Chapter

Modulation of Host Cell Apoptosis by *Trypanosoma cruzi*: Repercussions in the Development of Chronic Chagasic Cardiomyopathy

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Abstract

Trypanosoma cruzi is an intracellular parasite, which causes Chagas disease, affecting millions of people throughout the world. *T. cruzi* can invade several cell types, among which macrophages and cardiomyocytes stand out. Chagas disease goes through two stages: acute and chronic. If it becomes chronic, its most severe form is the chagasic chronic cardiomyopathy, which accounts for most of the fatalities due to this disease. For parasites to persist for long enough in cells, they should evade several host immune responses, one of these being apoptosis. Apoptosis is a type of programmed cell death described as a well-ordered and silent collection of steps that inevitably lead cells to a noninflammatory death. Cells respond to infection by initiating their own death to combat the infection. As a result, several intracellular microorganisms have developed different strategies to overcome host cell apoptosis and persist inside cells. It has been shown that *T. cruzi* has the ability to inhibit host cells apoptosis and can also induce apoptosis of cells that combat the parasite such as cytotoxic T cells. The aim of this chapter is to present up-to-date information about the molecules and mechanisms engaged by *T. cruzi* to achieve this goal and how the modulation of apoptosis by *T. cruzi* reflects in the development of chronic chagasic cardiomyopathy.

Keywords: apoptosis, cardiomyocytes, chronic chagasic cardiomyopathy, macrophages, T lymphocytes, *Trypanosoma cruzi*

1. Introduction

The long-neglected disease called American trypanosomiasis or Chagas disease (CD) was first described in 1909 by the Brazilian doctor Carlos Chagas and it currently affects millions of people throughout the world. Its causative agent is the protozoan parasite *Trypanosoma cruzi* (Kinetoplastidae: Trypanosomatidae) found in more than 150 species of domestic and wild mammals that act as reservoirs [1, 2]. There are several transmission routes to humans such as the inoculation of parasites present in the feces of a hematophagous insect vector of the Triatominae subfamily or consumption of contaminated food with these feces, blood transfusions, organ transplants, and congenital [3]. The infection has a self-limiting acute phase with evident or absent parasitemia and may go unnoticed in many infected individuals. Some patients succumb during the acute phase of the disease, while others develop an adaptive immune response that generally controls the infection. If the parasite cannot be completely eradicated, the infection can last a lifetime and, if left untreated, presents potentially life-threatening complications such as chronic chagasic cardiomyopathy (CCC), which can present itself in its acute form or after a latency period that can extend for decades [4]. The persistence of the parasite in the host for such long periods indicates that it must surpass the host's immune response mechanisms, of which apoptosis is among the most important. Interestingly, *T. cruzi* is capable of inducing or preventing apoptosis of host cells as needed [5]. In this work, we describe the basic aspects of Chagas disease and analyze the role of apoptosis in the infection by this protozoan parasite.

2. Epidemiology

CD is endemic in 21 countries in Latin America, from Mexico to the south of Argentina and Chile. Nevertheless, due to migrations and climate change, the disease has spread alarmingly to other parts of the world [6]. The World Health Organization (WHO) classifies this parasitic disease as a VBD (Vector-Borne Disease) that is among the unattended tropical diseases associated to extreme poverty in rural areas where the vectors are distributed, which favors their transmission route [7]. VBDs can be caused by bacteria, virus, and parasites, are usually transmitted by bloodsucker arthropods, represent 17% of all infectious diseases, and are prevalent in tropical and subtropical regions [8]. During the past three decades, the epidemiological overview of this disease has experienced important changes due to the implementation of vector control measures such as the use of pesticides and housing improvements [9]. In this respect, there has been a clear descent in the number of people infected by *T. cruzi*, going from 30 million in 1990 to 6–7 million currently infected. The incidence has also decreased from 700,000 estimated cases in 1990 to 30,000 cases per year in 2018. Furthermore, the mortality has gone down from 45,000 to 12,000 deaths in the same years [10, 11]. Despite all these efforts, CD continues to be an important health problem. Migration of asymptomatic people in chronic stage, who are unaware of their infection, has led to the spread of the disease to urban areas and nonendemic regions, increasing the frequency of cases in countries such as the United States, Japan, Australia, Spain, Italy, the United Kingdom, and other European countries where it is considered an emerging disease [6, 12]. In Latin America, Argentina (1,535,235), Brazil (1, 156, 821), and Mexico (876, 458) are the countries with the highest number of cases, followed by Bolivia with 607, 186 cases, making it the

country with the highest prevalence, with an estimate between 6.8 and 18% of the population seropositive for *T. cruzi* [13]. Recent estimations suggest that there could be 300,000 infected people with *T. cruzi* in the United States [14]. A study performed in Los Angeles reported a seroprevalence of 5.2% among Latin American migrants who also presented cardiac abnormalities [15]. Outside of America, Spain is the most affected country. It is estimated that only in this country there could be more than 50,000 infected individuals, most of them South American immigrants [16].

3. *Trypanosoma cruzi* life cycle

T. cruzi goes through several developmental stages. Within the vector, it is possible to find epimastigotes and metacyclic trypomastigotes, while blood trypomastigotes and amastigotes are found in vertebrates [17]. The cycle starts when a triatomine acquires blood trypomastigotes by sucking blood from an infected mammal [2]. Inside the vector, in the medial posterior gut, trypomastigotes differentiate to epimastigotes and duplicate. Afterward, epimastigotes migrate to the rectum, where they differentiate to metacyclic trypomastigotes. During the bloodmeal, vectors defecate and expel metacyclic trypomastigotes in their droppings that infect the mammal through the site of the bite, fissures in the skin, or mucous membranes [3, 18]. Parasites invade macrophages and connective tissue cells close to the site of entry due to the action of surface glycoproteins involved in the anchoring/penetration process such as gp82, gp83, gp85, and Tc-85, as well as glycoinositolphospholipids (GIPLs) [19–23]. Once inside cells, parasites have the ability to escape from the phagolysosome because of the action of enzymes such as a trans-sialidase (TS) and a low pH-dependent pore-forming protein, which allow them to reach the cytosol, where metacyclic trypomastigotes differentiate to amastigotes, duplicate, and finally transform to blood trypomastigotes that lyse the cell [24, 25]. They have the ability to surpass host effector mechanisms due to the expression of surface molecules such as GP160 and calreticulin that allow them to evade the action of complement, and GPI-mucins, which act as an antigenic protective coat. This antigenic coat presents variations that partially explain the differences in virulence and immunomodulation among strains [24, 26]. Trypomastigotes disseminate via lymph and blood to other tissues being able to infect any nucleated cell, but with certain tropism to macrophages, muscle cells (cardiac, smooth, and skeletal), and nervous cells. The cycle is completed when another vector ingests infected blood [17].

4. Genetic diversity

Initially it was thought that *T. cruzi* was a highly clonal species that had experienced little genetic mixing during its evolution [27]. However, due to increasingly detailed studies of the structure of populations and nuclear and mitochondrial DNA of *T. cruzi,* it has been suggested that in addition to clonal propagation, there have been more recent and frequent hybridizations and genetic exchange than previously thought [28]. At present, there are six discrete typification units (DTU TcI-VI) accepted by international consensus [29] that include at least two hybrid lineages (TcV and TcVI) and an additional one mainly found in bats (TcBat) [30, 31], closely related to TcI. Among these DTUs, TcI is the most diverse and widely distributed lineage with the smallest genome, the least amount of aneuploidy, and probably

related with some hybrid lineages [28]. The association of a particular *T. cruzi* strain with the diverse spectrum of the disease has not been completely established [32], although it has been possible to show some correlations. For example, Chagas disease megasyndrome is mainly found in South America where TcV and TcVI predominate. On the other hand, in North and Central America, cardiomyopathy is more common with Tc1 being strongly associated with human infection [33].

