help to describe a trajectory of discovery that, like that of science in general, is not linear in time. Because several events overlap in time and relevance and therefore cannot be dissociated, references to particular findings may appear more than once. It is noteworthy that some of these events were initially thought to be of general importance in the biology of *T. cruzi*-host cell interactions, yet later turned out to be restricted to a particular parasite strain, clone or even target host cell. It has become increasingly apparent that a complex interplay of signalling cascades, involving both parasitic and cellular components, seem to operate in the infection process (Burleigh & Andrews 1995b, 1998, Burleigh & Woolsey 2002, Yoshida 2006, Alves & Colli 2007, Scharfstein & Lima 2008, Yoshida & Cortez 2008). The parasite infective forms considered herein are metacyclic (MT) and tissue-culture derived trypomastigotes (TCTs), as well as extracellular amastigotes (Fig. 2); bloodstream trypomastigote forms have also been studied by several laboratories, and their in vitro and in vivo behaviour is more similar to that of TCTs than MTs (Brener 1969, Gutteridge et al. 1978, Kipnis et al. 1979, Krettli et

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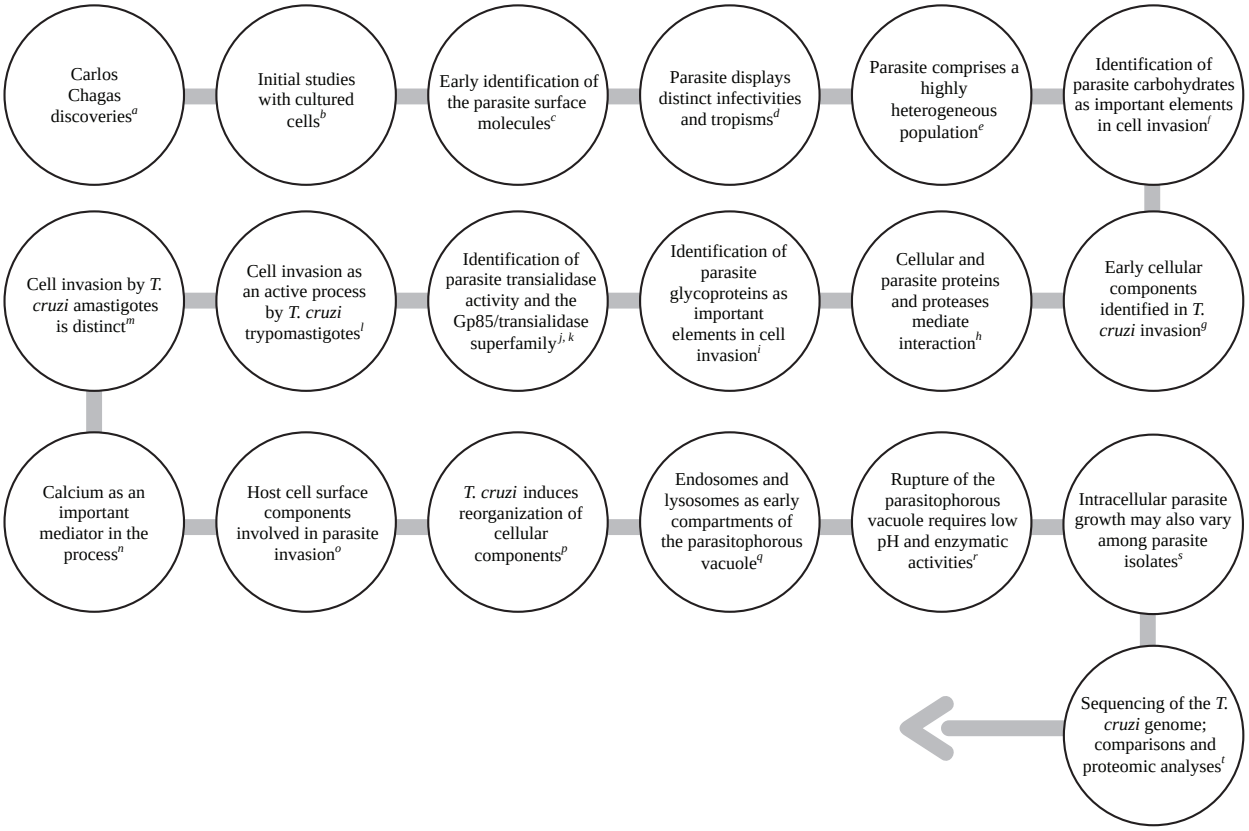

