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Chapter 2

Biochemical, Cellular, and Immunologic Aspects during Early Interaction between Trypanosoma cruzi and Host Cell

Rosa Lidia Solís-Oviedo, Víctor Monteon, Ruth López and Ángel de la Cruz Pech-Canul

Abstract

The close parasite-host relationship involves different aspects such as the biochemical, physiological, morphological, and immunological adaptations. Studies on parasite-host interaction have provided a myriad of information about its biology and have established the building blocks for the development of new drug therapies to control the parasite. Several mechanisms for the parasite invasion have been proposed through in vivo or in vitro experimental data. Since the first histological studies until the studies on the function/structure of the involved molecules, this complex interaction has been roughly depicted. However, new recent strategies as genetic and proteomic approaches have tuned knowledge on how the host reacts to the parasite and how the parasite avoids these host’s reactions in order to survive.

Keywords: Trypanosoma cruzi, immune system, parasite interactions, animal model studies, in vitro models, phagocytic, non-phagocytic

1. Introduction

The life cycle of Trypanosoma cruzi comprises several morphological transformations involving both mammalian and vector hosts, where three different major developmental stages are identified: epimastigotes, trypomastigotes, and amastigotes (Figure 1). The developmental stages of T. cruzi alternate between non-infective and infective forms. Epimastigote and amastigote are non-infective but replicative stages in the gut of the triatomine vector and inside the
mammalian cell, respectively. Trypomastigote stage is infective but non-replicative and can also be considered as two different developmental stages: the bloodstream trypomastigotes, found in the blood of the mammalian host, and the metacyclic trypomastigotes, found in the rectum of the triatomine vector.

*Trypanosoma cruzi* is internalized by phagocytic and non-phagocytic nucleated host cells via multiple pathways. The first general steps through the interaction process of the *T. cruzi* and its mammalian host cell can be divided into three stages: (1) adhesion/recognition, (2) signalling, and (3) invasion [2, 3]. During the adhesion/recognition stage, diverse molecules with cell-adhesion properties are expressed on the membrane surface of the metacyclic trypomastigotes from of the parasite; these molecules bind to receptors of the target host cells and are able to trigger signals pathway, toward the parasite invasion [4]. That invasive process allows *T. cruzi* internalization and involves the engulfment of the parasite, the formation of a *T. cruzi* parasitophorous vacuole (TcPV) [5], as well as the late disruption and the dispersion of the TcPV, thereby the parasite is released to the host cytoplasm where its replication and differentiation starts until the infective stage [6, 7]. The aim of this chapter is to discuss and to outline the interaction models during the early interaction between *T. cruzi* and its mammalian host cells.

*Figure 1.* The different stages of *Trypanosoma cruzi*. The image depicted the amastigote, epimastigote, and trypomastigote stages from *T. cruzi* and their membrane domains: Nucleus (N), Kinetoplast (K), Flagellum (F), Flagellar Pocket (FP), and Cell Body (CB). Reprinted with permission from Ángel de la Cruz Pech-Canul et al. [1], Copyright © 2017.
2. An overview of parasite interaction

One of the first barriers faced by *T. cruzi* during host cell invasion is the complexity of the host defence system. The skin and mucous membranes act as physical barriers which prevent penetration by microbes. Undoubtedly, they are the site for multiple and diverse types of chemical, physical, and biological contacts. Lipids and proteins are among the main components of the innate immune system in these tissues. Lipids comprise linoleic acid, oleic acid, squalene, ceramides, and sphingolipids, whereas proteins are more diverse, such as keratin on the surface of the skin or the cationic peptides alpha- and beta-defensins produced by neutrophils and mucosa tissue, respectively [8]. Furthermore, saliva produced by salivary glands of the vector contains a sort of proline-rich proteins and histidine-rich proteins both with antibiotic properties, lysozyme, peroxidase, lactoferrin, cystatins, and mucins [9]. Due to the rich protein content, both pH and salt concentration play a significant role as inhibitory factors during the parasite/host interaction.

