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Review articles

Trypanosoma cruzi: The early contact between insect-derived metacyclic trypomastigotes and the mammalian cells

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ABSTRACT. Natural transmission of *Trypanosoma cruzi* to human is established when feces of hematophagous triatomines contaminated with insect-derived from metacyclic trypomastigotes get in contact with the skin, conjunctiva or even oral route. Article is aimed at updating the knowledge about the early interaction between insect-derived metacyclic trypomastigotes at the port of entry and the host. There are few works in the literature describing this first contact between host and natural insect-derived metacyclic trypomastigote. Although it is currently accepted that *T. cruzi* parasites can penetrate through the lesion left by the insect´s bite, pioneer data do not support this hypothesis as the main via; however, once in the dermis metacyclic trypomastigotes can spread rapidly and likely escape from inoculation site through endothelial cells and disseminate to the body via the bloodstream. A moderate inflammatory reaction took place in the skin at the port of entry within hours, the cytokines induces recruit of neutrophils predominantly, probably because triatomine feces microbiota is present in the inoculum that in some way, its presence modify the progress of the infection.

Keywords: *Trypanosoma cruzi*, metacyclic trypomastigotes, inoculation site, early interaction, *Triatoma*

Introduction

Natural transmission of *Trypanosoma cruzi* to human is established when hematophagous triatomines deposit feces contaminated with insect-derived metacyclic trypomastigotes (MT) on the skin as well as oral and conjunctiva routes. The success of maintaining the biological cycle of *T. cruzi* transmission in nature rely on the vector competence, such as metacyclogenesis, bite rate, inoculum size, vector density, infection rate, vector species, and vector survival. In this context, become important to know the natural infection rate of vectors with *T. cruzi*. Among the 150 species of vector triatomines in Latin America, its natural infection rate vary enormously between genera and species, for example, in *Rhodnius neglectus* and *Rhodnius prolixus* is 50% and 37% respectively; whereas in some *Triatoma* species can vary dramatically. The natural infection rate in *Triatoma sordida*, *Triatoma brasileinsis* and *Triatoma pseudomaculata* is 5%, 7% and 1% respectively. However, in *Triatoma infestans*, *Triatoma rubrovaria* and *Triatoma dimidiata* can reach up to 42%, 27% and 26% respectively whereas in *Pastrongylus megistus* around 27% [1,2]. Therefore, in the endemic zone, humans can be exposed several times to bites and insect's feces before being infected.

Other factors that influence the success of infection is the inoculum size. The number of metacyclic trypomastigotes excreted can be so variable depending on triatomine species and nymph and adult stage. In *T. dimidiata* we have observed up to 30,000 metacyclic trypomastigotes /mL in excreted feces, similar was observed recently in *Rhodnius prolixus*, but in a very recently published data in sylvatic vector *Mepraia spinolai* collected in Chile was observed that a single fecal drop might contain between 3 and 25,140 parasites, [3,4]. Thus, it is probable that the natural inoculum range around hundreds to few thousands of parasites if we consider that the host might be in contact with 10–100 µL of contaminated feces. Above consideration, result very important to have into account in experimental studies.

Furthermore, the metacyclogenesis or the proportion of metacyclic trypomastigotes in triatomine's feces is another factor affecting the success of infection. The proportion of metacyclic trypomastigotes/epimastigotes also is affected by the genera and species of triatomine in conjunction with the vector stage. It has been recognized that metacyclogenesis in *R. neglectus* can be up to 50% whereas in *R. prolixus* 37%. In some *Triatoma* species such as *T. sórdida*, *T. brasiliensis*, and *T. pseudomaculata* 5%, 3% and 0% respectively. In our experience in *T. dimidiata* the metacyclogenesis can be so variable among triatoma individuals. We have found individual triatomine with high metacyclogenesis up to 95% whereas other individuals not more than 5%, in spite of capture was made in the same locality and season.

In brief, the inoculum size might be from some dozen to several thousands of parasites content in no more than 100 microliters, and the proportion of infective phase "the metacyclic trypomastigotes" in the contaminated feces, in some cases as low as 3% to very high density up to 95%.