Fig. 1: major events in *Trypanosoma cruzi*-host cell interactions. *a*: Chagas (1909, 1911); *b:* Meyer (1942), Meyer and Xavier de Oliveira (1948); *c*: Alves and Colli (1974, 1975), de Lederkremer et al. (1980), Lima and Kierszenbaum (1982), Zingales et al. (1982), de Lederkremer and Colli (1995); *d*: Brener (1969), Silva and Nussenzweig (1953), Howells and Chiari (1975), Brener (1977), Melo and Brener (1978), Bice and Zeledon (1970); *e*: Miles (1974), Brener (1977), Alcantara and Brener (1978), Melo and Brener (1978), Souto et al. (1996), Briones et al. (1999), Brisse et al. (2000), Robello et al. (2000), Devera et al. (2003), Gaunt et al. (2003), Elias et al. (2005), Junqueira et al. (2005); *f*: Alves and Colli (1974, 1975), Araujo-Jorge and De Souza (1984), Avila et al. (1989), Andrade et al. (1991), Ouaissi et al. (1991), Travassos and Almeida (1993), Previato et al. (1994), Souto-Padrón et al. (1994), Parodi et al. (1995), Kahn et al. (1996), Silva et al. (1998, 2006); *g*: Nogueira and Cohn (1976), Henriquez et al. (1981), Lima and Kierszenbaum (1982), Piras et al. (1982, 1983, 1985), Nogueira (1983), Villalta and Kierszenbaum (1983, 1984), Ouaissi et al. (1985), Snary (1985), Schenkman et al. (1991a**)**, Schenkman and Mortara (1992); *h*: Piras et al. (1985), de Titto and Araujo (1987), Mbawa et al. (1991), McKerrow (1991), Schenkman et al. (1991b**)**, (1992, 1993), Eakin et al. (1992), Meirelles et al. (1992), Chaves et al. (1993), Colli (1993), Cross and Takle (1993), Burleigh et al. (1997), Santana et al. (1997), Procópio et al. (1998), Grellier et al. (2001), Santos et al. (2005); *i*: Alves and Colli (1975), Zingales et al. (1982), Parodi et al. (1983), Andrews et al. (1984, 1988), Araujo-Jorge and De Souza (1984), Alves et al. (1986), Scharfstein et al. (1986), Piras et al. (1987), Avila et al. (1989), Couto et al. (1990, 1993), Yoshida et al. (1990), Andrade et al. (1991), Ouaissi et al. (1991), Mortara et al. (1992), Villalta et al. (1992a**)**, Ming et al. (1993), Ruiz et al. (1993), Schenkman et al. (1993), Travassos and Almeida (1993), Previato et al. (1994), Souto-Padrón et al. (1994), Parodi et al. (1995), Kahn et al. (1996), Yoshida et al. (1997), Silva et al. (1998, 2006), Manque et al. (2000), Almeida and Gazzinelli (2001), Magdesian et al. (2001), Baida et al. (2006); *j*: Pereira (1983), Previato et al. (1985), de Titto and Araujo (1987), Schenkman et al. (1991b), (1992, 1993), Chaves et al. (1993), Colli (1993), Cross and Takle (1993); *k*: Previato et al. (1985), Frevert et al. (1992), Parodi et al. (1992), Schenkman et al. (1992), Kahn et al. (1993), Frasch (1994), Chuenkova and Pereira (1995), Manque et al. (2000), Malaga and Yoshida (2001), Atayde et al. (2004), Yoshida (2006), Alves and Colli (2008); *l*: Behbehani (1973), Dvorak and Hyde (1973), Alexander (1975), Tanowitz et al. (1975), Nogueira and Cohn (1976), Kipnis et al. (1979), Meirelles et al. (1982a), Schenkman et al. (1988, 1991), Mortara (1991), Schenkman and Mortara (1992); *m*: Nogueira and Cohn (1976), Pan (1978b), Carvalho et al. (1981, 1999), Hudson et al. (1984), Umezawa et al. (1985), Carvalho and De Souza (1986), Ley et al. (1988), Kahn et al. (1995), Fernandes and Mortara (2004), Mortara et al. (2005, 2008), Fernandes et al. (2006, 2007), Silva et al. (2006), da Silva et al. (2009, unpublised observations); *n*: Moreno et al. (1994), Tardieux et al. (1994), Burleigh and Andrews (1995a), Dorta et al. (1995), Wilkowsky et al. (1996), Rodriguez et al. (1997, 1999), Caler et al. (2000, 2001), Scharfstein et al. (2000), Yoshida et al. (2000), Tan and Andrews (2002), Garzoni et al. (2003), Yoshida and Cortez (2008); *o*: Henriquez et al. (1981), Lima and Kierszenbaum (1982), Nogueira (1983), Piras et al. (1983), Villalta and Kierszenbaum (1983, 1985), Ouaissi et al. (1985), Snary (1985), Schenkman et al. (1988, 1991), von Kreuter and Santos Buch (1989), Mortara (1991), Ortega-Barria and Pereira (1991), Scharfstein et al. (2000), Magdesian et al. (2001), Woolsey et al. (2003), Fernandes et al. (2007a); *p*: Nogueira and Cohn (1976), Henriquez et al. (1981), Meirelles et al. (1982b), Nogueira (1983), Piras et al. (1983), Colli (1984), Schenkman et al. (1991), Schenkman and Mortara (1992), Tardieux et al. (1992), Vieira et al. (1994), Carvalho et al. (1999), Procópio et al. (1999), Cortez et al. (2006), Ferreira et al. (2006), Bartholomeu et al. (2008); *q*: Milder and Kloetzel (1980), Meirelles et al. (1986), Carvalho and De Souza (1989), Andrews (1995), Wilkowsky et al. (2002), Andreoli and Mortara (2003), Stecconi-Silva et al. (2003), Woolsey et al. (2003), Andrade and Andrews (2005); *r*: Milder and Kloetzel (1980), Meirelles et al. (1987), Andrews and Whitlow (1989), Ley et al. (1990), Hall et al. (1992), Stecconi-Silva et al. (2003), Andrade and Andrews (2004), Rubin-de-Celis et al. (2006); *s*: Baker and Price (1973), Behbehani (1973), Dvorak and Hyde (1973), Tanowitz et al. (1975), Nogueira and Cohn (1976), Pan (1978c), Milder and Kloetzel (1980), Franke de Cazzulo et al. (1994), Aoki et al. (1995), Bertello et al. (1996), Garg et al. (1997), Almeida-de-Faria et al. (1999), Tonelli et al. (2004); *t*: Atwood et al. (2005), El Sayed et al. (2005a, b).