The cellular composition of skin and mucous membranes is a fundamental barrier for permissive or refractory colonization/infection. In the skin, the epidermis is composed by 95% of keratinocytes and other cells present at low concentration, such as melanocytes, Langerhans cells, intra-epithelial lymphocyte, and Merkel cells. Keratinocytes express Toll-like receptors (TLRs) 1–6, 9, and 10 which are able to recognize basically all pathogen-associated molecular patterns (PAMPs) with exception of flagenin; as a consequence, they can secrete an array of mediators such as nitric oxide, leukotrienes, cyclooxygenase, metalloprotease 1 and 9, classical cytokines IL-1, IL-6, IL-8, TNF-alpha, and chemokines CXCL1 and CXCL8. Keratinocytes also express receptors for different cytokines (IL-1, IL-3, TNF-alpha, IL-17, IL-21, IL-22) and chemokines (CXCL9, CXCL10, CXCL11, and CCL20). Other skin cells present at low concentration have also a broad array of receptors that are able to respond to physical and chemical stimulus. In addition, a dense protein layer is found between epidermis and dermis which is composed by collagen type IV, laminin fibronectin, iodogen, and heparan sulfate; together, they structure the basement membrane [10]. The cellular composition of dermis is more complex and diverse. Fibroblast, myofibroblasts, macrophages, adipocytes, dendritic cells, mast cells, and mesenchymal stem cells are found among resident cells in the dermis (Figure 2), whereas transitory cells include lymphocytes, polymorphonuclear cells and monocytes. In addition, dermis presents an intricate network of nerves, lymph, and blood system. As skin, mucosal tissue has the property to react with a complex array of mediators required for immune surveillance and inflammatory response to tissue injury and infection. A remarkable differential feature between skin and mucosa tissue is the bias to immune tolerance and anti-inflammatory response in mucosal compartments [11, 12].

In natural conditions, *T. cruzi* infection is established when metacyclic trypomastigotes are deposited on injured skin or mucosa host tissue by blood feeding triatomine. Thus, metacyclic trypomastigotes has to face the above innate immune responses at the portal entry in order to survive (Figure 2). Since the pioneer work published by Romañá [13], where a histology description was done, limited information on this area of concern exists. It is very critical to take into account different factors in the relationship between parasite and host. For example, factors
as specie of vector are involved in the transmission, inoculum size, *T. cruzi* phase, portal of entry, *T. cruzi* strain, host immune responses, and microbiota presented in the vector.

### 3. Specie of vector and *Trypanosoma cruzi*

Firstly, there are many triatomine vector species that transmit the Chagas disease. Some of them have a wide geographical distribution and others are confined to restricted geographical areas. However, all of them can transmit *T. cruzi* infection with different efficacy, a feature that relies on biological behaviour and physiological condition itself. For example, metacyclogenesis involves the process of parasite transformation into the vector; this step is fundamental in order to accomplish the life cycle. The basic transformation that takes place inside the vector is from bloodstream trypomastigote phase to epimastigote and to metacyclic trypomastigotes. This last phase is essential for mammalian infection in as much as epimastigotes are vulnerable to innate immune mechanism. Thus, the metacyclogenesis that takes place into the vector is fundamental in order to switch to mammalian host. Perlowagora-Szumlewicks and Carvalhio-Moreira [14] described triatomine vector species influencing metacyclogenesis with remarkable observation.
They pointed out higher metacyclogenesis rates in *Rhodnius neglectus* and *R. prolixus* (50 and 37%, respectively), whereas in some *Triatoma* species, metacyclogenesis rates were dramatically lower in comparison (5% in *Triatoma sordida*, 7% in *T. brasiliensis*, and 1% in *T. pseudomaculata*). However *T. infestans* can reach up to 42%, in *T. rubrovaria* 27%, in *T. dimidiata* 26%, and *Panstrongylus megistus* metacyclogenesis rates can reach 27%. Other remarkable observation is that metacyclic trypomastigotes rate is not continuous along vector life span. In some cases, it can reach a plateau, but in other cases, it can reach several peaks before metacyclogenesis drops. In natural conditions, *T. barberi* can reach up 76%, in *T. pallidipennis* 15%, whereas in *T. dimidiata* 26% [15].

The metacyclogenesis of *T. dimidiata* in laboratory conditions is similar to natural conditions; in addition, metacyclogenesis is also influenced by the *T. cruzi* strain and the rate of metacyclic parasites change along the age of triatomine vectors [16]. Furthermore, *T. cruzi* strains can moderately influence the rate of metacyclogenesis that take place inside the same triatomine specie but have less impact when compared across triatomine specie [16, 17]. Altogether, the above data highlight the importance of triatomine species and *T. cruzi* strains in the development of metacyclic trypomastigotes: the natural parasite phase that will face mammalian host to complete its life cycle. Due to its importance, this variable should be taken into account for experimental design. Besides, the parasite strains show different virulence relying on virulence factors such as trans-sialidase activity, complement resistance, and cysteine protease cruzipain (TCC) [18]. Trans-sialidase removes and transfers sialic acid from host cells to parasite mucin-like glycoprotein. It is known that trans-sialidase activity is a virulence factor which allows parasite to invade and to escape from parasitophorous vacuole. This enzyme is more expressed in bloodstream and tissue-culture trypomastigotes than in metacyclic trypomastigotes. Trans-sialidase activity also depends on *T. cruzi* lineage and consequently its virulence [19].