It is accepted, at least in an animal model, that metacyclic trypomastigotes are not able to cross intact skin, but if there are small wounds in the skin some parasites can invade the organism. Once, contaminated feces with MT gain access to the body, through wounds in the skin an inflammatory reaction take place at the inoculation site. It is important to have in mind, that subjects might be in contact with feces that are free of parasites likely several times before being infected with *T. cruzi.*

The first description of early contact between metacyclic trypomastigotes and the host was made in the 70s and 80s recognized that triatome`s bite is not the best choice for metacyclic trypomastigotes to gain access to the body. In a murine experimental model, we have studied at the inoculation site the early immune response to inoculum size of 1000 metacyclic trypomastigotes; the inflammatory reaction at the inoculation site is predominantly neutrophils, they appear as soon as 1 h post, then infiltration turns into mononuclear cells in the following 24 h post-inoculation. [5]. Later Schuster and Schaub [6] described the skin-penetration kinetics in a murine model with an inoculum of 1.5×10^4 metacyclic trypomastigotes on scratched skin and established that infection can be established as soon as 5 min after contact with inoculum and some parasites are immediately transported away from the inoculation site.

In this context, it is important to remember that parasite once in the epithelial tissue have to face an innate immune response and adaptive immune responses.

The first barrier is the epidermis that is basically constituted with keratinocytes, and less in abundance Langerhans, intraepithelial lymphocyte and Merkel cells, whereas the dermis, poses great diversity of cells, such as fibroblasts, miofibroblasts, macrophages, adipocytes, dendritic cells, mast cells, endothelial cells, free nerve ending and mesenchymal stem cells.

In parallel to innate cellular immune defense, the humoral innate immune responses such as complement and chemokines should be overcome by metacyclic trypomastigotes.

In this context, *T. cruzi* metacyclic trypomastigotes have evolved to be able to survive in this complex scenario.

Metacyclic trypomastigotes virulence factors

Since metacyclic trypomastigotes are very difficult to isolate directly from triatome's feces or urine, there are few reported works, the mayor information comes from epimastigotes forms that are easier to be cultured *in vitro*, or from tissue-culture trypomastigotes or metacyclic trypomastigotes artificially obtained *in vitro.*

In this section, present author is going to review the most important virulence factors that allow metacyclic trypomastigotes to survive and invade mammalian cells, MT has different virulence factors, some of them are on the cellular membrane, others are secreted.

An enzyme known as **trans-sialidase** (TS), removes and transfers sialic acid from host glycoconjugates to parasite mucin-like glycoproteins, the most abundant surface components of infective forms [7–9]. Although metacyclic trypomastigotes express a reduced level of TS in comparison to cell cultured trypomastigotes (TCT), both are able to invade mammalian cells, but TCT escapes their parasitophorous vacuole earlier than metacyclic trypomastigotes [10]. These findings suggest that TS helps trypomastigotes to invade and escape from the parasitophorous vacuole. In addition, the negative charge provided by sialic acid on the parasite surface can help the parasite to evade complement lysis and immune response recognition. The presence of TS without enzyme activity

anchored to parasite surface or secreted as extracellular microvesicle can interfere with sialylated peripheral homing receptors, giving a reduction of T cells to the parasitized tissues and promoting cell parasitization [11,12].

The **Gp82 and Gp90** are both members of the trans-sialidase superfamily genes and are the two main metacyclic stage-specific surface proteins. The Gp82 is an adhesion molecule that binds and promotes internalization of MT by inducing Ca++ signal in the host cells, promoting cytoskeleton disruption and lysosome spreading. In addition, Gp82 has very low ability in binding extracellular matrix components such as collagen, laminin and heperan sulfate [13–15], whereas Gp90 exerts a down-regulatory internalization and reduce ability to invade host cells, its surface expression depends on *T. cruzi* strain, giving, as a result, a broad differences ability to infect cells [13]. Thus, the presence of members of trans-sialidase proteins helps metacyclic trypomastigote to infect mammalian cells and evade immune responses.

The *T. cruzi* **mucins** are the most abundant of glycosylated protein displayed a great array of heterogeneity on the parasite membrane anchored via glycosylphosphatidylinositol (GPI). They are classified according to the domain and tandem repeats, with consensus sequences for *O*-glycosylation sites (TcMUC I-III). The small mucin gene (TcSMUG) family is predominant in metacyclic trypomastigotes "the insect stages of the parasite's life", resulting very important to the infectivity on the insect host. The mucins have different roles in the infection process, on one hand, they trigger the inflammatory reaction at the site of infection. In the other side, this mucin coat provides immune evasion mechanism, because of the great array of GPI-mucin genes, there are so many different *T. cruzi* clones within the parasite population with high variability of surface protein mucins and consequently high antigenic variability did exist among *T. cruzi* population. Furthermore, these mucins inhibiting clonal expansion of $CD4^+$ lymphocytes, rendering the cells anergic [11].

