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**Specialty section:**

This article was submitted to  
Parasite and Host,  
a section of the journal  
Frontiers in Cellular and  
Infection Microbiology

**Received:** 31 August 2021

**Accepted:** 05 October 2021

**Published:** 28 October 2021

**Citation:**

D'Avila H, Souza NP, Albertoni ALS,  
Campos LC, Rampinelli PG, Correa JR  
and Almeida PE (2021) Impact  
of the Extracellular Vesicles  
Derived From *Trypanosoma cruzi*:  
A Paradox in Host Response  
and Lipid Metabolism Modulation.  
*Front. Cell. Infect. Microbiol.* 11:768124.  
doi: 10.3389/fcimb.2021.768124

# Impact of the Extracellular Vesicles Derived From *Trypanosoma cruzi*: A Paradox in Host Response and Lipid Metabolism Modulation

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Chagas disease is a major public health problem, especially in the South and Central America region. Its incidence is related to poverty and presents a high rate of morbidity and mortality. The pathogenesis of Chagas disease is complex and involves many interactive pathways between the hosts and the *Trypanosoma cruzi*. Several factors have been implicated in parasite-host interactions, including molecules secreted by infected cells, lipid mediators and most recent, extracellular vesicles (EVs). The EVs of *T. cruzi* (EVsT) were reported for the first time in the epimastigote forms about 42 years ago. The EVsT are involved in paracrine communication during the infection and can have an important role in the inflammatory modulation and parasite escape mechanism. However, the mechanisms by which EVs employ their pathological effects are not yet understood. The EVsT seem to participate in the activation of macrophages *via* TLR2 triggering the production of cytokines and a range of other molecules, thus modulating the host immune response which promotes the parasite survival. Moreover, new insights have demonstrated that EVsT induce lipid body formation and PGE<sub>2</sub> synthesis in macrophages. This phenomenon is followed by the inhibition of the synthesis of pro-inflammatory cytokines and antigen presentation, causing decreased parasitic molecules and allowing intracellular parasite survival. Therefore, this mini review aims to discuss the role of the EVs from *T. cruzi* as well as its involvement in the mechanisms that regulate the host immune response in the lipid metabolism and its significance for the Chagas disease pathophysiology.

**Keywords:** extracellular vesicles, *T. cruzi*, infectious diseases, inflammation, lipid droplets, prostaglandin, parasite replication, Chagas disease

## INTRODUCTION

Chagas disease is a neglected disease caused by infection with *Trypanosoma cruzi*, in which the persistence of the parasite and the prolonged activation of the immune system lead to a chronic inflammatory process and cardiomyopathies (D'Avila et al., 2011). The pathogenesis of Chagas disease is a multifactorial complex mechanism that involves a large number of molecules and

vesicles, among them, the extracellular vesicles (EVs) – small lipid vesicles released from the host- cell and/or parasite-cell into the extracellular space, potentially modulating the immune response (Cestari et al., 2012; Torró et al., 2018).

The EVs traffic has been the target of important mechanisms of cellular communication. Moreover, the EVs can mediate parasite-parasite and host-parasite interactions. Infected cell-derived EVs induce the communication between distant parasites and facilitate the dissemination of virulence factors (Mantel and Marti, 2014). In addition, EVs from parasitic protozoa are important in the pathogenicity and disease progression (Gonçalves et al., 1991; Nogueira et al., 2015).

The persistence of parasitemia through immune evasion mechanisms demonstrates the success of *T. cruzi* in the chronic development of Chagas disease where the EVs play an important role in modulating the immune response to the parasite. The purpose of this mini review is to present the recent progress in elucidating the origin, morphology and functions of EVs from the host cells and *T. cruzi*, as well as their impact on the parasite escape mechanism.

## EXTRACELLULAR VESICLES ORIGIN AND MORPHOLOGY

The term extracellular vesicles (EVs) is commonly used to indicate different membrane-bound structures delimited by a lipid bilayer, released in the extracellular environment. The EVs are heterogeneous, with different biogenesis, molecular composition, sizes (from 20 nm to 5 µm) and functions (Van Niel et al., 2018; Witwer and Théry, 2019). They are secreted by either prokaryotic or eukaryotic cells, thus extending their phenotype (Campos et al., 2010; Torrecilhas et al., 2012). Moreover, EVs can carry and transfer molecules for the maintenance of homeostasis, respond to cellular imbalance and help the rapid modulation and/or evasion of the immune response during different pathogenic infections (Oliveira et al., 2010; Marcilla et al., 2014; Vargas et al., 2015; De Pablos et al., 2016; Dong et al., 2019; Babatunde and Subramanian, 2020; Palacios et al., 2021).