Once metacyclic trypomastigotes have overcome the first nonspecific immune mechanical barrier (skin/mucosal tissues), they need to swing into the extracellular matrix proteins in order to find cells to invade for replication and then accomplish their life cycle. GP82, a surface glycoprotein found in both bloodstream and tissue-culture trypomastigotes, has the ability to bind to matrix extracellular proteins such as fibronectin, heparan sulfate, and laminin, serving as bridges for parasite-target cell association and leading to enhanced infection. However, this interaction inhibits cell invasion. The presence of the major cysteine proteinase cruzipain (TCC) helps to degrade these extracellular matrix proteins enabling cell invasion [20]. These surface glycoproteins are very polymorphic among *T. cruzi* strains resulting in different grades of virulence.

The complement system, another unspecific immune mechanism that is essential for inflammation and cellular lysis, can be activated by three pathways. The lectin triggered by mannose-binding lectins (mannose-binding proteins, ficolins, and CL-K1 proteins) that binds to pathogen-associated molecular pattern (PAMPs) rich in D-mannose, L-fucose, glucose, and N-acetyl-glucosamine, O-acetylated, and glycan compounds containing sialic acid which activate MASP-1 and MASP-2. The alternative pathway is triggered when the complex C3\((H_2O)B\) factor is stabilized on a surface allowing the formation of C3 convertase (C3 \((H_2O)Bb\)). Whereas the classical pathway activation depends on C1 complex interaction with antibodies or LPS and porins present in Gram-negative bacteria, but also with phosphatidylserine on apoptotic cells or via C-reactive proteins synthetized in liver as stress proteins [21].
The four phases of _T. cruzi_ (amastigote, epimastigote, metacyclic, and bloodstream trypomastigote) can activate the complement system, but only epimastigotes are susceptible to lysis. However, some strains on metacyclic trypomastigote phase are more vulnerable [22, 23]. Some _T. cruzi_ surface molecules enable parasite to evade innate and adaptive immune responses. There are other mechanisms to circumvent the action of complement system such as the presence of calreticulin (TcCRT), the complement regulatory protein (Gp160/TcCRP), the complement C2 receptor inhibitor trispanning (TcCRIT), and the presence of GP58/68 protein and T-DAF. For a comprehensive review, see [21].

Finally, it has been observed that in animal models, metacyclic trypomastigotes induce an inflammatory response at the site of inoculation, as early as 1 h, and it is composed basically of neutrophils while mononuclear infiltrate begins at 24 h with a maximum infiltration at day 15. Nonetheless, poor cytokine expression such as IL-2, IL-4, IL-10, IL-12, and IFN-gamma persists over a 2-week post-inoculation, whereas at the regional lymph node to the site of inoculation, it was evident as early as 1 h. The induced pattern of cytokine at the inoculation site is permissive to establishing infection, despite the appropriate immune response in other lymph secondary organs [24–26]. Our group recently reported that pre-exposure to faeces of triatomine decreases parasitemia in mice challenged with metacyclic trypomastigotes. This finding suggests that inflammatory reaction to bacteria faeces in immune individuals helps to control parasite load _in vivo_ [27].

4. _In vitro_ models

Diverse _in vitro_ studies on the _T. cruzi_ /host cell interaction process have been described through the years [28]. These studies have included a wide variety of eukaryotic cell lines and parasite strains, as well as the different parasite phases able to infect cells: amastigotes, metacyclic trypomastigotes, or both, bloodstream and tissue-culture trypomastigotes [2, 29]. _T. cruzi_ is capable to invade phagocytic or non-phagocytic cells via endocytic mechanisms. Currently, three models for _T. cruzi_ invasion have been proposed: lysosomal-dependent, lysosomal-independent, and actin-dependent [3, 6, 30].

Cortez and co-workers [30] recently showed that the participation of lysosomes in the parasite entry site depends on the source of the trypomastigote. They found that the metacyclic trypomastigotes invasion occurs mainly by the lysosome-dependent mechanism, whereas the tissue-culture trypomastigote invasion takes place mostly by the lysosome-independent mechanism. Interestingly, it has been reported that amastigotes are capable of invading host cells by the actin-dependent phagocytic mechanism probably due to their motionless nature [29, 31].

