(REVIEW ARTICLE)

Modulation of apoptotic pathways by *Trypanosoma cruzi* and its relationship with the progression of heart disease in the host

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Abstract

Chagas disease, a parasitic infection caused by the intracellular protozoan *Trypanosoma cruzi*, affects millions of people worldwide, and South American countries are among the most affected. This disease has a clinical course that varies from the acute asymptomatic phase to the chronic phase with the presence of important alterations that compromise the cardiac and digestive systems. Studies have shown that the life cycle of the parasite impacts on the modulation of apoptosis, revealing a complex pathogen-host interaction that can substantially influence the development of cardiac alterations. This intriguing strategy used by *Trypanosoma cruzi* has been increasingly explored and thus it is expected to be able to better clarify the events that precede the development of chagasic cardiomyopathy.

Keywords: Chagas' disease; Myocarditis; Cardiomyopathy; Apoptosis, *T. cruzi*

1. Introduction

Chagas disease is an infection caused by the intracellular parasite *Trypanosoma cruzi* (*Protozoa, Sarcomastigophora, Kinetoplastida, Tripanosomatidae*) that affects about 13 million people in the world and is highly endemic in the southern cone countries. Due to globalization, this disease has spread across European countries like Austria, Belgium, France, Germany, Italy, Netherlands, Portugal, Spain, Sweden, Switzerland, and the United Kingdom [1]. Data from the World Health Organization show 28,000 new cases per year and 8,000 newborns infected during pregnancy. Currently, Chagas disease affects about 8 million people, 65 million of whom are in areas at risk of acquiring the disease [2].

There are several means of transmission of the disease, the vector being the main one, although the increase in oral transmission has brought the attention of researchers in recent years [3; 4]. In addition to these, infection through organ transplantation, blood and congenital transfusion are also responsible for a significant portion of cases of Chagas' disease [5; 6].

Vector transmission occurs with the participation of triatomic insects (*Triatominae, Hemiptera, Reduviidae*); that carry the parasites in their rectal ampulla [7]. At the site of the bite, the vector releases infectious forms of the parasite that are present in its feces and urine, which penetrate the injured skin, quickly reaching the blood and lymphatic system [8]. At these sites, the metacyclic trypomastigotes (infecting forms) interact immediately with cells of the phagocytic mononuclear system, initiating a complex process of cell invasion [4].

Once inside the cells, there is a rapid and massive differentiation of the metacyclic trypomastigotes forms into replicant forms (amastigotes). After some cycles of binary division of the amastigote forms, the transformation occurs again into trypomastigotes that will escape from the cell having access to several other organs through hematological dissemination [4; 9]

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Chagas' disease has two clinically distinct phases, the acute phase and the chronic phase. In the acute phase, the majority of the patients, around 90%, will present asymptomatic or non-specific symptoms that can be confused with other pathologies. Thus, the presence of fever, edema, hepatosplenomegaly and lymphadenopathy alone are not suggestive of Chagas' disease. When there is a condition of heart failure and the patient is a resident of a risk area, it becomes easier for the doctor to suspect Chagas disease [10; 11].

Once the acute phase is over, the patient will enter a long period classified as a chronic asymptomatic phase. Such period is considered the indeterminate form of the disease, being benign and unapparent. Many individuals who have gone through the acute asymptomatic phase, enter the indeterminate chronic phase without even knowing they are infected [10]. Regarding laboratory findings, the indeterminate form is marked by the presence of positive serology and positive parasitological tests, with no specific clinical picture of Chagas' disease. On the other hand, cardiac evaluations, such as echocardiography, Holter and ergometric test, may present discrete alterations that may be originated from the acute phase or signs of progression of the chronic phase [12]. However, it is important to note that a good portion of this population will spend the rest of their lives without developing any clinical complications [13]

However, about 30% of patients will reach the chronic symptomatic phase, with severe cardiac complications. This phase is characterized by a wide spectrum of manifestations, ranging from subclinical abnormalities to the most severe forms with the presence of refractory heart failure, myocarditis, fibrosis, myocardial hypertrophy, thromboembolism, complex arrhythmias and sudden death [14]). These alterations describe the condition of chronic chagasic cardiomyopathy (CCC) which is the main complication found in the chronic symptomatic phase of Chagas' disease [15,16].

Thus, according to these observations, CCC can be considered a progressive, fibrotic disease in which myocardial inflammation plays a fundamental role [14; 16; 17; 18].

2. Cellular death: overview

As long known, in a pathological context, cell death is the result of a persistent injury to the cell, with varying degrees of severity [19]. The non-observance of the removal of the stimulus that gave rise to the lesion is a key factor in understanding the irreversible lesion that can culminate in the death of the cell as previously mentioned.

From then on, death by necrosis will occur, where the injury is usually of high magnitude with rupture of the cellular plasma membrane and presence of inflammatory response, an event that affects several cells at the same time [20,21] or results in programmed cell death by apoptosis in which, the slow and progressive stimulus allows the cell to trigger controlled mechanisms of self-destruction individually [22].

Apoptosis is crucial for maintaining tissue homeostasis and for modulating the immune response in metazoan [23]. It was discovered in 1972 when Kerr, Willey and Currie described a death pattern that was distinct from necrosis, both biochemical and morphologically. From a physiological point of view, apoptosis was demonstrated as an event contrary to mitosis in order to regulate the cell population [24].