5. Chagas disease

In humans, Chagas disease progresses through two phases, acute and chronic, with the development of a symptomatic or an asymptomatic form [34]. The initial acute phase evolves within 1–2 weeks after the inoculation, lasts for 4–8 weeks, and is characterized for high levels of blood parasitemia. Affected individuals show moderate and unspecific symptoms such as fever, general discomfort, and hepatosplenomegaly. Sometimes it is possible to observe a cutaneous node (inoculation chagoma) or a unilateral bipalpebral edema (Romaña sign) that indicates the parasite inoculation site [34, 35]. The majority of acute infections are never detected. Only in less than 1% of the cases, encephalitis or myocarditis occurs that can be fatal mainly in children and the elderly [36]. The initial increase in parasitemia is contained by proinflammatory cytokines (IL-12, TNF-α, IFN-γ) and microbicidal substances synthesized by macrophages (reactive oxygen and nitrogen species) and NK cells (perforins). This initial innate immune response is followed by the development of an acquired immune response characterized by a polyclonal lymphocyte activation against the parasite mediated by T $CD4^+$, $CD8^+$, and B lymphocytes, which together reduce the parasite load, but do not completely eliminate *T. cruzi*, which still survives in the host tissues, thus initiating the chronic phase [37, 38]. Observations from research conducted before 1990 show that after being in the acute phase for 10–30 years, 30–40% of patients will turn to the symptomatic chronic phase and present digestive and/or cardiac compromise, while the rest will remain in an indeterminate phase for the rest of their lives [39–41]. Recent studies performed in children and teenagers from endemic areas in Mexico suggest that the time for the outcome of the CCC symptoms is shorter, months in some cases. Therefore, more research is required to clarify this aspect [42, 43].

5.1 Gastronitestinal form of Chagas disease

The gastrointestinal form of Chagas disease affects the whole digestive tract, with the esophagus and colon being the most altered. They present anatomic and motor disturbances as a result of chronic inflammatory lesions, focal myositis, fibrosis, and damage to intramural neurons [44], which in the esophagus could lead to dysphagia, odynophagia, epigastric or retrosternal pain, cough, and regurgitation. In advanced stages, the denervated esophagus is unable to transport the bolus, retaining it at the level of the cardia, which can cause weight loss, malnutrition, and repetitive aspiration pneumonitis [46]. On the other hand, the megacolon is characterized by prolonged constipation that could lead to fecaloma, abdominal distension, and intestinal obstruction. Despite treatment with laxatives, patients worsen and may suffer from volvulus due to intestine torsion [45, 47].

5.2 Chagasic chronic cardiomyopathy

The chronic chagasic cardiomyopathy is the most critical clinical manifestation of Chagas disease due to its high morbidity and mortality in endemic regions [48, 49]. It is characterized by a complex pathogenesis, and many aspects are still under investigation. The causes for the colossal cardiac damage experienced by CCC patients have not been thoroughly explained, and different theories have been exposed throughout the years. It was previously thought that the cardiac damage was only caused by the direct action of the parasite over cardiomyocytes; however, it is now known that there are other contributing factors such as inflammatory response, autoimmunity, microvascular abnormalities, and nervous damage [50]. The cardiac tissue damage is progressive and characterized by chronic inflammation, myocytolysis, and fibrosis [51]. A recent study revealed that during a *T. cruzi* infection, activated macrophages release the metalloproteinases MMP2 and MMP9 that activate TGF-β signaling for cardiac extracellular remodeling and thus fibroblast differentiation to myofibroblasts that is a cellular phenotype present during damage and possesses characteristics that make it suitable for healing functions [51].

The most critical heart lesions are located in the myocardium and can lead to heart failure, arrhythmias, and thromboembolism. The heart excite-conducting system is also affected, blocking completely or incompletely some of the branches of the bundle of His (mainly the right) and sometimes a complete blockade of the atrioventricular node [50, 52].

The number of parasites in the cardiac tissue of CCC patients is scarce. This, together with the finding of immunoglobulins with affinity to muscarinic receptors and β-1 adrenergic expressed in the cardiomyocyte surface and the appearance of cytotoxic T lymphocytes with reactivity toward myocardial fibers, suggested that the etiology of CCC was autoimmune [53–57]. Investigations performed during the last 30 years in animal models infected with *T. cruzi* and patients with Chagas disease have modified this theory. Nowadays it is known that the amount of *T. cruzi* antigens correlates with the intensity of the inflammatory infiltrate that acts against the parasites that reside in the tissue [58]. Such infiltrate is mainly composed of macrophages and $CD8^+$ and $CD4^+$ T lymphocytes (2:1 ratio) that show a Th1 cytokine profile (TNF-α, IFN-γ, IL-1, IL-2) [59–61]. At first, this Th1 response protects the host, but its exacerbation produces diffuse myocarditis that over time causes myocytolysis and reparative fibrosis with interstitial deposition of collagen fibers, whose progression is directly correlated with cardiac dilatation and deterioration of systolic function. The growth of the left ventricle is common, although it is also possible to observe it in the right ventricle and auricles [52, 62]. In advanced stages of the disease, it is possible to find a cardiac apical aneurism, pathognomonic of CCC [49]. Studies carried out in patients and postmortem analysis have demonstrated the presence of intracavitary thrombi accompanied by infarcts in several organs such as the lungs, kidneys, and brain [63–65].

At present, the factors that contribute to the progression of patients from the indeterminate phase to the determinate phase of Chagas disease have not been fully elucidated. As for other infectious diseases, the prognosis of CD depends on factors attributable to the host and the pathogen. Being an intracellular parasite, *T. cruzi* needs to modulate the defense mechanisms of the host cell in order to guarantee its survival. One of these mechanisms is apoptosis.

6. Apoptosis

The word apoptosis has its etymological origin in the Greek apó, which means "from" and ptosis, which means "falling off." The term encompasses a genetically regulated process of cell death through which a cell destroys itself [66] with the key participation of cysteine-dependent proteases called caspases that are specific for aspartic acid [67]. Caspases are functionally divided in initiator (caspases 8, 9, and 10) and executioner (caspases 3, 6, and 7) [68]. In metazoans, apoptosis is an essential step in a great variety of physiological events such as embryogenesis, tissue remodeling, and the elimination of damaged or non-functional cells [69].

The sequence of events that lead to apoptosis can be unchained by two main routes: the extrinsic and the intrinsic pathways [66]. The intrinsic pathway initiates with the binding of death ligands (TNF-α, Fas-L, among others), present in soluble form or in the surface of effector cells, to their respective death receptors localized in the membrane of target cells. This binding results in the recruitment of cytosolic factors into the cytoplasmic domains of the death receptor, forming the death-inducing signaling complex or DISC. Initiator caspases such as procaspase 8, 10, or both are recruited to the DISC where they are activated. Caspases 8 and 10 in turn activate the following caspases in the pathway [70].

On the other hand, the intrinsic pathway, also known as the mitochondrial pathway of apoptosis, can be induced by various factors such as environmental stress or absence of growth factors. In this pathway, the formation of pores in the outer mitochondrial membrane allows the release of cytochrome-c into the cytosol, as well as other molecules such as endonuclease-G and Bcl-2 proteins. The interaction between cytochrome c, procaspase 9, and protease activating factor 1 or APAF 1 stimulates the formation of a heptameric complex known as apoptosome, which recruits procaspase 9 molecules, activating them. Caspase 9 molecules proceed to activate the following procaspases that execute the pathway, inducing apoptosis [66, 70].