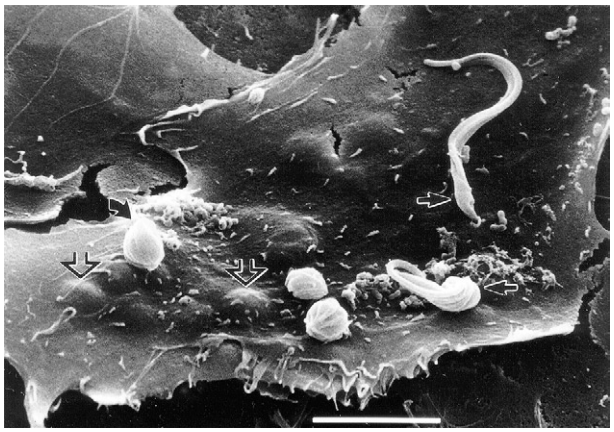

Fig. 2: *Trypanosoma cruzi* trypomastigotes as well as extracellular amastigotes are capable of infecting mammalian cells in culture. Scanning electron micrograph shows *T. cruzi* trypomastigotes (black arrows) and extracellular amastigotes (curved black arrow) invading Vero cells. Opened downward arrows show surface protrusions compatible in size with internalised amastigotes. Bar = 5 µm. Image from reference Procópio et al. (1999).

al. 1979, Alcantara & Brener 1980, Meirelles et al. 1982b, Wirth & Kierszenbaum 1984, Zwirner et al. 1994, Gomes et al. 1995, Alves & Colli 2007). More recently, it was discovered that there are differences in the invasion mechanisms engaged by the distinct infective forms of the parasite from the two major phylogenetic lineages, an observation that has opened new avenues to study this already intricate process (Mortara et al. 2005, 2008, Yoshida 2006, 2008).