The EVs populations are usually classified according to their origin and size into three different types: exosomes (20–100 nm), microvesicles (MVs) (ectosomes like EVs - 100–1,000 nm) and apoptotic bodies (>1000 nm) (Akers et al., 2013; Witwer and Théry, 2019). The main type of EVs are categorized according to their intracellular origin in eukaryotic cells: the exosomes, formed inside multivesicular bodies and released upon fusion of these endosomal compartments with the plasma membrane and the microparticles, formed by direct budding and constriction from the plasma membrane (Akers et al., 2013). In EVs from trypanosomatids, the vesicles have been described as larger vesicles that bud from the plasma membrane and smaller vesicles that bud within the flagellar pocket. They are released through exocytosis of multivesicular bodies carrying components of the parasite membrane and intracellular environment (Silveira et al., 1979; Lovo-Martins et al., 2018). In general, EVs from parasites or hosts cells are isolated from the

culture after spontaneous secretion, then the supernatants containing EVs are filtered for a total exosome isolation (Cestari et al., 2012; Lovo-Martins et al., 2018) (**Table 1**).

The EVs participate in intercellular communication (parasite-parasite, parasite-host cell or host cell-host cell), modulate the immune response and act as pro-inflammatory mediators (Lovo-Martins et al., 2018). Thus, they can potentiate the course of the infection from the delivery or capture of its content by the host cells, through distinct routes: endocrine, paracrine, juxtacrine or autocrine signaling (Torró et al., 2018). These pathways, however, can be influenced by several factors such as the phase of the disease, immunocompromised patient, levels of parasitemia and parasite life cycle (Ramirez et al., 2017; Torró et al., 2018).

## ROLE OF EVS FROM *T. cruzi* (EVsT) IN THE IMMUNE SYSTEM MODULATION

For years, numerous studies have been conducted on EVs in the context of diseases (Silveira et al., 1979; Lovo-Martins et al., 2018; Cronemberger-Andrade et al., 2020). The EVs are constantly shedding and sharing their products with the extra- or intracellular milieu which is correlated with the niche for proliferation and survival used by the different pathogens. The role and impact of EVs secreted by parasites during infection have been highlighted in several studies. However, the capacity of EVs to modulate the host-cell response is not clear.

The EVsT are produced in the different life cycle phases of the parasite and participate in the host cell infection process (Torrecilhas et al., 2020). In addition, several signaling cascades are activated by EVsT components modulating host-cell responses (Torrecilhas et al., 2020) (**Figure 1A**). They can modulate the cytoskeleton as well as the invasion of the metacyclic trypomastigotes by inducing tyrosine kinase phosphorylation and the actin nucleation (Yoshida and Cortez, 2008; Torrecilhas et al., 2020).

In *T. cruzi* infection, it is proposed that the intracellular life cycle should be responsible not only for parasite-parasite transmission but also for parasite-host cells, since the EVsT develop in a compartmentalized environment which increases their probability of coupling and delivery within the host cell (Torró et al., 2018). Thus, the EVsT can function as effectors in the host-parasite interaction mechanisms modulating the host immune response, increasing the number of cells infected as well as the parasitaemia (Moreira et al., 2019).