Cells that die by apoptosis undergo characteristic morphological and biochemical changes that include a reduction in size, the collapse of the cytoskeleton, the disassembly of the nuclear envelope, and the fragmentation and condensation of chromatin. There are changes in the cell membrane composition and structure. Phosphatidylserine (PS) is translocated to the outer face of the membrane and protrusions are formed that finally break into membrane-enclosed fragments called apoptotic bodies. The mitochondrial membrane also changes with the formation of pores in the outer sheath that provokes the loss of the membrane potential of this organelle. These traits are used to quantitatively assess apoptosis [71, 72]. In metazoans, apoptotic cells and cell fragments are rapidly recognized and phagocytosed by cells of the immune system such as macrophages, which efficiently recognize PS expressed on the membrane of apoptotic cells. This early removal of cellular debris prevents the inflammatory response [73].

7. Apoptosis-like death in *T. cruzi*

It may seem odd to think that single-celled organisms can undergo apoptosis themselves, but it is believed that this has benefits for the survival of the population and therefore of the species. Shortly after apoptosis was described in metazoans, Docampo et al., using transmission electron microscopy, observed cytoplasmic and nuclear features suggestive of apoptosis in epimastigotes of *T. cruzi* treated with β-lapachone,

an o-naphthoquinone that inhibits the synthesis of DNA. Such alterations included plasma membrane blebbing, chromatin condensation, and mitochondrial membrane alterations [74]. In addition to Trypanosoma, apoptotic death has been described in other protozoan taxa such as *Plasmodium*, and *Leishmania* that show distinctive characteristics of apoptosis similar to those described in multicellular organisms; however, the mechanisms involved are not fully understood [75]. Since then, other research groups have reported the appearance of stress- and drug-induced apoptotic traits (DNA fragmentation, PS externalization, loss of mitochondrial membrane potential, and cytochrome-c release) in *T. cruzi* [76, 77] with phenotypic similarities between metazoan cell apoptosis and *T. cruzi* cell death, but also with important differences in the processes and molecules that participate in them. Although PS translocation is a remarkable apoptotic trait in mammalian cells, in *T. cruzi* seems to be a parasite strategy to counteract macrophage activation [78]. On the other hand, caspases, key participants in apoptosis, are not present in *T. cruzi*. Nevertheless, its genome contains the TcMCA3 and TcMCA5 genes that code for metacaspases, cysteine proteases structurally similar to caspases but, unlike the latter, they have specificity for basic amino acid residues and are dependent on millimolar concentrations of calcium [79, 80]. The TcMCA3 protein is expressed in the four main stages of the parasite (epimastigotes, metacyclic trypomastigotes, blood trypomastigotes, and amastigotes), while TcMCA 5 is only expressed in epimastigotes. Analysis performed by immunofluorescence has shown that the treatment of *T. cruzi* with human serum, to induce programmed cell death, provokes a change in the subcellular localization of both metacaspases translocating them to the nucleus [81]. It has been observed that the increase in the expression of TcMCA5 augments the sensitivity of epimastigotes to programmed cell death as compared with parasites that express it at physiological levels. For its part, TcMCA3 protects epimastigotes from natural cell death and seems to play an important role in the process of differentiation to infectious metacyclic trypomastigotes [81].

Another molecule that could play an important role in the regulation of apoptotic cell death in *T. cruzi* is the elongation factor-1 (EF-1). This molecule, usually found in the nucleus and cytoplasm of eukaryotic cells, is formed of two subunits (EF-1α and EF-βγδ), and plays an important role in protein synthesis and in processes such as mitosis and cell proliferation [82]. Using anti-TcEF-1α antibodies coupled with fluorescein isothiocyanate, it was possible to observe that TcEF-1α accumulates in the nucleus of *T. cruzi* epimastigotes cultured for more than 13 days, which also showed apoptotic traits [83]. Subsequent investigations revealed that changes in the expression levels of EF-1α modify the rate of apoptosis in a murine erythroleukemic cell line [84], which suggests that TcEF-1α could be a marker of apoptosis in *T. cruzi*. The similarities that have been found between mammalian apoptosis and the phenomenon observed in *T. cruzi* have led some researchers to call it "apoptosis-like cell death" [85]. Nevertheless, other authors have concluded that the type of death observed in *Trypanosoma* and other protozoan parasites does not have the characteristics of a regulated death, and it is more an incidental cell death or necrosis [86].

8. Apoptosis modulation by *T. cruzi*

8.1 Apoptosis induction

Being an obligate intracellular parasite, *T. cruzi* needs to modulate the immune response mechanisms of its host to complete its life cycle and guarantee its survival

and propagation. One of these mechanisms is apoptosis that can be modulated by this parasite on several cell types such as lymphocytes, macrophages, and cardiomyocytes. Macrophages are one of the most important niches in the mammalian host for *T. cruzi* replication and they are crucial for the immune response against the parasite because, depending on the stimulus, can be classically or alternatively activated. Classically activated macrophages (M1) produce nitric oxide (NO) that has the ability to kill *T. cruzi*, whereas alternatively activated macrophages, belonging to the M2 spectrum, synthesize polyamines that participate in parasite proliferation [87, 88]. Thus, one of the most important mechanisms of protective immunity against *T. cruzi* is a Th1-type immune response mediated by $CD4^+$ and $CD8^+$ T lymphocytes that produce IFN-γ, which in turn activates macrophages toward a classical phenotype for the control of parasitemia [89]. In response to this, *T. cruzi* displays outstanding strategies to control the activation of macrophages and inhibit apoptosis such as a reduction in the production of toxic molecules, including NO and its derivatives [90, 91], and the escape from the parasitophorous vacuole [92]. One of the molecules involved in the interference with NO is phosphatidylserine (PS). Experimental evidence indicates that PS exposure is connected to the survival and reproduction of obligate intracellular parasites by inhibiting NO production from macrophages [93, 94]. This strategy has been demonstrated in the infection of murine macrophages activated with IFN-γ and LPS with *T. cruzi* where trypomastigotes expose PS in their membrane. PS expression promotes parasite engulfment by phagocytic cells, a significant decrease in the expression of NOS2, and an increase in the production of the anti-inflammatory cytokine TGF-β by the infected macrophages. This suggests that the exposure of PS by *T. cruzi* (an apoptotic trait) could be responsible for the induction of the anti-inflammatory response similar to that induced by apoptotic cells [78].

In addition to the exposure of PS in the surface of *T. cruzi*, other membrane components participate in the immune evasion strategies and are considered virulence factors. All stages of *T. cruzi* have in their plasma membrane glycoconjugates attached to the membrane via glycosylphosphatidylinositol (GPI) anchors such as GIPLs and GPI-anchored glycoproteins [95], transialidase, mucins, mucin-associated proteins, and gp63 metalloproteases. It has been shown that GIPLs, in the presence of IFN-γ, induce apoptosis in macrophages through the ceramide portion by a NO production-independent pathway [96]. Also, it has been revealed that the sialylation of GPI-mucins protects trypomastigotes from lytic antibodies and, most likely, from the action of complement [97]. The alpha galactosylceramide expressed by *T. cruzi* induces anergy in NK cells and an increase in IL-33 [5]. On the other hand, the transsialidase (TS) of *T. cruzi* is an enzyme that transfers sialic acid from glycoconjugates present in mammalian cells to the parasite surface favoring its pathogenesis due to the formation of adhesive and protective structures on its surface. Also, TS has been shown to induce apoptosis of cells of the immune system [96]. In the infections with *T. cruzi*, TS can be located on the surface of the parasite or it can be secreted away from the site of infection [98]. Experiments performed in mice where recombinant TS was administered showed that the enzyme is capable of inducing apoptosis of the thymus. Contrarily, mice treated with anti-TS neutralizing antibodies did not show abnormalities in the organ [99, 100]. Later on, these observations were corroborated with the TUNEL assay and found that apoptosis was activated by sialylation of the CD43 mucin, which is constitutively expressed on the surface of T lymphocytes and monocytes [97]. This effect was not observed in mice that were given lactitol, an inhibitor of the transferase activity of the enzyme [101].