4.1. Lysosomal-dependent

The lysosomal-dependent model is also known as the lysosome exocytosis pathway. Tardieux et al. visualized the recruitment of lysosomes at the parasite entry site during the early event of internalization of tissue-culture trypomastigotes into their mammalian host cells, and they proposed that this process is required for parasite internalization [32]. PGTF is a soluble factor
proteolytically generated from trypomastigote which is capable to induce Ca\textsuperscript{2+} signaling in mammalian cells. The addition of PGTF during the host cell invasion of tissue-culture trypomastigotes showed that Ca\textsuperscript{2+} signalling plays a role in the parasite invasion through the reorganization of host cell microfilaments as well as in the migration and fusion of lysosomes [15, 33]. In addition, the increase of Ca\textsuperscript{2+} is required to trigger a form of endocytosis to repair the mechanically injured host cell membrane due to T. cruzi invasion [17]. The elevation of intracellular Ca\textsuperscript{2+} concentration triggers the exocytosis of lysosomes. The lysosomal enzyme acid sphingomyelinase (ASM) is released to the host plasma membrane where ASM converts sphingomyelin into ceramide: a lipid capable of forming ceramide-enriched endosomes [34, 35]. Ceramides are also capable to coalesce and to accumulate into the parasitophorous vacuoles, which suggest that this lipid plays an important role in the membrane deformation process required to allow the large trypomastigotes entry into the host cells [32, 36].

4.2. Lysosomal-independent

The lysosomal-independent mechanism depends on phosphatidylinositol-3 (PI 3)-kinase (PI3K) which is activated in the presence of T. cruzi bloodstream trypomastigotes. This mechanism is correlated to an efficient parasite invasion of non-phagocytes and phagocytic cells. In vitro analysis during T. cruzi infection of phagocytic cells has shown the presence of vacuoles enriched with lipids derived from the PI 3-kinase activities: phosphatidylinositol 3-phosphate (PI\textsubscript{3}P), phosphatidylinositol 3,4-bisphosphate (PI\textsubscript{3,4}\textsubscript{2}P\textsubscript{2}), and phosphatidylinositol PI 3,4,5-triphosphate (PI\textsubscript{3,4,5}\textsubscript{3}P\textsubscript{3}) [37–39].

The inhibition of the class I and III PI 3-kinase activities abolishes the parasite entry into macrophages which suggests a prominent role of the host PI 3-kinase activities during the T. cruzi infection process [37]. A class III PI 3-kinase located in T. cruzi (TcVps34) is able to produce phosphatidylinositol 3-phosphate, and it has been shown that it plays an important role in vital processes for the parasite survival such as osmoregulation, acidification, and vesicular trafficking [40].

4.3. Actin-dependent

Amastigotes are also capable to penetrate host cell through its plasma membrane via the actin-dependent mechanism. This mechanism contrasts notably from the two models described previously in which trypomastigotes are involved [41, 42]. The invasion capability of amastigotes depends on the T. cruzi lineage. Amastigotes from the T. cruzi I lineage (G strain) have a remarkable ability to invade non-phagocytic cells [29, 43], while the less-infective amastigotes belonging to T. cruzi II lineage (such as the Y strain) are largely engulfed by phagocytic cells (macrophages) and occasionally by other cell types [43, 44].

Once inside the host cell, amastigotes show the same ability as trypomastigotes to disrupt the parasitophorous vacuole, to replicate in the cytosol, and to differentiate into the infective trypomastigote form. There is also evidence that trypomastigotes are able to differentiate into amastigotes extracellularly while circulating in the bloodstream [45]. This remarkable observation has unravelled an additional mechanism through which the parasite can move among intracellular compartments, elude the host immune system, and sustain the infection.
5. Conclusions

Chagas disease is a potentially life-threatening illness caused by *T. cruzi*. Currently, there are no vaccines which prevent the parasite infection; hence, vector control is still the most useful method to prevent such illness. Although the mammalian host has developed a fine battery of physical and biochemical defences, the parasite has adapted its metabolism to overcome the host defences. *T. cruzi* exhibits multiple strategies to evade the host defenses in order to survive, as summarized here; diverse studies have been conducted trying to unravel the basics of *T. cruzi* infection during the early interaction with its mammalian host. The different *in vivo* and *in vitro* experimental approaches showed a complex interaction depending on both, the parasite and the host characteristics. For example, the amastigote form was relatively recently described as a potentially infective form for host cells. Despite the fact that amastigote form is generally known as a replicative form in the mammalian host, it is capable to infect host cells within the host system in a completely different manner than the one described for the typical infective trypomastigote form. Despite the amount of studies on this topic, the comprehensive understanding of the parasite invasion mechanisms is still incomplete. More efforts should be followed for the elucidation of the early steps of parasite–host interaction as they are crucial for the development of future drugs to prevent the Chagas disease.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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