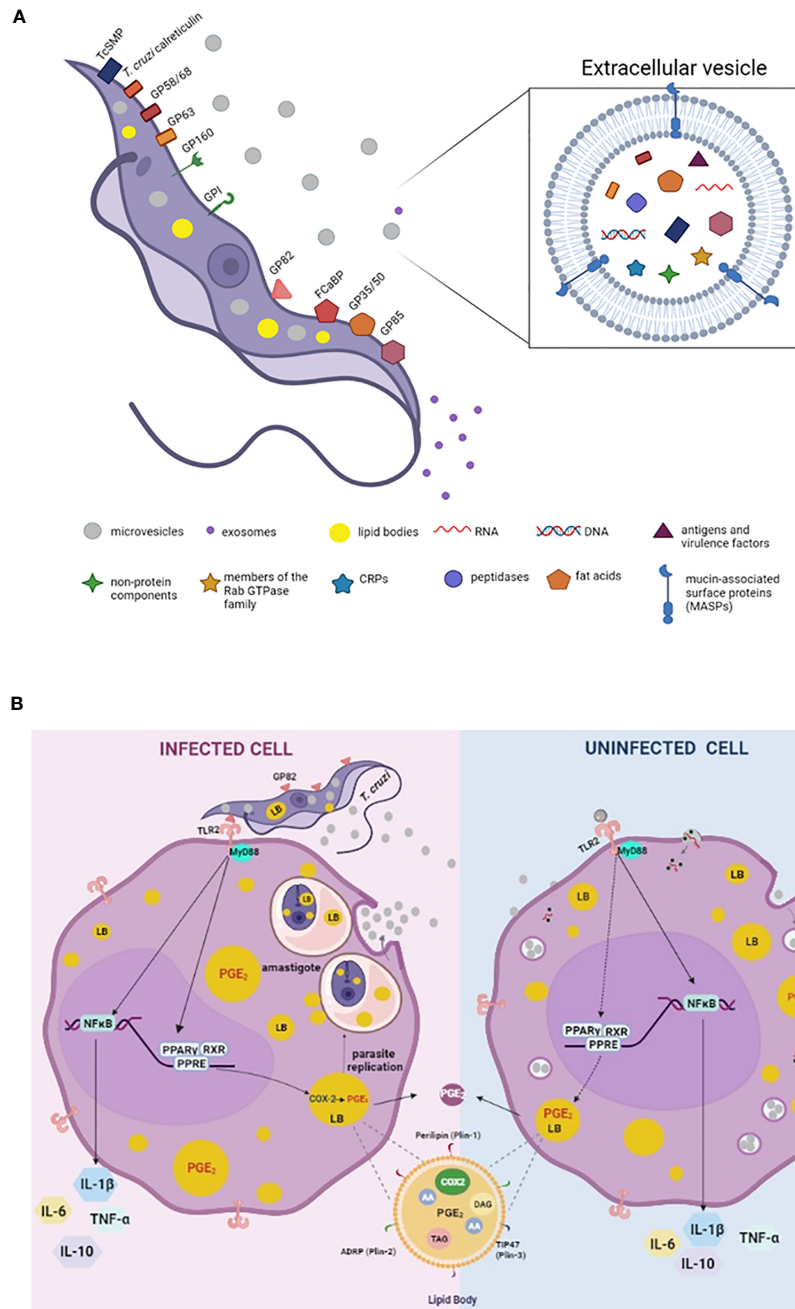
The role of the EVs in the immune system modulation is controversial. In general, some studies have demonstrated that the EVs present a protective role (Oliveira et al., 2010; Cronemberger-Andrade et al., 2014; Nogueira et al., 2015; Ribeiro et al., 2018). However, in other studies, the EVsT have a role in the evasion mechanism of the immune response, followed by an increase (50% to 250%) in parasitaemia in mice cells (Lovo-Martins et al., 2018; Moreira et al., 2019).

In the early stages of *T. cruzi* infection, the parasites promote the release of vesicles from the host cell plasma membrane,