The induction of apoptosis in macrophages infected with *T. cruzi* may represent a proliferative strategy. It has been shown that when macrophages are infected with *T. cruzi* and phagocytose apoptotic $CD4^+$ T cells, there is an increase in parasite replication inside macrophages, which in turn undergo apoptosis releasing more infective forms of the parasite such as trypomastigotes and spheromastigotes [5]. As a response to infection, macrophages release TGF-β, IL-10, and PGE2, which together deactivate them, allowing the survival of the intracellular forms of the parasite, as has also been observed with *Leishmania* [102].

For decades, the immunological suppression that *T. cruzi* exerts on cells of the immune system such as $CD4^+$ and $CD8^+$ T lymphocytes has been studied in detail, both in murine models and in patients with Chagas disease. This suppression can be macrophage-dependent or independent. In the first case, the unfavorable environment for T $CD4^+$ cell proliferation during the acute phase of *T. cruzi* infection is due to the production of IFN-γ and NO by activated macrophages. In the second case, the suppression of $CD4^+$ is through the interaction of TCR and CD3. The elimination of these cells inhibits the production of IFN-γ, thus preventing classical macrophage activation and favoring the development of an M2 phenotype, which favors the persistence of the parasite [88].

Likewise, apoptosis triggers the release of anti-inflammatory cytokines such as IL-10 and TGF-β by phagocytes, allowing the parasite to survive and continue with the infection.

It has been observed that in patients with chagasic cardiomyopathy there is no proliferation of peripheral blood mononuclear cells due to the activation of induced-apoptosis (AICD) by *T. cruzi* antigens. This has been complemented by the observation of a higher percentage of apoptotic cells in the hearts of patients with cardiac damage as compared with asymptomatic patients. Experimental evidence shows that the induction of apoptosis in $CD4^+$ T cells occurs through the induction of Fas/FasL and the activation of the executioner caspases 3 and 8 [103]. The participation of Fas/Fas-L was verified by *in vivo* injection of anti-FasL antibodies, which blocked the induced apoptosis of $CD8^+$ T lymphocytes, improving the Th1 response against the parasite. Interestingly, the blockade of TNF-α and TRAIL with antibodies did not have the same effect [104]. The increase in Fas-L has also been observed in the serum of patients in a chronic phase with CCC symptoms indicating that apoptosis also takes place during this phase [105]. Patients with chagasic cardiomyopathy showed a reduction in the proliferative response of T lymphocytes, in addition to a high production of $CD4^+CD62L^-$ T cells and an increase in the intracellular production of TNF-α, as well as the expression of genes of the TNF/TNFR superfamilies and caspases [106].

8.2 Apoptosis inhibition

T. cruzi is also capable of inhibiting apoptosis in infected cells. In addition to macrophages and T cells, the parasite also infects cardiomyocytes. *In vitro* experiments performed on murine cardiomyocytes incubated with *T. cruzi* or cruzipain (a parasite cysteine protease) showed that both trypomastigotes and cruzipain promote cardiomyocyte survival when cultured in media containing minimal serum concentrations. This phenomenon was associated with increased phosphorylation of Akt kinase and increased expression of the antiapoptotic protein Bcl-2. Furthermore, cultures that were treated with cruzipain showed less caspase-3 activation despite serum deprivation, suggesting that this enzyme might be responsible for the antiapoptotic effect [107]. Additionally, Chuenkova and Pereira observed that Schwann cells infected with

T. cruzi trypomastigotes are capable of surviving the proapoptotic stimulus induced by TNF-α, TGF-β, and H_2O_2. They found that the neurotrophic factor derived from the parasite (PNDF, a GPI-anchored neuraminidase and TS) interacts with the Akt kinase, increasing the expression levels of this protein and inhibiting the expression of at least 3 genes encoding proapoptotic proteins such as Bax, caspase-9, and the FOXO transcription factor, which together promoted cell viability [108].

It has been recently shown that *T. cruzi* amastigotes induce apoptosis in cardiomyocytes due to overexpression of Bax and reduced expression of Bcl-2 linked to trypomastigotes and amastigotes, respectively. The transcription factor STAT3, but not STAT1, was found to be active in cardiomyocytes due to trypomastigote infection. In addition, the TLR7 gene was observed to be overexpressed in cardiomyocytes incubated with trypomastigotes, which indicates that the TLR receptor is involved in intracellular recognition [109].

8.3 Apoptosis modulation by *T. cruzi* in other tissues

In addition to the modulation of apoptosis by *T. cruzi* of its main host cells, similar effects have been observed in other cell types. It has been shown that during the infection with *T. cruzi*, the parasite is able to induce apoptosis in human chorionic villi [110]. Also, the parasite has been shown to have an antiproliferative activity on the malignant melanoma cell line B16-BL6 [111].

The coinfection of *T. cruzi* with HIV can cause meningoencephalitis since both pathogens infect astrocytes. Interestingly, infected astrocytes with *T. cruzi* or *T. cruzi* and HIV, but not with HIV alone, showed a decrease in cell death and IL-6 secretion, which has a protective effect against death. This suggests that *T. cruzi* can reduce the induction of cell death by HIV and even more affect the replication of the virus [112].

9. Conclusion

Numerous studies have been performed with the aim to understand the molecular basis of the survival of *T. cruzi* in its host. *T. cruzi* is an intracellular parasite that needs to surpass different defense mechanisms and a hostile environment to persist inside host cells. To achieve this goal, this protozoan has developed a wide array of strategies that target different mechanisms and molecules in the host. One of the mechanisms that *T. cruzi* modulates is apoptosis. It has been shown that the parasite has the ability to induce or inhibit apoptosis of different cells. The profound knowledge of these targets will allow the development of strategies capable of interfering with the development of the disease. It remains to be elucidated which signal transduction pathway or pathways are responsible for establishing this relationship.

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Conflict of interest

The authors declare no conflict of interest.