The **cruzipain** (CZ), the major cysteine proteinase in MT is involved in the host cell invasion, its mechanism relies on kininogen and bradykinin release, this cysteine protease cleaves plasma kininogens and complement factor 5, which is increased in the present heparan sulfate; CZ is an enzyme present in the lysosome, plasma membrane and can be secreted. [16–21]. As a consequence of CZ action, pro-oedematogenic substances are generated, the metacyclic trypomastigotes might spread more easily to other tissues.

In recent years it has been reported that, that CZ mediates proteolysis of NF-κB P65 an important signaling factor for IL-12 expression in infected macrophages, leading to unresponsiveness at least during <60 minutes, in this way CZ can modulate immune response [20].

Complement evasion molecules are another sort of molecules that metacyclic trypomastigotes have developed, which can interfere with the three complement activation pathway lectin (LP), classical (CP), and alternative (AP).

The following molecules are important in evasion of the complement system. The *T. cruzi* **calreticulin** (TcCRT), the complement regulatory protein **TcCRP**, the *T. cruzi* complement C2 receptor inhibitor trispanning (**TcCRIT**), the **gp58/68** , the *T. cruzi* decay accelerating factor (**T-DAF**) and in addition the membrane-derived vesicles which interact with C3 convertase resulting in complement inactivation or inhibition of the above complement pathway [22–24].

The surface of trypanosomas is rich in GalNAc and GlcNAc anchored by glycosylphosphatidylinositol (GPI), thus can be easily recognised by manosa binding lectins (MBL), ficolins, C3 and C1q activating LP leading to the activation of MASP-1 and MASP-2 and consequently to complement activation [25–27]. However, in spite of complement activation, the MT can circumvent the crucial activity of the complement pathway and survive [28].

TcCRT is a Ca++ binding protein that can interfere with C1q and MBL interaction to host pattern-recognition molecules inhibiting lectin and classical complement pathway; TcCRT interacts with L-Ficolin inhibit the action of MBL, through its TcCRT central domian interacts with C1, inactive C1 that remains bound to the surface parasite. In the recent paper, it was demonstrated that the distribution of TcCRT is differentially expressed in trypomastiogotes and epimastigotes. While TcCRT is preferentially located in the kinetoplast and nucleus in trypomastigotes, suggesting a secretory action; in epimastigotes, its distribution is nuclear [29]. However, there is no work using the metacyclic trypomastigotes, the great source of information comes from epimastigotes and tissue culture trypomastigotes.

T-DAF interferes in the assembly and

Table 1. Some metacyclic trypomastigotes virulence factors

Factor	Action	Consequence	References
Trans-sialidase	Transfer a sialic acid to parasite mucin plasma membrane	– Avoid early complement-mediated host response – Allow escape from parasitophora vacuole – Allow host cell adhesion	[9–11]
Gp82	– Activate a Ca2+ signaling pathway in host cells – Mediates protein tyrosine phosphorylation	– Allow internalization of parasites – Allow host-cell invasion	[13–14]
Gp90	Glycosidase activity	Antiphagocytic effect mediated by the removal of sugar residues	[15]
Cruzipain	Papain-like cysteine protease	– Host cell invasion – Proteolysis of NF-κB P65 leading to inhibition of IL-12 synthesis in macrophages	[16–20]
Calreticulin	Ca++ binding protein	Interfere C1q and MBL interaction inhibiting lectin and clasical complement pathway	[29]
T-DAF (Gp160)	Glycosylphosphatidylinositol-linked membrane protein inactivate and prevent stabilization of C3 convertases	Prevents formation of the membrane attack complex	[29]
TcCRP	Glycosylphosphatidylinositol-linked membrane protein inactivate and prevent stabilization of C3 convertases	Interfering the stabilization and formation of the C3 convertases of the alternative and clasical complement parthway	[29]
TcCRIT	No enzymatic inhibition protecting the C1s cleavage site on C2	Preventing formation of the CP C3 convertase	[29]
Complement regulatory gp58/68	Fibronectin/collagen receptor. Inhibit the formation of factor B with C3	Interfering alternative C3 convertase	[29]
Tryparedoxin peroxidase	Catalyses the reduction of peroxides	Neutralized the action of macrophage derived peroxynitrite (ONOO-)	[30]
Proline racemases	catalyze the interconversion of L- and D-proline enantiomers	Resistant against host proteolitic enzymes	[33–34].

stabilization of C3 convertases of the AP, CP, and LP. In a similar way, TcCRP is able to bind to C3a and C4b interfering with the stabilization and formation of the C3 convertases of the alternative and classical complement pathway. The expression of these molecules are *T. cruzi* strain dependent, we have observed metacyclic trypomastigotes strains more susceptible to complement than others and recently was reported in tissue culture derived trypomastigotes that expression of CRP, T-DAF and CRIT is three-fold lower messenger RNA in the susceptible strain [30,31].