**TABLE 1 |** Extracellular vesicles (EVs) in *T. cruzi* and other parasites.

Pathogen	Reference	EV	Origin	Study model	Methodology
<i>T. cruzi</i>	Bautista-López et al., 2017	M / E	Trypomastigotes (Tulahuen strain) / Vero cells	<i>in vitro</i>	UC, proteomic, WB, SEM
	Bayer-Santos et al., 2013	M / E	Epimastigotes and trypomastigotes (Dm28c clone)	<i>in vitro</i>	UC, proteomic, NTA, Sucrose-density gradient, TEM
	Cestari et al., 2012	M	THP-1 and mouse blood (BALB/c mice)	<i>in vitro</i> and <i>in vivo</i>	UC, FC, TEM
	Choudhuri and Garg, 2020	M	Trypomastigotes (SylvioX10/4, ATCC 50823) / C2C12, Raw 264.7, blood samples (WT and Parp1 <sup>-/-</sup> )	<i>in vitro</i> and <i>in vivo</i>	UC, NTA, ZetaView, PCR, CL-ELISA, WB
	Cronemberger-Andrade et al., 2020	M / E	Trypomastigotes (Y strain) / THP-1	<i>in vitro</i>	UC, SEC, proteomic, SEM, NTA
	De Pablos et al., 2016	M / E	Trypomastigotes (CL- Brener, PAN4 strains)	<i>in vivo</i>	UC, TEM
	Garcia-Silva et al., 2014	M / E	Trypomastigotes (Dm 28c clone)	<i>in vitro</i>	UC, TEM, Bradford
	Lovo-Martins et al., 2018	M / E	Trypomastigotes (Y strain)	<i>in vitro</i> and <i>in vivo</i>	UC, NTA
	Lozano et al., 2017	M / E	Amastigote, epimastigotes, trypomastigotes (PAN4 strain) / Vero cells	<i>in vivo</i>	UC, SEM, TEM, confocal laser scanning microscopy, Micro-BCA, WB
	Madeira et al., 2021	M / E	Trypomastigotes (Y strain) and Plasma of chronic Chagas disease patients	<i>in vitro</i> and <i>in vivo</i>	UC, CL-ELISA, NTA, SEM
	Moreira et al., 2019	E	Trypomastigotes (PAN4 strain)	<i>in vitro</i>	UC, TEM, NTA, DLS, WB, Micro-BCA
	Neves et al., 2014	M / E	Trypomastigotes (Y strain and CL-Brener clone)	<i>in vitro</i> and <i>in vivo</i>	UC, SEM, TEM, Bradford
	Nogueira et al., 2015	M	Trypomastigotes (Colombiana, YuYu, Y and CL-14 strain)	<i>in vitro</i> and <i>in vivo</i>	UC, SEC, SEM, NTA, CL-ELISA, Micro BCA
	Ramirez et al., 2017	M	Epimastigotes and trypomastigotes (Sylvio X10/6, Y, CL strains) / THP-1	<i>in vitro</i> and <i>in vivo</i>	UC, FC, proteomic, Bradford
	Ribeiro et al., 2018	M / E	Trypomastigotes (Y and YuYu strains)	<i>in vitro</i>	UC, SEC, SEM, NTA, CL-ELISA, WB, proteomic
Others					
<i>Toxoplasma gondii</i>	Beauvillain et al., 2007	E	Dendritic cell (SRDC)	<i>in vivo</i>	UC, SEM, Micro-BCA
<i>Leishmania amazonensis</i>	Cronemberger-Andrade et al., 2014	M / E	Macrophages (Bone marrow cells - BALB/c mice )	<i>in vitro</i>	UC, FC, TEM, Micro-BCA
<i>Mycobacterium bovis</i> BCG and <i>M. tuberculosis</i>	Giri and Schorey, 2008	E	<i>M. tuberculosis</i> and <i>M. bovis</i> BCG-infected macrophages (J774 cell line)	<i>in vitro</i> and <i>in vivo</i>	UC, Sucrose-density gradient, FC, Micro-BCA, WB, TEM
<i>Leishmania infantum</i> , <i>L. braziliensis</i> and <i>L. amazonensis</i>	Nogueira et al., 2020	M / E	<i>L. infantum</i> (MCAN/BR/89/BA262 strain), <i>L. braziliensis</i> (MHOM/BR/01/BA788 strain), and <i>L. amazonensis</i> (MHOM/BR/87/BA125 strain) promastigotes	<i>in vitro</i> and <i>in vivo</i>	UC, NTA, SEM, Micro-BCA
<i>Cryptococcus neoformans</i>	Oliveira et al., 2010	M / E	<i>C. neoformans</i> (HEC3393, B3501, Cap 67 strains)	<i>in vitro</i>	UC, confocal laser scanning microscopy, quantitative fluorimetric Amplex Red sterol assay kit
<i>Leishmania donovani</i> and <i>L. major</i>	Silverman et al., 2010	E	<i>L. donovani</i> (Sudan S2, 1SR, 1SR HSP1002/2, Bob, BobLPG22/2 strains) and <i>L. major</i> (Fredlin strain) amastigotes	<i>in vitro</i> and <i>in vivo</i>	UC, mass spectrometry, Sucrose-density gradient, WB
<i>Candida albicans</i>	Vargas et al., 2015	M / E	<i>C. albicans</i> (11, ATCC 90028, ATCC SC5314 strains)	<i>in vitro</i>	UC, TEM, DLS, SDS-PAGE, immunoblotting, proteomic, quantitative fluorimetric kit

M, Microvesicles; E, Exosomes; UC, Ultracentrifugation; WB, Western Blot; SEM, Scanning Electron Microscopy; TEM, Transmission Electron Microscopy; FC, Flow Cytometry; DLS, Dynamic Light Scattering; NTA, Nanoparticle Tracking Analysis; SEC, Size-exclusion Chromatography; CL-ELISA, Chemiluminescent Enzyme-linked Immunosorbent Assay; Micro-BCA, bicinchoninic acid assay; Micro-BCA, protein assay kit; PCR, Polymerase Chain Reaction; CLSM, Confocal Laser Scanning Microscopy.