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References

[1] Lewinsohn R. Carlos Chagas and the discovery of Chagas's disease (American tripanosomiasis). Journal of the Royal Society of Medicine. 1981;**74**:451-455. DOI: 10.1177/014107688107400612

[2] Rassi A, Rassi A, Marcondes de Rezende J. American Trypanosomiasis (Chagas disease). Infectious Disease Clinics of North America. 2012;**26**: 275-291. DOI: 10.1016/j.idc.2012.03.002

[3] Rassi A, Rassi A, Marin-Neto JA. Chagas disease. Lancet. 2010;**375**:1388-1402. DOI: 10.1016/S0140-6736(10)60061-X

[4] Gascón J, Albajar P, Cañas E, Flores M, Prat JG, Herrera RN, et al. Diagnosis, management, and treatment of chronic Chagas' heart disease in areas where *Trypanosoma cruzi* infection is not endemic. Revista Española de Cardiología. 2007;**60**:285-293

[5] Decote-Ricardo D, Nunes MP, Morrot A, Freire-de-Lima CD. Implications of apoptosis for the phathogenesis of *Trypanosoma cruzi* infection. Frontiers in Immunology. 2017;**8**:518. DOI: 10.3389/fimmu.2017.00518

[6] Freitas-Lidani KC, Andrade FA, Bavia L, Damasceno FS, Beltrame MH, Messias-Reason IJ, et al. Chagas disease: From discovery to a worldwide health problem. Frontiers in Public Health. 2019;**49**:1-13. DOI: 10.3389/fpubh.2019.00166

[7] World Health Organization (WHO). 2019. Chagas disease (American trypanosomiasis). Available from: https://www.who.int/health-topics/chagas-disease#tab=tab_1

[8] Vieira CB, Praça YR, Bentes KLS, Santiago PB, Silva SMM, Silva GS, et al. Triatomines: Trypanosomatids, Bacteria, and viruses potential vectors? Frontiers in Cellular and Infection Microbiology. 2018;**8**:1-12. DOI: 10.3389/fcimb.2018.00405

[9] Ramsey JM, Schofield CJ. Control of Chagas disease vectors. Salud Pública de México. 2003;**45**:123-128. DOI: 10.1590/s0036-36342003000200010

[10] Rodríguez-Morales AJ, Von A, Franco-Paredes C. Achievements and challenges in controlling Chagas disease. Boletín Médico del Hospital Infantil de México. 2011;**68**:102-109

[11] Organización Panamericana de la Salud (OPS). 2020. Enfermedad de Chagas. Available from: https://www.paho.org/es/temas/enfermedad-chagas

[12] Klein N, Hurwitz I, Durvasula R. Globalization of Chagas disease: A growing concern in nonendemic countries. Epidemiology Research International. 2012;**7**:1-13. DOI: 10.1155/2012/136793

[13] World Health Organization. Chagas disease in Latin America: An epidemiological update based on 2010 estimates. Relevé Épidémiologique Hebdomadaire. 2015;**90**:33-43

[14] Traina MI, Hernandez S, Sanchez DR, Dufani J, Salih M, Abuhamidah AM, et al. Prevalence of Chagas disease in a U.S. population of Latin American immigrants with conduction abnormalities on electrocardiogram. PLoS Neglected Tropical Diseases. 2017;**11**:e0005244. DOI: 10.1371/journal.pntd.0005244

[15] Bern C, Montgomery SP. An estimate of the burden of Chagas disease in the United States. Clinical Infectious Diseases. 2009;**49**:e52-e54. DOI: 10.1086/605091

[16] Blasco-Hernández T, García-San Miguel L, Navaza B, Navarro M, Benito A. Knowledge and experiences of Chagas disease in Bolivian women living in Spain: A qualitative study. Global Health Action. 2016;**9**(1):30201. DOI: 10.3402/gha.v9.30201

[17] Onyekwelu KC. Life cycle of *Trypanosoma cruzi* in the invertebrate and the vertebrate hosts. In: Biology of *Trypanosoma Cruzi*. 1st ed. Rijeka: IntechOpen; 2019. DOI: 10.5772/intechopen.84639

[18] García ES, Genta FA, Azambuja P, Shaub GA. Interactions between intestinal compounds of triatomines and *Trypanosoma cruzi*. Trends in Parasitology. 2010;**26**:499-505. DOI: 10.1016/j.pt.2010.07.003

[19] Tardieux I, Nathanson MH, Andrews NW. Role in the host cell invasion of *Trypanosoma cruzi* induced cytosolic free Ca^{2+} transients. Journal of Experimental Medicine. 1994;**179**:1017-1022. DOI: 10.1084/jem.179.3.1017

[20] Lima MF, Villalta F. Host-cell attachment by *Trypanosoma cruzi* identification of an adhesion molecule. Biochemical and Biophysical Research Communications. 1988;**155**:256-262. DOI: 10.1016/s0006-291x(88)81077-5

[21] Mattos EC, Tonelli RR, Colli W, Alves MJ. The Gp85 surface glycoproteins from Trypanosoma cruzi. Sub-cellular. Biochemistry. 2014;**74**:151-180. DOI: 10.1007/978-94-007-7305_9_7

[22] Colli W, Alves MJ. Relevant glycoconjugates on the surface of *Trypanosoma cruzi*. Memórias do Instituto Oswaldo Cruz. 1999;**94**:37-49. DOI: 10.1590/s0074-02761999000700004

[23] de Souza W, de Carvalho TM, Barrias ES. Review on *Trypanosoma cruzi*: Host cell interaction. International Journal of Cell Biology. 2010;**2010**:295394. DOI: 10.1155/2010/295394

[24] Cardoso MS, Reis-Cunha JL, Bartholomeu DC. Evasion of the immune response by *Trypanosoma cruzi* during acute infection. Frontiers in Immunology. 2016;**6**:659. DOI: 10.3389/fimmu.2015.00659

[25] Sibley LD, Andrews NW. Cell invasion by un-palatable parasites. Traffic. 2000;**1**:100-106. DOI: 10.1034/j.1600-0854.2000.010202.x

[26] Soares RP, Torrecilhas AC, Assis RR, Rocha MN, Moura E, Castro FA, et al. Intraspecies variation in *Trypanosoma cruzi* GPI-mucins: Biological activities and differential expression of α-galactosyl residues. American Journal of Tropical Medicine and Hygiene. 2012;**87**:87-96. DOI: 10.4269/ajtmh.2012.12-0015

[27] Tibayrenc M. Genetic subdivisions within *Trypanosoma cruzi* (discrete typing units) and their relevance for molecular epidemiology and experimental evolution. Kinetoplastid Biology and Disease. 2003;**2**:12. DOI: 10.1186/1475-9292-2-12

[28] Messenger LA, Miles MA. Evidence and importance of genetic exchange among field populations of *Trypanosoma cruzi*. Acta Tropica. 2015;**151**:150-155. DOI: 10.1016/j.actatropica.2015.05.007

[29] Zingales B, Miles MA, Campbell DA, Tibayrenc M, Macedo AM, Teixeira MM,

et al. The revised *Trypanosoma cruzi* subspecific nomenclature: Rationale, epidemiological relevance and research applications. Infection, Genetics and Evolution. 2012;**12**:240-253. DOI: 10.1016/j.meegid.2011.12.009

[30] Marcili A, Lima L, Cavazzana M, Junqueira AC, Veludo HH, Maia Da Silva F, et al. A new genotype of *Trypanosoma cruzi* associated with bats evidenced by phylogenetic analyses using SSU rDNA, cytochrome b and histone H2B genes and genotyping based on ITS1 rDNA. Parasitology. 2009;**136**:641-655. DOI: 10.1017/S0031182009005861

[31] Ramirez JD, Hernandez C, Montilla M, Zambrano P, Florez AC, Parra E, et al. First report of human *Trypanosoma cruzi* infection attributed to TcBat genotype. Zoonoses and Public Health. 2014;**61**:477-479. DOI: 10.1111/zph.12094

[32] Lewis MD, Francisco AF, Taylor MC, Jayawardhana S, Kelly JM. Host and parasite genetics shape a link between *Trypanosoma cruzi* infection dynamics and chronic cardiomyopathy. Cellular Microbiology. 2016;**18**:1429-1443. DOI: 10.1111/cmi.12584