The **Tc C2 receptor inhibitor traspanning is a transmembrane** protein that has C4 sequence homology that is able to bind to C2 molecule, as consequence inhibits C1 and MSP-2 protease activity preventing the formation of C3 convertase of CP and LP cascades. There is no work on metacyclic trypomastigotes, but in tissue cultured derived trypomastigotes susceptible strain showed lower expression than resistance strains [31].

The *T. cruzi* **complement regulatory gp58/68** is a fibronectin/collagen receptor important in the parasite: cell interaction, but also inhibits the

formation of factor B with C3, thus interfering alternative C3 convertase for more details, see an excellent review [28].

Tryparedoxin peroxidase is an enzyme that neutralized the action of macrophage-derived peroxynitrite (ONOO-) a potent cytotoxic and strong oxidant molecule against *T. cruzi* [32]. This enzyme allows *T. cruzi* to survive inside the parasitophora vacuole and favoring escape from it. In MT, the levels of cytosolic and mitochondrial tryparedoxin are higher in virulent strains than in non-virulent [33]. In addition, MT contains superoxide dismutase (Fe-SOD) that degrade oxygen singlets (O-2) [34].

Proline racemases (PRs) are enzymes that catalyze the interconversion of L- and D-proline enantiomers, thus allowing that membrane glycoproteins bearing D-proline become more resistant against host proteolitic enzymes [35]. In addition, it is a B cell mitogen that triggers a polyclonal B cell stimulation [34]. In general, information about proline racemases is almost missing (Table 1).

In vivo studies

If we return to the beginning point, where the triatomine`s feces are deposited with MTs on the injured skin, the parasite encounters a set of cells in the epidermis and dermis.

Studies about the early events of this primary contact, between metacyclic trypomastigotes and skin tissue cells at the inoculation site are very scarce. One explanation is because the generation of metacyclic trypomastigotes for experimental studies require infected triatomines. MT is different in many aspects of other parasite phases such as TCT, epimastigotes or metacyclic trypomastigotes derived from culture. For example, the gp82 metacyclic stage-specific surface protein on MT has