**FIGURE 1** | Schematic model summarizing the molecules involved on parasite-host cell interaction process and EVs secreted by trypomastigotes of *Trypanosoma cruzi* (A). Mechanisms of action triggered by *T. cruzi*, EVs in macrophages (B). (A) Parasite exhibiting major components of its membrane associated with EVsT. Note the parasite cytoplasm containing microvesicles, exosomes, and lipid bodies (LBs). In detail, EVsT showing a lipid bilayer containing the main macromolecules carried by these structures, such as DNA, RNA, fatty acids, enzymes, mucin-associated surface proteins (MASP), among others. (B) *T. cruzi* internalized by macrophage (infected cell) induces LB formation and PGE<sub>2</sub> derived from LBs contributing to amastigote replication, as well as release of microvesicles from the host cell (EVs). We suggest that the EVsT from *T. cruzi* and EVs from infected host cells are also recognized by uninfected macrophages via TLR2 inducing LB formation and PGE<sub>2</sub> synthesis (dashed lines). However, further studies are needed to define the receptors and signaling responses induced upon EVs and EVsT-macrophage interaction and how these interactions/responses change as the exosome composition is modified during an infection. In general, *T. cruzi* and/or EVs/ EVsT via TLR2 active PPAR-γ to translocate to nucleus, heterodimerize with RXR and binding to specific DNA response elements (PPRE) in target genes, altering the lipid metabolism and inducing LB formation. The PGE<sub>2</sub> (produced in LBs via COX-2 enzyme activation) is a potent lipid mediator which reduces the host Th1 immune response (by inhibition of the TNF-α and IL-6 and induction of the IL-10) and down modulates the microbicidal function of the macrophage. In parallel, the activation of Nf-κB also modulates the synthesis of cytokines. In detail, representation of the structure and composition of LB. Arachidonic acid (AA); cyclooxygenase -2 (COX-2), triacylglycerol (TAG), diacylglycerol (DAG), peroxisome proliferator-activated receptor-γ response element (PPER).



which may contribute to their survival (Cestari et al., 2012; Bautista-López et al., 2017). According to Ramirez et al., 2017, the close contact between the membranes results in the bidirectional fusion of microvesicles (host-parasite and vice-versa), thus facilitating the interaction between the parasite and the host cell plasma membranes. In metacyclic, tissue culture-derived trypomastigote and noninfective epimastigote, *T. cruzi* forms were shown to induce different levels of EVs release from host cells (Ramirez et al., 2017). Furthermore, the EVs released during the interaction of the parasite with host cells were able to increase (around 40-50%) the host cell invasion by metacyclic trypomastigotes (Ramirez et al., 2017).

In the *T. cruzi* infection, the mobilization of intracellular  $\text{Ca}^{2+}$  deposits by the host cell during the cell invasion leads to the depolarization of the host plasma membrane, the depolymerization of F-actin and to lysosomal recruitment to the point of infection (Tardieux et al., 1994; Caler et al., 2000; Scharfstein et al., 2000). The family of small membrane proteins of *T. cruzi* (TcSMP) detected in the EVsT trigger  $\text{Ca}^{2+}$  signaling and mobilization/exocytosis of lysosomes, events that induces parasitophorous vacuoles formation and invasion of the parasite (Neves et al., 2014; Martins et al., 2015). Thus, contributing to the invasion of host cells and the increase in the percentage of cellular parasitism (Moreira et al., 2019).

Furthermore, the evasion of the complement-mediated response is triggered by the formation of EVs in host cells that are induced by metacyclic trypomastigotes (Cestari et al., 2012; Lidani et al., 2017). The release of EVs from the parasitized cells is done as an escape mechanism of the innate immunity response, by invading host cells and inhibiting complement-mediated lysis and also facilitating host cell invasion. The parasite has been shown to be able to escape the immune system by depositing host cell-derived EVs on its surface, which inhibits the action of C3 convertase (Cestari et al., 2012). Once trypomastigotes reach the bloodstream, the parasite bypasses complement-mediated lysis and opsonization with the aid of surface proteins such as calreticulin and the complement regulatory protein (CRP) also called GP160. The Gp160 and the conserved regions of the N- and C-terminal of the mucin-associated surface proteins (MASPs) were found in the EVs secreted by trypomastigotes (De Pablos et al., 2016). The Gp160 is a trypomastigote GPI anchor surface protein that binds to C3b and C4b dissociating the classical and alternative C3 convertase from complement (Norris et al., 1991; Norris, 1998).