It is generally accepted that in order for cell invasion to occur, *T. cruzi* infective forms need to physically attach to the host cell surface. This process usually takes a few minutes in vitro, and it is not uncommon to observe trypomastigotes attaching and detaching from a target cell, as if 'probing' it before invasion (Dvorak & Hyde 1973, Dvorak & Howe 1976, Nogueira & Cohn 1976, Andrews & Colli 1982, Meirelles et al. 1982a, Lima & Villalta 1988, Schenkman et al. 1991c, Villalta et al. 1992b). Attachment can be separated from invasion by lowering the temperature or fixing target cells (Andrews & Colli 1982, Meirelles et al. 1982a, Schenkman et al. 1991c). Several lines of evidence suggest that motile trypomastigotes promptly attach to and invade live cells through an active mechanism that does not require intact host cell microfilaments (Kipnis et al. 1979, Schenkman et al. 1991c, Schenkman & Mortara 1992), but instead depends on parasite energy (Schenkman et al. 1991c). In contrast, extracellular amastigotes do not attach to fixed cells, and their invasion depends on functional host cell microfilaments (Mortara 1991, Procópio et al. 1998, Mortara et al. 2005). Once attachment is established, signals exchanged (see above references and section below) activate parasite-driven invasion of trypomastigotes (Kipnis et al. 1979, Schenkman et al. 1991c, Schenkman & Mortara 1992) or induce "phagocytosis-like" entry of amastigotes (Nogueira & Cohn 1976, Procópio et al. 1998, 1999).

It has been demonstrated that a number of parasite and host cell components (such as proteins/glycoproteins and other glycoconjugates) participate in the attachment phase of the invasion process (Burleigh & Andrews 1995b, 1998, Burleigh & Woolsey 2002, Andrade & Andrews 2005, Alves & Colli 2007, Yoshida & Cortez 2008) and consequently play a role in signalling exchanges. More recently, it has also been shown that lipid rafts in the host membrane may also take part in this process (Barrias et al. 2007, Fernandes et al. 2007a). The complexity of the invasion process can be gleaned from the examples provided in the section dealing with the multitude of signalling events involving *T. cruzi*- host cell interactions.

Another important factor of the multitude of studies in this area is the extensive variety of host or target cells. These include macrophages, epithelial and endothelial cells, fibroblasts, dendritic cells, neurites as well as cardiomyocytes (Meyer 1942, Meyer & Xavier de Oliveira 1948, Nogueira & Cohn 1976, Henriquez et al. 1981, Meirelles et al. 1982a, 1987, 1999, Morris et al. 1988, 1990, Schenkman et al. 1988, Araujo-Jorge 1989, Ortega-Barria & Pereira 1991, Aprigliano et al. 1993, Procópio et al. 1998, 1999, Huang et al. 1999, Chuenkova & Pereira 2001, Garzoni et al. 2003, Melo et al. 2004, Taniwaki et al. 2006, Coimbra et al. 2007, Bartholomeu et al. 2008, Lu et al. 2008, Poncini et al. 2008, Scharfstein & Lima 2008). This single variable exemplifies the multitude of host cell and parasite components that, upon interaction, lead to activation of the signalling pathways discussed below. Moreover, the plethora of molecules involved increases when different strains of parasite are compared. The analysis of the invasion mechanism (or mechanisms) of host cells by *T. cruzi* based on data from the literature can be restricted to one cell type-one or strain until more general conclusions can emerge. However, in the case of in vivo studies, the infection route must also be considered (Hoft 1996, Hoft et al. 1996, Yoshida 2008).