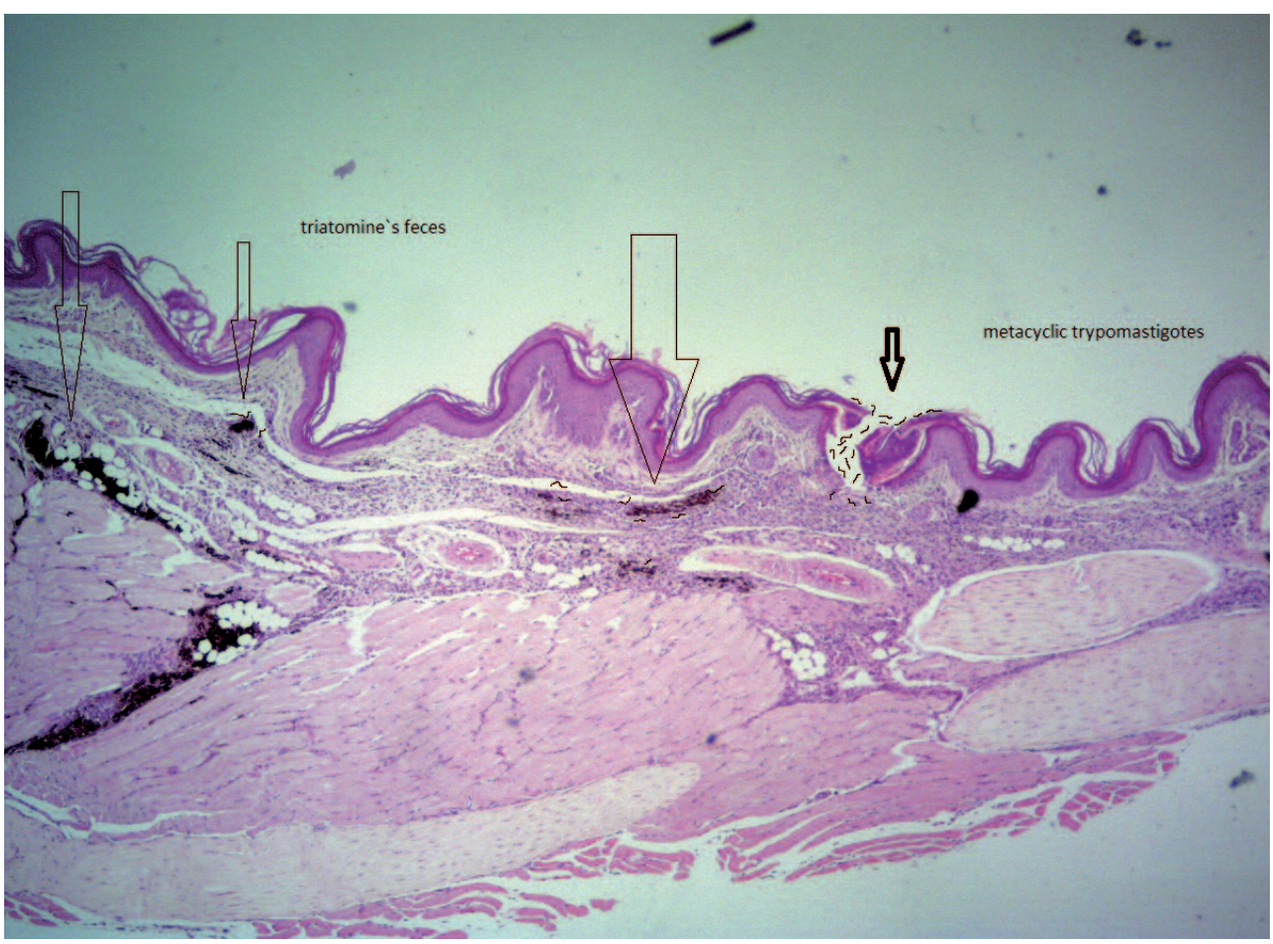

Fig. 1 a. Skin tissue inoculated with triatomine's feces. In the image can be see deposit of triatomine's feces in the dermis and the parasites drawn represents the entry of metacyclic trypomastigotes. Feces induce a strong induction of TNF-α, CXCL2 and CXCL3 at the inoculation site with recruitment of neutrophils and monocytes. This inflammatory reaction induces edema-favoring escape of metacyclic trypomastigotes via linfa or blood vessels, but in the other side, some parasites can be destroyed.

null very low binding ability for collagen, laminin, and heperan sulfate while gp 80–85-kDa glycoproteins on TCT is able to bind to these components. There are biochemical, biological and immunological differences between the different parasite phases.

In the early 80s observational study based on epidemiological evidence where people in the endemic area are exposed to contamination with infected feces do not acquire the disease. This observation gave support for the hypothesis of a possible trypanocidal effect of human sweat. Experimental studies showed that MT lost their motility after 30 minutes of incubation in the presence of human sweat. However, there was not enough support for this hypothesis by others, but instead, the desiccation was recognized as a factor that severely affect the viability and infectiveness.

In general, it is accepted that MT parasites can penetrate through bug bite site, however, the data published in regard to this issue have demonstrated that entry of MT through bug bite is an inefficient route, in comparison with mucosal via [37]. In the 80s was published that mice skin exposed to contaminated feces only 24% infection resulted. The above data might explain why the low antibody seroprevalence against *T. cruzi* in humans that live in areas of high triatomine abundance [38,39] (Fig. 1a).

In the 80s was reported using the hamster cheek pouch, a region that is devoid of lymphatic vessels, that trypomastigotes can replicate inside macrophages and resident cells in large numbers up to two weeks, but for the 28th day they were no longer seen. Whereas parasites inoculated into the footpad, a region with lymphatic vessels, it was found a diffuse inflammatory infiltrate and absence of parasites. This data suggest that the lymphatic system is the main route of parasite dissemination from the inoculation site. But in the 90's and 2000's using MT was recognized that some parasites are immediately transported away from the inoculation site, but others remain at the inoculation site from 24 h post-inoculum up to 15 days, these findings suggest that parasite can spread but others remain at the inoculation site for a brief period of time [5,6].