The EVs can also contribute to the activation of the immune response with the release of proinflammatory cytokines (Torró et al., 2018). Different strains of *T. cruzi* have been shown to release EVsT and promote the activation of macrophages via TLR2 (Nogueira et al., 2015). Cronemberger-Andrade et al., 2020, showed that THP-1 cells infected by EVsT were able to induce the activation and translocation of NF- $\kappa$ B via TLR2 signaling. In addition, the EVs released by uninfected THP-1 cells also activated the cells via TLR2 (Cronemberger-Andrade et al., 2020). Furthermore, the EVs enriched with  $\alpha$ -galactosyl triggered proinflammatory responses in macrophages via TLR2-signaling pathway (Nogueira et al., 2015; Ribeiro et al., 2018), (Figure 1B). Also, Choudhuri and Garg, 2020, demonstrated a

proinflammatory response in macrophages stimulated by EVsT and EVs from infected cells and plasma of acutely and chronically infected mice, in a mechanism dependent on PARP1 (a DNA repair enzyme). EVs containing oxidized DNA fragments are recognized by cytosolic DNA sensors, cyclic GMP-AMP synthase (cGAS) and as consequence they synergize with PARP1 inducing a NF- $\kappa$ B-mediated proinflammatory cytokine production (Choudhuri and Garg, 2020).

However, in infection-derived inflammatory processes, the pre-treatment with EVsT from Y strain (EVsY) can downmodulate the release of TNF- $\alpha$  and nitric oxide (around 50%) as well as the increase in cardiac parasitism (2.5 times) in mice (Lovo-Martins et al., 2018). These data were associated with a reduction in TNF- $\alpha$  in plasma, decreased production of TNF- $\alpha$  and IL-6 by the spleen cells of infected mice (Lovo-Martins et al., 2018). Also, macrophage stimulation with EVsY before infection by *T. cruzi* increased (around 100%) the internalization rate of the parasite and the release of infecting trypomastigotes by these cells (Lovo-Martins et al., 2018), (Figures 1A, B).

Recently, Madeira et al., 2021, observed a lower concentration of circulating EVs associated with differential activation of the immunological system in patients with chronic Chagas disease, with increased production of IFN- $\gamma$  when compared with uninfected healthy controls (Madeira et al., 2021). This data was associated with parasite persistence suggesting that the EVs can be potential candidates as biomarkers during the course of Chagas disease.

## COMPOSITION OF EVsT AND ITS IMPACT IN THE COURSE OF CHAGAS DISEASE

The EVs composition contains proteins involved in host-parasite interactions, signaling, traffic and membrane fusion, transporters, oxidation-reduction, oxidized DNA, small RNAs derived from tRNAs and rRNAs among others (Théry et al., 2002; Bayer-Santos et al., 2013; Bautista-López et al., 2017; Witwer and Théry, 2019; Choudhuri and Garg, 2020), (Figure 1A). Moreover, EVs contain specific proteins involved in vesicle formation and specific markers of the endosomal pathway, such as Rab GTPases, chaperones and tetraspanins (Ostrowski et al., 2010).

The EVsT also carry a wide range of potential virulence factors, such as peptidases (calpain cysteine peptidase, oligopeptidase, thermostable carboxypeptidase 1 or aminopeptidase P), responsible for the proteolysis of different peptide substrates (Alvarez et al., 2012), oxidized DNA (Choudhuri and Garg, 2020) and ribosomal subunit (Torró et al., 2018). The epimastigotes forms also release fragments of tRNA that can induce epigenetic changes in host cells, changing the expression profile of genes involved with cytoskeleton, extracellular matrix and immune response pathways (Garcia-Silva et al., 2014; Torrecilhas et al., 2020).

The probability of the EVsT to reach distant cells is related to the cellular microenvironment of the infected tissue and its distance to the appropriate means of transport (blood and

lymphatic fluid) (Rank et al., 2011; Torr o et al., 2018). In addition, different post-translational modifications in the EVs nucleus shared by the different forms of the parasite can also cause changes in the vesicle composition, protein targets and/or biological functions (Torr o et al., 2018). Bayer-Santos et al., 2013, analyzed the proteomic composition of EVsT, confirming that a large proportion of the *T. cruzi* secretome is constitutively released via EVs (Bayer-Santos et al., 2013).

It has been demonstrated that *T. cruzi* PAMPs are poorly detected by the innate immune system at the beginning of the disease, delaying the activation of the immune response of infected host cells (Torr o et al., 2018). By immuno electron microscopic analysis, on the surface of the EVsT it was found mucin-associated proteins (MSPs), that induced an insufficient switching from IgM to IgG during the infection in mice, allowing the parasite to escape the humoral response (De Pablos et al., 2016). Similar data was observed in chagasic patients (Lozano et al., 2017).