Biology of *T. cruzi*-host cell invasion

Paradigmatic studies have provided new insights into the invasion mechanisms of *T. cruzi*. Some of these studies have observed that calcium-dependent lysosomal recruitment takes place during trypomastigote invasion (Tardieux et al. 1992, Andrews 2002). According to this model, TCTs engage a signalling process that culminates with the formation of the parasitophorous vacuole (PV) (Burleigh & Andrews 1998, Burleigh & Woolsey 2002). Additional evidence suggests the participation of components of the early endocytic trafficking pathway, such as dynamin and Rab5, and indicates that the lysosomal process might be both more elaborate and downstream of earlier events (Wilkowsky et al. 2002). Previous studies (Todorov et al. 2000, Wilkowsky et al. 2001) have used a quantitative approach to identify the role of phosphatidyl-inositol 3-kinase (PI3-K) (Woolsey et al. 2003) in the lysosomal pathway and observed that this key cellular component is involved in a lysosome-independent *T. cruzi* internalisation pathway utilised by TCTs. Trypomastigotes that use this route mobilise phosphorylated inositides during the formation

of the PV. These molecules then mature and become enriched in the same compartments as the lysosomal marker LAMP-1 (Tardieux et al. 1992, Procópio et al. 1998, Woolsey et al. 2003, Andrade & Andrews 2004, Fernandes et al. 2007b). One important outcome of this work was to demonstrate for the first time the relative contributions of each mode of entry, namely PI3-K (50%), lysosome (20%) and endosomal routes (20%) (Woolsey et al. 2003). The available information about the mechanisms of amastigote penetration is comparatively scarcer than that of trypomastigotes. In studies on interactions with macrophages, it has been noted that members of the Gp85/TS antigen family engage mannose receptors to gain entry to professional phagocytes (Kahn et al. 1995). Carbohydrate epitopes seem to play a role in the initial steps of invasion in non-phagocytic cells (Silva et al. 2006). The relative roles of PI3-K, endosomal trafficking and LAMP-1 (Procópio et al. 1998) pathways in extracellular amastigote invasion are still not fully understood.

After entering host cells, parasites are usually found in an acidic membrane-bound compartment referred to as phagosome or PV, that may be comprised of host cell plasma membrane, or endosomal or lysosomal in its origin (Milder & Kloetzel 1980, Meirelles et al. 1986, Carvalho & De Souza 1989, Hall et al. 1991, Schenkman & Mortara 1992, Wilkowsky et al. 2002, Woolsey et al. 2003, Andrade & Andrews 2004, Fernandes et al. 2007b). The time of residence inside the PVs may vary between infective forms, ranging from 1-2 h in the case of amastigotes and TCTs (Meirelles et al. 1986, Ley et al. 1990, Hall et al. 1992, Stecconi-Silva et al. 2003, Andrade & Andrews 2004, Rubin-de-Celis et al. 2006) to several hours in the case of MT trypomastigotes (Stecconi-Silva et al. 2003, Rubin-de-Celis et al. 2006). These then eventually escape and differentiate into amastigotes in the cytoplasm (De Souza 1984, 2000, 2005, Andrews 2000).

Once inside the host cells, trypomastigotes and amastigotes secrete TcTOX, a complement 9 (C9) factor-related molecule that, at low pH, will destroy the PV membrane and allow the parasite access to the cytosol (Andrews & Whitlow 1989, Andrews 1990, Andrews et al. 1990, Ley et al. 1990, Manning-Cela et al. 2001, Rubin-de-Celis et al. 2006). Raising the intracellular pH with weak bases affects MT invasion and substantially delays escape from the PV, increasing the latency from about 2-10 h. By contrast, the kinetics of amastigote invasion and escape are not affected by this treatment (Stecconi-Silva et al. 2003). This lytic activity is likely to be facilitated by parasite transialidase activity on the lumenal glycoproteins that protect the PV (Hall et al. 1992, Stecconi-Silva et al. 2003, Rubin-de-Celis et al. 2006). In agreement with the idea that the glycosylation of lysosomal lumenal glycoproteins is relevant for the protection of the PV membrane, parasites promptly escape from PVs formed in CHO cells deficient in sialylation (Stecconi-Silva et al. 2003, Rubin-de-Celis et al. 2006). So far, TcTOX activity has been observed in extracellular amastigotes (Ley et al. 1990, Stecconi-Silva et al. 2003) and TCTs (Manning-Cela et al. 2001, Andreoli & Mortara 2003, Rubin-de-Celis et al. 2006). In contrast, MT trypomastigotes display both very weak transialidase activity and undetectable TcTOX (Andreoli & Mortara 2003, Stecconi-Silva et al. 2003). Therefore, whereas extracellular TCTs and amastigotes display a somewhat predictable behaviour regarding cell invasion and escape, at present we do not have a consistent model to fully understand how MT trypomastigotes actually escape from their PVs. Using polyclonal antibodies to C9, it has recently been shown that amastigotes express a TcTOX-related compound (Andreoli et al. 2006); this tool may be useful for mapping this compound throughout the intracellular traffic in the different infective forms. Recent work has shown that the kinetics of endosomal and lysosomal marker accumulation, and their subsequent loss - indicative of parasite escape into the cytoplasm - is not correlated with either the infective form or phylogenetic group of the parasite tested under these particular conditions (Fernandes et al. 2007b).