In a recently published data, the histological description at the portal of entry was described in a murine model. We observed an intense inflammation constituted by PMN cells in the acute phase and collagen fragmentation; in addition to fibroblast and mast cells activation at the inoculation site as soon as 1 h post-inoculation of triatomine's feces contaminated with MT. Furthermore, mice immunized several times with no contaminated feces for 1 or 3 months, and boosted with contaminated feces with metacyclic trypomastigotes showed intense inflammation rich in PMN and macrophages and less parasitemia than non-immunized mice [40]. The above information, suggest that microbiota present in the feces may have some role in the *T. cruzi* infection progression.

Recently, published data was reported in triatoma's microbiota feces the presence of bacterial community composed of *Kytococcus*, *Brevibacillus*, *Kocuria*, *Chryseobacterium*, *Pantoe*, *Proteus*, *Burkholderia*, *Acinetobacter* and *Staphylococcus*. This bacterial community is able to induce local inflammation and specific humoral IgG response [41]. Thus, it is likely this immune response against bacterial community might have a negative effect on the MT inoculum size, and consequently in the intensity/ progression of the disease (Fig. 1b).

The cells of the skin tissue are sentinels in the immune system, they are able to recognized *T. cruzi* PAMP in particular, GPI-anchored mucin-like glycoproteins through TLR2, the CpG motifs by TLR9 and RNA via TLR7, a N-lignoceroyl-sphinganine via TLR4, lipid moiety composition of different GPI anchors by TLR2 (alkylacylglycerol) or TLR4 (dihydroceramide [42–46]. This TLR-signaling activates pathways for the inflammation process, which may favor parasite survival or may be deleterious.

In general *T. cruzi* is able to induce immune suppression of macrophages and lymphocytes at an early phase of infection. So, the size of an inflammatory reaction at the inoculation site and the state of activation of infiltrated cells is very important. Also, because intense inflammatory reaction at the site of inoculation site may reduce the size of the inoculum parasite and the intensity of the infection [40]. Moreover, tissue damage may also activate TLR signaling by the so-called "damage-associated molecular patterns, promoting inflammation [47].

The cytokine response at the inoculation site in mice infected intradermally with MT, showed that there were no cytokines detected during the first week, they appeared by day 10th such as IL-10 and IL-12; however in regional lymph node to inoculation site IL-2, IL-10, IL-12 and TGF-β were detected as soon as 24 h post-inoculation [48]. Similar cytokine profile was noticed in re-infected