[33] Gruendling AP, Massago M, Teston AP, Monteiro WM, Kaneshima EN, Araujo SM, et al. Impact of benznidazole on infection course in mice experimentally infected with *Trypanosoma cruzi* I, II, and IV. American Journal of Tropical Medicine and Hygiene. 2015;**92**:1178-1189. DOI: 10.4269/ajtmh.13-0690

[34] Bern C. Chagas' disease. New England Journal of Medicine. 2015;**373**:456-466. DOI: 10.1056/NEJMral410150

[35] Malik LH, Singh GD, Amsterdam EA. The epidemiology, clinical manifestations, and management of Chagas heart disease. Clinical Cardiology. 2015;**38**:565-569. DOI: 10.1002/clc.22421

[36] Pérez-Molina JA, Molina I. Chagas disease. Lancet. 2018;**391**:82-94. DOI: 10.1016/S0140-6736(17)31612-4

[37] Acevedo GR, Girard MC, Gómez KA. The unsolved jigsaw puzzle of the immune response in Chagas disease. Frontiers in Immunology. 2018;**9**:1929. DOI: 10.3389/fimmu.2018.01929

[38] Reyes AC, Rosales JLE. *Trypanosoma cruzi* infection: Mechanisms of evasion of immune response. In: Biology of *Trypanosoma Cruzi*. 1st ed. Rijeka: IntechOpen; 2019. DOI: 10.5772/intechopen.84359

[39] Espinosa R, Carrasco HA, Belandria F, Fuenmayor AM, Molina C, González R, et al. Life expectancy analysis in patients with Chagas' disease: Prognosis after one decade (1973-1983). International Journal of Cardiology. 1985;**85**:45-56. DOI: 10.1016/0167-5273(85)90262-1

[40] Gascón J, Albajar P, Cañas E, Flores M, Gómez i Prat J, Herrera RN, Lafuente CA, Luciardi HL, Moncayo A, Molina L, Muñoz J, Puente S, Sanz G, Treviño B, Sergio-Salles X. Diagnosis, management and treatment of chronic Chagas' heart disease in areas where *Trypanosoma cruzi* is not endemic. Revista Española de Cardiología. 2007;**60**:285-293

[41] Rodriguez CJ, Borges-Pereira J. Chronic phase of Chagas disease: Why should it be treated? A comprehensive review. Memórias do Instituto Oswaldo Cruz. 2011;**106**:641-645. DOI: 10.1590/s0074-02762011000600001

[42] Salazar-Schettino PM, Cabrera-Bravo M, Vazquez-Antona C, Zenteno E,

de Alba-Alvarado M, Torres-Gutiérrez E, et al. Chagas disease in México: Report of 14 cases of Chagasic cardiomyopathy in children. The Tohoku Journal of Experimental Medicine. 2016;**240**:243-249. DOI: 10.1620/tjem.240.243

[43] Méndez-Dominguez N, Chi-Méndez C, Canto-Losa J, Peniche-Echazarreta A, Canto-Losa JP, Gómez-Carro S. Chagasic Cardiopathy in a child: Case report. Revista Chilena de Pediatría. 2017;**88**:647-651. DOI: 10.4067/S0370-41062017000500012

[44] Bilder CR, Goin JC. Gastrointestinal involvement in Chagas disease. Neurogastro Reviews LATAM. 2017;**1**:168-179

[45] Carrada-Bravo T. *Trypanosoma cruzi*: historia natural y diagnóstico de la enfermedad de Chagas. Revista Mexicana de Patología Clínica. 2004;**51**:205-219

[46] Matsuda NM, Miller SM, Evora PR. The chronic gastrointestinal manifestations of Chagas disease. Clinics (São Paulo, Brazil). 2009;**64**:1219-1224. DOI: 10.1590/S18075932200900 1200013

[47] Dias JC. The indeterminate form of human chronic Chagas'disease. A clinical epidemiological review. Revista da Sociedade Brasileira de Medicina Tropical. 1989;**22**:147-156. DOI: 10.1590/s0037-86821989000300007

[48] Hotez P, Bottazzi ME, Strub-Wourgaft N, Sosa-Estani S, Torrico F, Pajín L, et al. A new patient registry for Chagas disease. PLoS Neglected Tropical Diseases. 2020;**14**:e0008418. DOI: 10.1371/journal.pntd.0008418

[49] Pech-Aguilar AG, Haro-Álvarez AP, Rosado-Vallado ME. Updated review on the pathophysiology of Chagas cardiomyopathy. Revista Médica del Instituto Mexicano del Seguro Social. 2020;**58**:1-8. DOI: 10.24875/RMIMSS.M20000037

[50] Benck L, Kransdorf E, Patel J. Diagnosis and management of Chagas cardiomyopathy in the United States. Current Cardiology Reports. 2018;**20**:131. DOI: 10.1007/s11886-018-1077-5

[51] Choudhuri S, Garg NJ. *Trypanosoma cruzi* induces the parp1/ap-1 pathway for upregulation of metalloproteinases and transforming growth factor β in macrophages: Role in cardiac fibroblast differentiation and fibrosis in Chagas disease. MBio. 2020;**11**:e01853-e01820. DOI: 10.1128/mBio.01853-20

[52] Texeira AR, Nascimento RJ, Sturn NR. Evolution and pathology in Chagas disease-a review. Memórias do Instituto Oswaldo Cruz. 2006;**101**:463-491. DOI: 10.1590/s0074-02762006000500001

[53] Cossio PM, Diez C, Szarfman A. Chagasic cardiopathy. Demonstration of a serum gamma globulin factor which reacts with endocardium and vascular structures. Circulation. 1974;**49**:13-21. DOI: 10.1161/01.cir.49.1.13

[54] Higuchi ML, Lopes EA, Saldanha LB, Barretto AC, Stolf NA, Bellotti G, et al. Immunopathologic studies in myocardial biopsies of patients with Chagas disease and idiopathic cardiomyopathy. Revista do Instituto de Medicina Tropical de São Paulo. 1986;**28**:87-90. DOI: 10.1590/s0036-46651986000200004

[55] Borda EP, Cossio P, Veja MV, Arana R, Sterin-Borda L. A circulating IgG in Chagas disease which binds to β-adrenoreceptors of myocardium and modulates their activity. Clinical and Experimental Immunology. 1984;**57**:679-686

[56] Sterin-Borda L, Gorelik G, Borda ES. Chagasic IgG binding with cardiac

muscarinic cholinergic receptors modifies cholinergic-mediated cellular transmembrane signals. Clinical Immunology and Immunopathology. 1991;**61**:387-397. DOI: 10.1016/s0090-1229(05)80010-8

[57] Santos-Buch CA, Texeira AR. The immunology of experimental Chagas' disease. III. Rejection of allogeneic hearth cells in vitro. Journal of Experimental Medicine. 1974;**140**:38-53. DOI: 10.1084/jem.140.1.38

[58] Higuchi MDL, Reis MM, Aiello VD, Benvenuti LA, Gutierrez PS, Belloti G, et al. Association of an increase in $CD8^+$ T cells with the presence of *Trypanosoma cruzi* antigens in chronic, human, chagasic myocarditis. American Journal of Tropical Medicine and Hygiene. 1997;**56**:485-489. DOI: 10.4269/ajtmh.1997.56.485

[59] Higuchi MDL, Gutiérrez VDA. Immunohistochemical characterization of infiltrating cell in human chronic chagasic myocarditis: Comparison with myocardial rejection process. Virchows Archiv. A, Pathological Anatomy and Histopathology. 1993;**423**:157-160. DOI: 10.1007/BF01614765