Once free in the cytoplasm, trypomastigotes differentiate into amastigotes; these forms then begin to grow by binary fission for up to nine cycles (Dvorak & Hyde 1973). During the course of intracellular growth, the parasite disrupts host cellular structure, attachment to the substrate becomes loose and basic functions such as contractility are impaired (Meyer & Xavier de Oliveira 1948, Dvorak & Hyde 1973, Low et al. 1992, Pereira et al. 1993, 2000, Carvalho et al. 1999, Hall et al. 2000, Taniwaki et al. 2005, 2006). Usually, with the cytoplasm loaded with a couple of tens of amastigotes, host cell division becomes arrested (Meyer & Xavier de Oliveira 1948, Low et al. 1992). During the differentiation of amastigotes into trypomastigotes, preceding cell rupture and the release of the parasite into the surrounding medium, intermediate epimastigote-like forms have been observed (Meyer & Xavier de Oliveira 1948, Almeida-de-Faria et al. 1999, Tonelli et al. 2004). When the cell becomes filled with trypomastigotes, the plasma membrane ruptures and significant degenerative processes can be observed, probably due to the intense mechanical movement of the parasites (Meyer & Xavier de Oliveira 1948, Low et al. 1992, Pereira et al. 1993, Taniwaki et al. 2005, 2006). Interestingly, the intracellular cell cycle of *T. cruzi* seems to be independent of the of the host cell nucleus, as all developmental stages can be found within enucleated host cells infected with trypomastigotes (Coimbra et al. 2007). Although the precise mechanism underlying cell rupture has been inferred as being mostly mechanical in nature, it has been known since the original studies by Hertha Meyer that different cell types present distinct susceptibilities to cellular rupture by the intracellular parasites (Meyer 1942, Meyer & Xavier de Oliveira 1948).

The precise mechanisms that govern the intricate signalling exchanges between the parasite and the host cell are discussed in a separate section. As indicated above, several studies have found that amastigotes, prematurely released from infected cells or generated by the extracellular differentiation of released TCTs, can also infect cultured cells and animals (Behbehani 1973, Nogueira & Cohn 1976, Hudson et al. 1984, Carvalho & De Souza

1986, Ley et al. 1988, Mortara 1991). Systematic studies on cell invasion and PV escape carried out in several laboratories have reinforced the notion that each infective form of the parasite has a unique interplay with the specific target host cell with which it interacts. Not only the parasite infective form, but also the strain (and phylogenetic origin) will determine the outcome of this interaction (Milder & Kloetzel 1980, Carvalho & De Souza 1986, 1989, Meirelles et al. 1986, Ley et al. 1990, Hall et al. 1992, Andrews 1994, Ochatt et al. 1997, Stecconi-Silva et al. 2003, Rubin-de-Celis et al. 2006, Fernandes et al. 2007b). The variety of mechanisms used for cell invasion and escape from the PVs by amastigotes and trypomastigotes is consistent with the complex repertoires of both infective forms and surface molecules that the parasite has evolved to ensure host colonisation (Ley et al. 1990, Hall et al. 1992, Andrews 1994, Yoshida 2002, Mortara et al. 2005, Yoshida & Cortez 2008, da Silva et al. 2009). Adding to this already complex scenario, less canonical host cell invasion mechanisms should also be mentioned. These include the phagocytosis of apoptotic *T. cruzi* infected lymphocytes (Freire-de-Lima et al. 2000, Luder et al. 2001, Lopes et al. 2007, De Meis et al. 2008) and the less-studied autophagic pathway (Romano et al. 2009). It is similarly worth mentioning that there are a variety of receptors linked to the host immune system, such as Toll-like receptors that parasite molecules engage with during in vivo infections, and which therefore may also play a key role in the host-parasite interplay (Tarleton 2007).