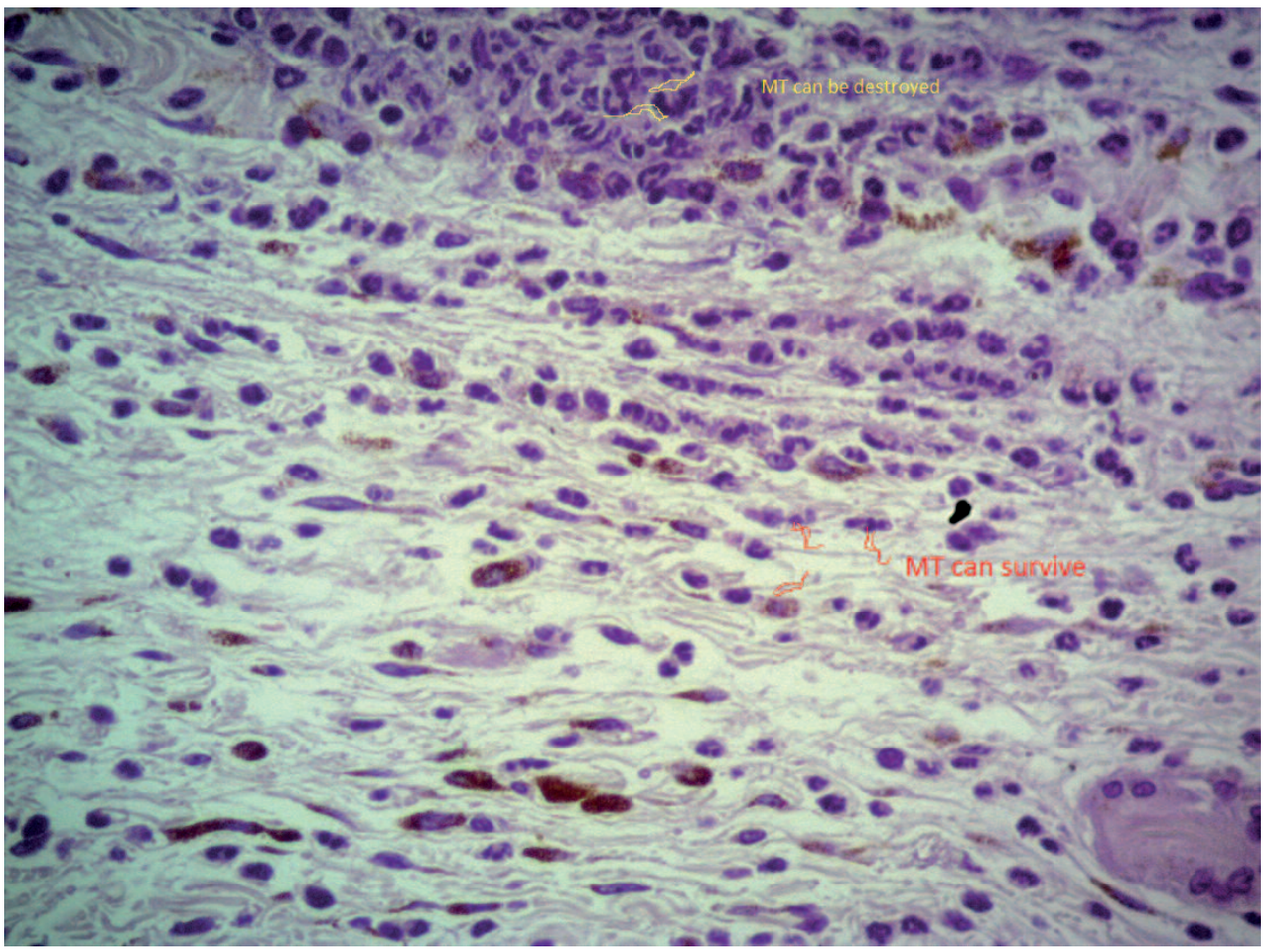

Fig. 1 b. Skin tissue inoculated with triatomine´s feces. In the image can be seen polymorphonuclear cells foci in the upper side and macrophages with ingested material in the dermis. The parasites drawn represent metacyclic trypomastigotes destroyed by neutrophils cells and in the other side a metacyclic trypomastigotes able to infect macrophages and fibroblasts. The production of TNF-α, CXCL1, CXCL2, CXCL8 and CXCL3 and the neutrophil extracellular traps (NETs) may favoring destruction of some parasites, but parasite virulence factors allow them to survive and infect some cells at the inoculation site.

animals but infiltrated inflammatory cells increased dramatically for 24h post-inoculation render no parasite DNA detection and lower parasitemia [49]. In other studies, using the same model, but tissue culture trypomastigotes instead, a type I IFN response was noticed at the site of inoculation as soon as 24 h post inoculation. This cytokine is typically involved as an antiviral mechanism, however in *T. cruzi* infection type I IFNs may result in major susceptibility and disease [50,51].

In natural conditions, metacyclic trypomastigotes are contained in the triatoma' feces, thus when a *T. cruzi* infection takes place, it is inherent that feces are also inoculated. We have observed that triatoma' feces induce a strong induction of TNF-α, CXCL2 and CXCL3 at the inoculation site at as soon as 1h post inoculum, however, when metacyclic trypomastigotes are contained in the feces the cytokine expression is lower (unpublished data).

Other important issue not fully resolved is about how *T. cruzi* escape from the dermis site and crosses the endothelial barrier. The endothelium restricts the transit of virtually large macromolecules and cells. In a recently published paper using confluent, primary endothelial cells grown on top of a collagen matrix, tissue-derived trypomastigotes were left in for 2–3 h incubation, finally, the depth relative to the monolayer was determined. They found that 10–30% of parasites in planes below the endothelial cell monolayer, suggesting a transmigration process similar to leukocytes transmigration between endothelial cell junctions [52]. However, we do not know if this mechanism can be the same for triatoma-derived metacyclic trypomastigotes.

In an animal model, MT or TCT have

demonstrated the development of prominent edema; this reaction is likely to favor parasite dissemination and invasion of endothelial cells by up-regulation of Bradikinin2 receptor and endothelin in endothelial cells at the inoculation site [40,53]. Nevertheless, virtually very scarce information exists in how *T. cruzi* crosses the endothelial barrier and escape from site inoculation.

The above data suggest that infection with metacyclic trypomastigotes may modulate the immune response and microbiota feces favor immune inflammation and likely help in controlling inoculum size.