[60] Morato MJF, Colley DG, dos Reis MA. Cytokines profiles during experimental Chagas' disease. Brazilian Journal of Medical and Biological Research. 1998;**31**:123-125. DOI: 10.1590/s0100-879x1998000100016

[61] Rocha-Rodrigues DB, Dos Reis MA, Romano A, de Lima SA, Antunes V, Tostes S, et al. In situ expression of regulatory cytokines by heart inflammatory cells in Chagas' disease patients with hearth failure. Clinical and Developmental Immunology. 2012;**2012**:361730. DOI: 10.1155/2012/361730

[62] Rossi MA. The pattern of myocardial fibrosis in chronic Chagas' heart disease. International Journal of Cardiology. 1991;**30**:335-340. DOI: 10.1016/0167-5273(91)90012-e

[63] Bezerra da Silva G, Hörbe Antunes V, Motta M, et al. Chagas disease-associated kidney injury – A review. Nefrología Latinoamericana. 2017;**22**:22-26

[64] Carod-Artal FJ. Stroke: A neglected complication of American trypanosomiasis (Chagas' disease). Transactions of the Royal Society of Tropical Medicine and Hygiene. 2007;**102**:1075-1080. DOI: 10.1016/j.trstmh.2007.06.007

[65] Samuel J, Oliveira M, Correa De Araujo RR, Navarro MA, Muccillo G. Cardiac thrombosis and thromboembolism in chronic Chagas' heart disease. The American Journal of Cardiology. 1983;**52**:147-151. DOI: 10.1016/0002-9149(83)90085-1

[66] Elmore S. Apoptosis: A review of programmed cell death. Toxicologic Pathology. 2007;**35**:495-516. DOI: 10.1080/01926230701320337

[67] Keller N, Grüter M, Zerbe O. Studies of the molecular mechanism of caspase-8 activation by solution NMR. Cell Death and Differentiation. 2010;**17**:710-718. DOI: 10.1038/cdd.2009.155

[68] Opdenbosch NV, Lamkanfi M. Caspases in cell death, inflammation, and disease. Immunity. 2019;**50**:1352-1364. DOI: 10.1016/j.immuni.2019.05.020

[69] Singh R, Letai A, Sarosiek K. Regulation of apoptosis in health and disease: The balancing act of BCL-2 family proteins. Nature Reviews Molecular Cell Biology. 2019;**20**:175-193. DOI: 10.1038/s41580-018-0089-8

[70] D'Arcy MS. Cell death. A review of the major forms of apoptosis, necrosis and autophagy. Cell Biology International. 2019;**43**:582-592. DOI: 10.1002/cbin.11137

[71] Saraste A, Pulkki K. Morphologic and biochemical hallmarks of apoptosis. Cardiovascular Research. 2000;**45**:528-537. DOI: 10.1016/s0008-6393(99)00384-3

[72] Galluzzi L, Vitale I, et al. Molecular mechanisms of cell death: Recommendations of the nomenclature committee on cell death. Cell Death and Differentiation. 2018;**25**:486-541. DOI: 10.1038/s41418-017-0012-4

[73] Henson PM, Bratton DL, Fadok VA. Apoptotic cell removal. Current Biology. 2001;**11**:R795-R805. DOI: 10.1016/S0960-9822(01)00474-2

[74] Docampo R, Lopes JN, Cruz FS, de Souza W. *Trypanosoma cruzi*: Ultrastructural and metabolic alterations of epimastigotes by β-lapachone. Experimental Parasitology. 1977;**42**:142-149. DOI: 10.1016/0014-4894(77)90071-6

[75] Kaczanowski S. Apoptosis: Its origin, history, maintenance and the medical implications for cancer and aging. Physical Biology. 2016;**13**:031001. DOI: 10.1088/1478-3975/13/3/031001

[76] Piacenza L, Peluffo G, Radi R. L-arginine-dependent suppression of apoptosis in *Trypanosoma cruzi*: Contribution of the nitric oxide and polyamine pathways. Proceedings of the National Academy of Sciences of the United States of America. 2001;**98**:7301-7306. DOI: 10.1073/pnas.121520398

[77] Zuma AA, Mendes IC, Reignault LC, Elias MC, de Souza W, Machado CR, et al. How *Trypanosoma cruzi* handles cell cycle arrest promoted by camptothecin, a topoisomerase I inhibitor. Molecular and Biochemical Parasitology. 2014;**193**:93-100. DOI: 10.1016/j.molbiopara.2014.02.001

[78] Damatta RA, Seabra SH, Deolindo P, Arnholdt ACV, Manhães L, Goldenberg S, et al. *Trypanosoma cruzi* exposes phosphatidylserine as an evasion mechanism. FEMS Microbiology Letters. 2006;**266**:29-33. DOI: 10.1111/j.1514-6968.2006.00495.x

[79] Kosec G, Alvarez VE, Agüero F, Sánchez D, Dolinar M, Turk B, et al. Metacaspases of *Trypanosoma cruzi*: Possible candidates for programmed cell death mediators. Molecular and Biochemical Parasitology. 2006;**145**:18-28. DOI: 10.1016/j.molbiopara.2005.09.001

[80] Uren AG, O'Rourke K, Aravind L, Pisabarro MT, Seshagiri S, Koonin EV, et al. Identification of paracaspases and metacaspases: Two ancient families of caspase-like proteins, one of which plays a key role in MALT-lymphoma. Molecular Cell. 2002;**9**:911-917. DOI: 10.1016/s1097-2765(00)00094-0

[81] Laverrière M, Cazzulo JJ, Alvarez VE. Antagonic activities of *Trypanosoma cruzi* metacaspases affect the balance between cell proliferation, death and differentiation. Cell Death and Differentation. 2012;**19**:1358-1369. DOI: 10.1038/cdd.2012.12

[82] Sasikumar AN, Perez WB, Kinzy TG. The many roles of the eukaryotic elongation factor 1 complex. Wiley Interdisciplinary Reviews: RNA. 2012;**3**:543-555. DOI: 10.1002/wrna.1118

[83] Billaut-Mulot O, Fernández-Gómez R, Loyens M, Ouaissi A.

Trypanosoma cruzi elongation factor 1α: Nuclear localization in parasites undergoing apoptosis. Gene. 1996;**174**:19-26. DOI: 10.1016/0378-1119(96)00254-5

[84] Kato MV. The mechanisms of death of an erythroleukemic cell line by p53: Involvement of the microtubule and mitochondria. Leukemia and Lymphoma. 1999;**33**:181-186. DOI: 10.3109/10428199909093740

[85] Menna-Barreto RFS. Cell death pathways in pathogenic trypanosomatids: Lessons of (over) kill. Cell Death & Disease. 2019;**10**:93. DOI: 10.1038/s41419-019-1370-2

[86] Proto WR, Coombs GH, Mottram JC. Cell death in parasitic protozoa: regulated or incidental? Nature Reviews Microbiology. 2013;**11**:58-66. DOI: 10.1038/nrmicro2929

[87] Mills CD, Kincaid K, Alt JM, Heilman MJ, Hill AM. M-1/M-2 macrophages and the Th1/Th2 paradigm. Journal of Immunology. 2000;**164**:6166-6173. DOI: 10.4049/jimmunol.164.12.6166

[88] Bastos KR, Alvarez JM, Marinho CR, Rizzo LV, Lima MR. Macrophages from IL-12p40-deficient mice have a bias toward the M2 activation profile. Journal of Leukocyte Biology. 2002;**71**:271-278