Signalling mechanisms and molecules involved in *T. cruzi* invasion

The number of identified interacting components that may play a role in *T. cruzi*-host cell interactions is continually growing. For recent reviews on this rapidly evolving field, the reader is referred to the following references: De Souza (2000, 2002), Burleigh and Woolsey (2002), Yoshida (2002), Andrade and Andrews (2005), Mortara et al. (2005), Alves and Colli (2007), Scharfstein and Lima (2008), Yoshida and Cortez (2008). Multiple interactions between molecules from the parasite and the host lead to the internalisation of the parasite and an increase of cytosolic Ca^{2+} in both the host cells and in the parasite during invasion (Morris et al. 1988, Moreno et al. 1992, 1994, Krassner et al. 1993, Burleigh & Andrews 1995b, 1998, Docampo et al. 1995, Dorta et al. 1995, Wilkowsky et al. 1996). From the point of view of various types of host cell, contact with TCTs (Tardieux et al. 1994), MTs (Dorta et al. 1995) and extracellular amastigotes (Fernandes et al. 2006), but not epimastigotes (Tardieux et al. 1994), gives rise to a transient calcium influx. The same phenomena was observed when either specific molecules involved in *T. cruzi* cell invasion or uncharacterised factors released by the parasite (Tardieux et al. 1994, Burleigh & Andrews 1995a) were incubated with the host cell.

Calcium influxes have been associated with the formation of PVs or with parasite evasion from the vacuoles and successful infection (Burleigh & Andrews 1998, Burleigh & Woolsey 2002, De Souza 2002, Andrade & Andrews 2004, Burleigh 2005). Among the *T. cruzi* components involved in invasion are molecules belonging to the Gp85/trans-sialidase superfamily (Gp85/TS) and mucin-like proteins present on the surface of the parasite. Both are encoded by large gene families (~1430 and ~863 gp85/TS and mucin-encoding genes, respectively) (Colli 1993, Frasch 1994, Schenkman et al. 1994). As interesting examples, members of the Gp85/TS family are developmentally regulated by postranscriptional mechanisms, with Gp82 and Tc85 glycoproteins expressed mainly in the MT and TCT forms, respectively. Gp82 binds to gastric mucin and Tc85 binds to members of the laminin and fibronectin families in the extracellular matrix (ECM); however, other receptors cannot be ruled out as Tc85 molecules have been described as multi-adhesion glycoproteins (Wirth & Kierszenbaum 1984, Ouaissi et al. 1984, 1985, Noisin & Villalta 1989, Santana et al. 1997, Magdesian et al. 2001, Ulrich et al. 2002, Nde et al. 2006, Yoshida 2008). It should be mentioned that other molecules expressed in *T. cruzi* bind to ECM elements such as heparin, heparan sulfate, collagen and thrombospondin-1 (Ortega-Barria & Pereira 1991). A synthetic peptide based on the conserved FLY domain (VTVXNVFLYNR) present in all members of the Gp85/TS family promotes dephosphorylation of an intermediate filament protein (cytokeratin 18) that leads to cytoskeleton reorganisation and activation of the ERK1/2 signalling cascade; as a result, there is an increase in the entry of parasites into epithelial cells (Magdesian et al. 2007). On the other hand, it has been shown that an inactive form of TS from TCT that binds sialic acid triggers NF-kB activation, the expression of adhesion molecules on endothelial cells and upregulation of parasite entry in a FLY-independent and carbohydrate-dependent way (Dias et al. 2008). Recently, TS has been linked to the invasion of TrkA (nerve growth factor receptor)-expressing cells (e.g., dendritic cells) by a mechanism that involves triggering TrkA-dependent and PI3-K/AkT kinase signalling events (Melo-Jorge & Pereira-Perrin 2007). The presence of Gp82 on MT trypomastigotes induces calcium transients that result in phosphorylation of a 175 kDa protein in MTs (CL strain) and Ca^{2+} mobilisation in the host cell through a sequence of events involving PTK, PLC and IP3 (Yoshida 2006, 2008, Yoshida & Cortez 2008). Interestingly, although gp35/50 mucins are the main MT surface components involved in the attachment phase of the G strain of *T. cruzi* (Ruiz et al. 1993) and Gp85/35 mucins are important acceptors of sialic acid catalysed by *trans*-sialidase, sialyl residues (Schenkman et al. 1993) are not involved in the invasion mechanism (Yoshida et al. 1997). On the other hand, the role of sialic acid in TCT invasion, together with other lectin-like interactions, has yet to be fully clarified (Libby et al. 1986, Ming et al. 1993, Schenkman et al. 1993, 1994, Yoshida et al. 1997, Stecconi-Silva et al. 2003, Rubin-de-Celis et al. 2006, Dias et al. 2008).