In general, these studies suggest that EVsT are able to modulate the inflammatory response by inhibition of pro-inflammatory cytokine and NO production, as well as alteration on the humoral response in favor of the parasite (Torrecilhas et al., 2009; De Pablos et al., 2016; Lozano et al., 2017; Lovo-Martins et al., 2018).

In summary, the studies show that there is no consensus on the role of EVs during the acute and chronic phases of Chagas disease. Most of the studies discussed here during the acute phase of the disease suggest that EVs and EVsT collaborate with the development of the parasite escape mechanism. In the chronic phase there is insufficient IgG exchange during infection in mice, which allows the parasite to escape the humoral response, Choudhuri and Garg, 2020, demonstrating that EVs contained damaged DNA, thus collaborating to a pro-inflammatory profile during the chronic phase. (Choudhuri and Garg, 2020).

## ***T. cruzi* AND EVsT ELICITS LIPID BODY BIOGENESIS AND LIPID MEDIATOR SYNTHESIS IN MACROPHAGES DURING INFECTION**

Lipid body (LB) accumulation within macrophages is a common feature observed in models of Chagas disease and other parasite infections (D'Avila et al., 2008; Almeida et al., 2018). In *in vitro* and *in vivo* *T. cruzi* infection, LBs are present in both, host and parasite cells (Toledo et al., 2016). Moreover, EVsT induce LB formation and lipid mediator synthesis that modulate the host response in favor of the parasite (Lovo-Martins et al., 2018).

The mechanism of biogenesis of LBs is a regulated event highlighting the role of peroxisome proliferator-activated receptor- $\gamma$  (PPAR- $\gamma$ ), a member of the nuclear receptor family. When activated, PPAR- $\gamma$  acts as a transcription factor, translocating to the nucleus and heterodimers with the retinoid X receptors in target genes. (Figure 1B) The PPAR- $\gamma$  is involved in the mechanisms of synthesis of inflammatory mediators, fatty acid uptake and lipid storage in macrophages. Also, the activation of

the TLR2 initiates a signaling cascade that culminates in LB biogenesis through the PPAR- $\gamma$  activation (Almeida et al., 2009; Almeida et al., 2014). This data suggests that the TLR2 activation by EVsT may also activate PPAR- $\gamma$  translocation during the mechanism of LB formation in Chagas disease (Figure 1B).

In unstimulated cells, LBs are present in small numbers. However, in cells involved in inflammatory processes, infectious or non-infectious, the number of LBs can increase considerably, depending on the type of cell and the stimulus (Murphy, 2012). In the experimental infection by *T. cruzi* in rats and mice, it was demonstrated that the parasite promotes an intense inflammatory response characterized by monocytes migration to the infectious sites (Freire-De-Lima et al., 2000; Melo et al., 2003). In these sites, LBs formation in macrophages was associated with enhancement of parasitism, characterized by increased numbers of parasite nests in cardiomyocytes (Melo et al., 2003; Toledo et al., 2016).

Although not presenting a typical membrane, LBs are delimited by an electron-dense hemi-membrane formed by a hydrophilic monolayer composed of phospholipids and structural proteins. The hydrophobic core consists mainly of neutral lipids such as triacylglycerol (TAG – the major components of LBs), diacylglycerol (DAG) and cholesterol ester, as well as unsaturated fatty acids, such as arachidonic acid (AA) and oleic acid (OA) (Murphy, 2001; Walther et al., 2017).

The LBs hemi-membrane display structural proteins from perilipin family (PLIN), including perilipin/PLIN1, PLIN2/ADRP (for “adipose differentiation-related protein”), PLIN3/TIP47 (for “tail-interacting protein of 47 Kda”) (Figure 1B). The LBs present proteins involved in cell signaling processes, in vesicular transport, histones and cytokines in eukaryotic cells (Bozza et al., 2009; Li et al., 2012). Therefore, the LBs directly or indirectly act as hubs for many cell functions, such as metabolic processes, energy, store of neutral lipids for membrane synthesis, membrane traffic, intracellular signaling, lipid metabolism and the production of several inflammatory mediators (Walther et al., 2017; Almeida et al., 2018).

These organelles are considered intracellular sites of substrates and enzymes involved in the synthesis of lipid mediators biologically active, such as eicosanoids (D'Avila et al., 2006; D'Avila et al., 2011; Almeida et al., 2018). Arachidonic acid (AA) is the precursor of the eicosanoids, which is metabolized by enzymes, such as cyclooxygenase-2 (COX-2), to produce lipid mediators such as prostaglandins (PGs). Earlier studies have demonstrated that during *T. cruzi* infection, macrophages were positively immunostained for COX-2, (Freire-De-Lima et al., 2000; D'Avila et al., 2011). Although a COX-like enzyme has been reported in parasites, they do not express mammalian homologues COX-1 or COX-2. Thus, a synthesis of PGs is performed by PG synthases, which has already been identified in parasites with homology to humans (Kubata et al., 2007). Trypomastigotes stimulated with AA showed an increased number of LBs, representing sites of PGE<sub>2</sub> synthase (Toledo et al., 2016). In all these studies, both macrophages and parasite released large amounts of PGE<sub>2</sub> from new formed-LBs.