[89] Ghabdan-Zanluqui N, Fanini-Wowk P, Pinge-Filho P. Macrophage polarization in Chagas disease. Journal of Clinical and Cellular Immunology. 2015;**6**:2. DOI: 10.4172/2155-9899.1000317

[90] Vincendeau P, Daulouède S. Macrophage cytostatic efect on Trypanosoma musculi involves an L-arginine-dependent mechanism. Journal of Immunology. 1991;**146**:4338-4343

[91] Bogdan C. Nitric oxide synthase in innate and adaptive immunity: An update. Trends in Immunology. 2015;**36**:161-178. DOI: 10.1016/j.it.2015.01.003

[92] Nogueira N, Cohn Z. Trypanosoma cruzi: Mechanism of entry and intracellular fate in mammalian cells. Journal of Experimental Medicine. 1976;**143**:1402-1420. DOI: 10.1084/jem.143.6.1402

[93] Seabra SH, de Souza W, DaMatta RA. *Toxoplasma gondii* exposes phophatidylserine that induces a TGF-β autocrine effect orchestrating macrophages evasion. Biochemical and Biophysical Research Communications. 2004;**324**:744-752. DOI: 10.1016/j.bbrc.2004.09.114

[94] Wanderley JL, Moreira ME, Benjamin A, Bonomo AC, Barcinski MA. Mimicry of apoptotic cells by exposing phosphatidylserine participes in the establishment of amastigotes of *Leishmania (L) amazonensis* in mammalian hosts. Journal of Immunology. 2006;**176**:1834-1839. DOI: 10.4049/jimmunol.176.3.1834

[95] Figueiredo JM, Rodrigues DC, Silva RC, Koeller CM, Jiang JC, Jazwinski MS, et al. Molecular and functional characterization of the ceramide synthase from Trypanosoma cruzi. Molecular and Biochemical Parasitology. 2013;**182**:62-74. DOI: 10.1016/j.molbiopara.2011.12.006

[96] Booth L, Smith TK, Booth L, Smith TK. Lipid metabolism in *Trypanosoma cruzi*: A review. Molecular and Biochemical Parasitology. 2020;**240**:111324. DOI: 10.1016/j.molbiopara.2020.111324

[97] Mucci J, Risso MG, Leguizamón MS, Frasch ACC, Campetella O. The trans -sialidase from *Trypanosoma cruzi* triggers apoptosis by target cell sialylation. Cellular Microbiology. 2006;**8**:1086-1095. DOI: 10.1111/j.1462-5822.2006.00689.x

[98] Buschiazzo A, Amaya MF, Cremona ML, Frasch AC, Alzari PM. The crystal structure and mode of action of trans-sialidase, a key enzyme in *Trypanosoma cruzi* pathogenesis. Molecular Cell. 2002;**10**:757-768. DOI: 10.1016/s1097-2765(02)00680-9

[99] Leguizamón MS, Mocetti E, Garcia-Rivello H, Argibay PF, Campetella O. *Trans*-sialidase from *Trypanosoma cruzi* induces apoptosis in cells from the immune system *in vivo*. Journal of Infectious Diseases. 1999;**180**:1398-1402. DOI: 10.1086/315001

[100] Mucci J, Hidalgo A, Mocetti E, Argibay PF, Leguizamón MS, Campetella O. Thymocyte depletion in *Trypanosoma cruzi* infection is mediated by *trans*-sialidase induced apoptosis on nurse cells complex. Proceedings of the National Academy of Sciences of the United States of America. 2002;**99**:3896-3901. DOI: 10.1073/pnas.052496399

[101] Aoki M, Guiñazú NL, Pellegrini AV, Gotoh T, Masih DT, Gea S. Cruzipain, a major *Trypanosoma cruzi* antigen, promotes arginase-2 expression and survival of neonatal mouse cardiomyocytes. American Journal of Physiology. Cell Physiology. 2004;**286**:C206-C212. DOI: 10.1152/ajpcell.00282.2003

[102] Arcanjo AF, LaRocque-de-Freitas IF, Rocha JDB, Zamith D, Costa-da-Silva AC, Nunes MP, et al. The PGE2/IL-10 axis determines susceptibility of B-1 cell-derived phagocytes (B-1CDP) to *Leishmania major* infection. PLoS One. 2015;**10**:1-18. DOI: 10.1371/journal.pone.0124888

[103] Lopes MF, Nunes MP, Henriques-Pons A, Giese N, Morse HC III, Davidson WF, et al. Increased susceptibility of Fas ligand-deficient gld mice to *Trypanosoma cruzi* infection due to a Th2-biased host immune response. European Journal of Immunology. 1999;**29**:81-89. DOI: 10.1002/(SICI)1521-4141(199901)29:01<81::AID-IMMU81>3.0.CO;2-Y

[104] Guillermo LV, Silva EM, Ribeiro-Gomes FL, De Meis J, Pereira WF, Yagita H, et al. The Fas death pathway controls coordinated expansions of type 1 CD8 and type 2 CD4 T cells in Trypanosoma cruzi infection. Journal of Leukocyte Biology. 2007;**81**:942-951. DOI: 10.1189/jlb.1006643

[105] Rodrigues V Jr, Agrelli GS, Leon SC, Silva Texeira DN, Tostes S Jr, Rocha-Rodrigues DB. Fas/Fas-L expression, apoptosis and low proliferative response are associated with hearth failure in patients with chronic Chagas' disease. Microbes and Infection. 2008;**10**:29-37. DOI: 10.1016/j.micinf.2007.09.015

[106] Chaves AT, Assis J, De Gomes S, Fiuza JA, Carvalho AT, Ferreira KS, et al. Immunoregulatory mechanisms in Chagas disease: Modulation of apoptosis in T-cell mediated immune responses. BMC Infectious Diseases. 2016;**16**:1-11. DOI: 10.1186/s12879-016-1523-1

[107] Chuenkova MV, Pereira PM. *Trypanosoma cruzi* targets Akt in host cells as an intracellular antiapoptotic strategy. Science Signaling. 2009;**2**:ra74. DOI: 10.1126/scisignal.2000374

[108] Manning BD, Cantley LC. AKT/PKB signaling: navigating downstream. Cell. 2007;**129**:1261-1274. DOI: 10.1016/jcell.2007.06.009.PMID:17604717

[109] Stahl P, Ruppert V, Meyer T, Campos MA, Gazzinelli RT, Maisch B, et al. Trypomastigotes and amastigotes of *Trypanosoma cruzi* induce apoptosis and STAT3 activation in cardiomyocytes in vitro. Apoptosis. 2013;**18**:653-663. DOI: 10.1007/s10495-013-0822-x

[110] Duaso J, Rojo G, Jaña F, Galanti N, Cabrera G, Bosco C, et al. *Trypanosoma cruzi* induces apoptosis in ex vivo infected human chorionic villi. Placenta. 2011;**32**:356-361. DOI: 10.1016/j.placenta.2011.02.005

[111] Morillo DA, González BE, Sulbarán AJ. La infección por *Trypanosoma cruzi* disminuye el desarrollo del melanoma maligno e incrementa la supervivencia en ratones C57BL/6. Investigación Clínica. 2014;**55**(3):227-237

[112] Urquiza JM, Burgos JM, Ojeda DS, Pascuale CA, Leguizamón MS, Quarleri JF. Astrocyte apoptosis and HIV replication are modulated in host cells coinfected with *Trypanosoma cruzi*. Frontiers in Cellular and Infection Microbiology. 2017;7:1-16. DOI: 10.3389/fcimb.2017.00345