Another mechanism for the attachment-independent invasion of trypomastigotes phase involves the activation of the TGFβ signalling pathway (Silva et al. 1991, Ming et al. 1995, Araujo-Jorge et al. 2008). The agent

involved in mediating the signalling remains elusive, but it seems to be thermo-labile and hydrophobic in nature. It is likely that a protease secreted by the parasite might activate latent TGFβ-associated with ECM components, allowing activation of Smad 2/3 pathway through the TGFβ receptors (I and II) present on the surface of host cells. The pivotal role of this pathway in infectious of heart tissues and consequently in the chagasic myocardiopathy has been described (Araujo-Jorge et al. 2008).

Other molecules not involved directly in receptor-ligand interactions are nonetheless fundamental to establishing infection by *T. cruzi*. Inhibitors of the prolyl oligopeptidase (POP Tc80), a serine protease that hydrolyses human collagens types I and IV and fibronectin blocks the entry of TCTs into cultured cells (Harth et al. 1993, Santana et al. 1997, Grellier et al. 2001). Cruzipain, the major cysteine protease present in all stages of *T. cruzi*, has also been implicated in the internalisation process due to its ability to generate bradykinin and increase parasite entry through B2-type bradykinin receptors. A link between innate and adaptive immune responses through bradykinin has therefore been proposed. It is worth mentioning in this context that the invasion of host cells by MTs of the CL strain depends on tyrosine phosphorylation and the IP_3-dependent (1,4,5-inositol-triphosphate) release of calcium from endoplasmic reticulum (ER) stores, whereas MTs of the G strain engage adenylate cyclase and cause calcium to be mobilised from acidocalcisomes (Neira et al. 2002). No comparative data between both strains is evaluable for TCTs, but inhibitors of class I and class III PI3-K activities block the entry of the parasite into macrophages, suggesting the involvement of different isoforms of this kinase (Todorov et al. 2000). On the other hand, it seems that calcium mobilisation from acidocalcisomes, but not from the ER, is important for cellular invasion by extracellular amastigotes of either the G or CL strains (Mortara et al. 2005, Fernandes et al. 2006, Scharfstein et al. 2007, 2008, Scharfstein & Lima 2008).

A lot of attention was initially given to the signalling pathways active inside the host cell during *T. cruzi* infection, as well as to the identification of ligands and receptors involved in the infection process. Although a great deal has been learned from sequencing the *T. cruzi* genome (El Sayed et al. 2005a, b), including mapping its 190 kinases and 86 phosphatases (Parsons et al. 2005, Brenchley et al. 2007), knowledge about the signalling pathways active in the parasite is still scarce and, mostly, fragmented. It is evident that the complexity of the system has yet to be overcome.

Perspectives

It is clear that the mechanisms of invasion used by *T. cruzi* TCTs, MT trypomastigotes and extracellular amastigotes are divergent. Adding to this complexity is the finding that there are mechanistic variations between isolates of the two main phylogenetic groups that also depend on the type of host cell analysed. To circumvent this issue, specific host lineages and *T. cruzi* strains and/or clones could be chosen as models to be used by the scientific community in order to reveal urgently needed information about the general mechanisms that govern mammalian cell invasion.

A great deal more research has been done to establish the signalling pathways in the host cells than in the parasite during infection. The 190 kinases and 86 phosphatases identified in the *T. cruzi* genome should, hopefully, provide the necessary tools to increase interest in the field and provide more complete mechanistic explanations of the infection process.

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