In vitro studies

There are few works studying the interaction between insect-derived metacyclic trypomastigotes (MT) and mammals cells, many studies are conducted with media or chemically derived metacylic trypomastigotes (CMT) or tissue culture derived trypomastigotes (TCT). So, in this section, we are going to review the most recently published data.

On the interaction between epithelial cells and TCT, Chiribao et al. [54] demonstrated more than a thousand upregulated genes in the early interaction time. In particular, the inflammation-related genes; many of them are chemokines such as CXCL1, CXCL2, and CXCL8 involved in the recruitment of phagocytic cells, for instance, neutrophils. In our laboratory, we found that infection of Hela cells with MT induces a strong response of CXCL8 and the practically null or basal response of IL-6, IL-1β, TNF-α (data not published).

In vivo models as we reviewed in the previous section, the inflammatory process is rich in neutrophils in the early infection phase followed by cells mononuclear infiltration days after. Neutrophils have s short lifespan and may undergo apoptosis. One role of inflammatory and activated macrophages is to ingest apoptotic neutrophils, but this action turns off the production of pro-inflammatory cytokines. This process balances the inflammation and anti-inflammation steps through the secretion of pro-inflammatory and anti-inflammatory mediators.

In the 90s was found that, neutrophils can destroy *T. cruzi* amastigotes whereas macrophages in contact with live *T. cruzi* TCT but not epimastigotes induce IL-12 secretion and mediate resistance to infection in the early infection phase [55]. However, infected Balb/c macrophage with CMT in co-culture with inflammatory and apoptotic neutrophils increase *T. cruzi* proliferation through the participation of TGF-β and PGE2. [56].

Furthermore, TCT induces activation, decrease viability and apoptosis of neutrophils but not monocytes, allowing parasite proliferation in infected macrophages [57]. In addition, the neutrophil extracellular traps (NETs) against trypomastigotes can limit infection but could not kill them. But, pretreatment of parasites with NETs resulted in a significantly decreased number of parasites in the blood [58].

Macrophages, neutrophils and dendritic cells have a wide range of pattern recognition receptors such as TLR that efficiently recognize pathogen-associated molecular patterns that promotes the secretion of inflammatory cytokines such as IL-1β, IL-6, IL-8, IL-12, but in under specific circumstances, are able to secrete IL-10 and TGF-β for a regulatory function and increases the surface expression of HLA, CD40.

However, the dendritic cell-*T. cruzi* interaction promotes the reduction of surface molecules like CD80, CD86 and increases secretion of IL-10 and TGF-β in virulent *T. cruzi* strains which favor infection process [59,60].

Another important cell in the dermis is the fibroblasts, a recently published paper analysed transcriptoma in fibroblast at 4, 12, 20, 24, 30, 48, 72 and 96 hours post-infection with TCT [61]. They found upregulated genes in parasites between 4 and 24 hpi involved in lipid and sterol pathways while downregulated genes belong to synthesis of ubiquinone and coenzyme Q10, all part of lipid metabolism and adenine salvage pathway. Meanwhile, the fibroblasts respond strongly at 24 h with type I interferons and for 72 h TNF-related apoptosis-inducing ligand. In addition, Guimarães-Pinto et al. [62] found activation marker of the senescence-associated secretory phenotype (SASP) such as IL-6, TNF-α, IL-1β, and MCP-1 in NIH-3T3 fibroblasts.

In conclusion, thus, it is very important mimicking natural infection conditions in order to get appropriate information, for example by taking into account the inoculum size, parasite phase used, and route of infection. Since, insect *T. cruzi* phases epimastigotes and metacyclic trypomastigotes are so different between them, and in similar fashion the mammalian *T. cruzi* blood stream trypomastigotes and intracellular amastigotes.

In spite of current acceptance, that natural infection of humans happens when feces contained metacyclic trypomastigotes enter through insect bite lesion. This route seems to be an inefficient via, however it is epidemiologically important. Once metacyclic trypomastigotes gain access to the dermis layer, the infection can successfully establish. Although, there is very scarce information about the fate of metacyclic trypomastigotes once in the dermis, a moderate inflammation takes place, parasites remains for short time at the port of entry, edema and collagen fragmentation of the dermis result evident and probably parasites disseminate trough endothelial vessel. It is difficult found amastigotes nest at the port of entry or evidence of DNA parasite beyond 2 weeks post-infection.

It is necessary more studies using metacyclic trypomastigotes in the presence or not of triatomine`s feces, since natural infection occurs with triatomine`s feces contaminated with metacyclic trypomastigotes.

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