In summary, these data support the hypothesis that PGE<sub>2</sub> synthesis derived from LBs in infected cells are involved in the production of inflammatory mediators which can potentially inhibit the Th1 response in the host promoting the replication and survival of the parasite *via* enhancement of IL-10 production and a drastic reduction of TNF- $\alpha$  (D'Avila et al., 2011; Kalinski, 2012). Moreover, *T. cruzi* LBs are involved in the mechanism of release of the immunosuppressive inflammatory mediators, acting as an evasion strategy by the parasite (Toledo et al., 2016).

Interestingly, LBs biogenesis and PGE<sub>2</sub> synthesis have been observed in infected cells and in cells that do not contain internalized parasites, suggesting paracrine stimulation or a bystander amplification for the formation of these organelles and PGE<sub>2</sub> derived from LBs during *in vivo* and *in vitro* infections (Melo et al., 2003; D'Avila et al., 2011). This intercellular communication can occur through host-host cells or host-parasite mediators. Corroborating this fact, Lovo-Martins et al., 2018, demonstrated that EVsT from the Y strain alone were able to induce LBs and PGE<sub>2</sub> production by macrophages. In addition to LB formation, EVsT-stimulated macrophages showed higher PGE<sub>2</sub> production than non-stimulated macrophages (Lovo-Martins et al., 2018). PGE<sub>2</sub> derived from LBs inhibit the synthesis of TNF- $\alpha$  and antigen presentation, causing decreased NO production, thus allowing intracellular parasite survival (Freire-De-Lima et al., 2000; D'Avila et al., 2011).

Lovo-Martins et al., 2018, also demonstrated that infected macrophages primed with EVsT produced more PGE<sub>2</sub> (10 times) and less TNF- $\alpha$  and IL-6 (around 90% and 80%, respectively) than infected macrophages without prior EVsT exposure (Lovo-Martins et al., 2018). These authors hypothesized that EVsT could be down modulating the expression and activity of COX-2. As a result, the immune modulation exerted by PGE<sub>2</sub> production induced by EVsT seems to be important specifically in the beginning of the infection. In general, these data suggest that EVsT create a more favourable environment for *T. cruzi* infection, with a reduction in inflammatory cytokines and in the trypanocidal molecule NO.

These data support the role of EVsT in the complex pathogenesis of the acute phase of Chagas disease and provide new insights for a better understanding of the parasite-host interaction. However, the functionality of the EVs and the charges they carry in their compartments as well as the relevance of these products to the host cell should be further studied, as little is known about the ability of the EVs to modulate the conditions of the host cell.

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## CONCLUSION

The EVs shedding is a highly conserved parasite-host-cell interaction mechanism. The interaction between *T. cruzi* and its host cells is a bidirectional phenomenon, with thousands of EVs shared during the process. In this mini-review, we discuss the paradoxical role of EVs, which might coexist and affect differently the host response, presenting on one hand a protective role while in other studies, it contributes to the evasion mechanism, mainly through the modulation of the lipid metabolism for the production of PGE<sub>2</sub>. Also, in this mini-review, we mentioned several typical implications of EVs during *T. cruzi* infection, with an important impact in the host-lipid metabolism even in uninfected cells. Thus, the importance of EVs in the modulation of the host immune response presents a potential target for biomarkers of the Chagas disease progression.

## AUTHOR CONTRIBUTIONS

HD and PA drafted the manuscript. AA and LC edited figures. HD, AA, NS, LC, PR, JC, and PA wrote and approved the final version of the paper. PA and HD edited the manuscript. All authors contributed to the article and approved the submitted version.

## FUNDING

This work was supported by grants from Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG), Conselho Nacional de Desenvolvimento Científico e Tecnológico do Brasil (CNPq) (309523/2019-2), FAPDF and Programa de Pós-Graduação em Patologia Molecular/UNB. AA is PhD student supported by a UFJF (Federal University of Juiz de Fora) fellowship, NS and LC are PhD students supported by CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) fellowship.

## ACKNOWLEDGMENTS

The authors would like to thank Cassiana M. Boya for the English language revision.

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