Several pathological states can trigger apoptosis, such as DNA lesions by cytotoxic drugs and radiation, when the repair mechanisms are insufficient, in the infectious processes caused by various pathogens, including protozoa and tumor development [25; 23].

During apoptosis, characteristic morphological changes in cells are observed, as well as biochemical changes crucial for the establishment of energy-dependent cell disassembly [21]. In the early stages of death, cytoplasm shrinkage is observed, with condensation of the nucleus and fragmentation of the chromatin. In addition, organelles such as mitochondria, endoplasmic reticulum and Golgi complex may also undergo structural changes, with release of apoptosis mediators [26;27].

Unlike necrosis, in apoptosis there is no extravasation of cell content, because the integrity of the membrane is maintained. In addition, the formation of apoptotic bodies allows macrophages to phagocyte rapidly, thus avoiding the classic inflammatory response found in necrosis [24; 28]

2.1. Two pathways - same endings

Apoptosis is activated by two means, extrinsic and intrinsic, using extremely complex mechanisms. In given circumstances these pathways may not only converge, but also their molecules may interfere with each other's pathway

[29] (Figure 1). The extrinsic pathway begins with the activation of the so-called death binders, such as FasL and TNF-α to their respective receptors located on the surface of target cells, which triggers the activation of caspase 8 and subsequently the activation of caspase 3 effector [30] Death receptors are members of the tumor necrosis factor (TNFR) receptor family that contain a protein-protein domain, called the "death domain", essential for the transfer of apoptotic signals [30; 31; 32];

The intrinsic pathway is related to most apoptosis events and occurs in the absence of growth factors, in the presence of DNA damage and accumulation of free radicals, via p53 activation [33; 21]. Once activated, the intrinsic pathway is characterized by increased permeability of mitochondrial membranes that release apoptogenic proteins, such as cytochrome c, which will interact with other cytoplasmic apoptotic proteins. From then on, the activation of effector caspases such as caspases 3, 6 and 7 has begun [34].

Regardless of the route initially activated, the end is always the same and is determined by the activation of the effector caspases that will initiate the disassembly of the cell.
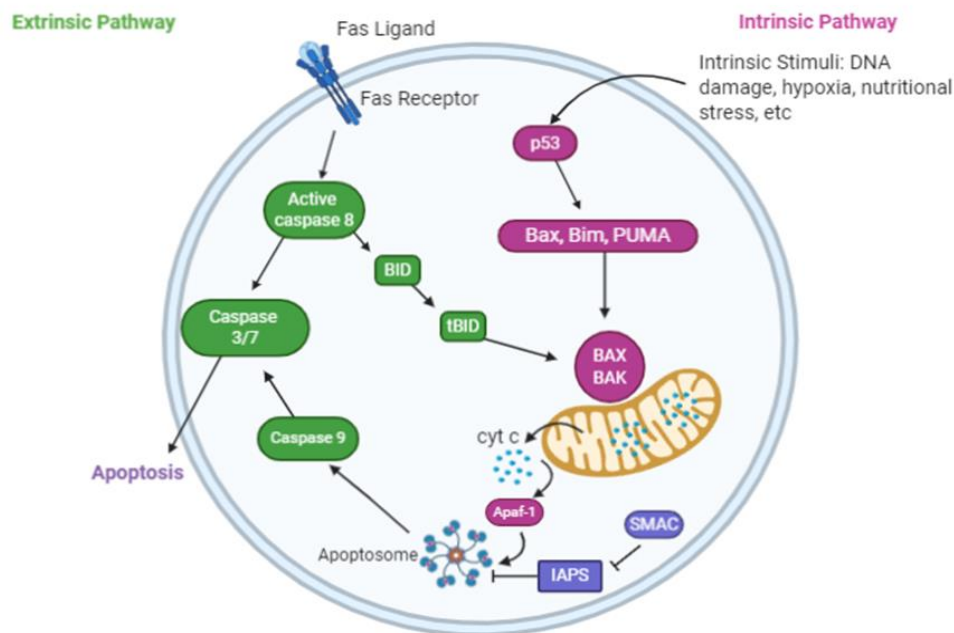

Figure 1 Summary diagram of the intrinsic and extrinsic pathways of apoptosis. Through specific death stimuli triggered by a significant increase in p53 expression a sequence of events begins. This dramatic increase is associated with the activation of pro-apoptotic proteins like Bax, Bim and PUMA, for example. Bax / Bak found in the target cell cytoplasm translocate to the mitochondrial membrane where they promote a change in permeability and consequent opening of channels with release of cytochrome c. In the cytoplasm, cytochrome c hydrolyzes scaffold proteins with the formation of the apoptosome that recruits the initiating caspase 9. The extrinsic pathway, in turn, is characterized by the active participation of death receptors from the TNF family in which, finally, caspases 3 and 7 are activated, which complete the cell destruction process, as shown in the figure.

2.1.1. Caspases - key proteases in the cell demolition process

Caspases are cysteine proteases that play a crucial role in the process of cell death, ensuring cell fragmentation into small units called apoptotic bodies, which are quickly eliminated by phagocytic cells avoiding and/or minimizing the local inflammatory response [35; 36; 37; 38].

These proteins are extremely specialized, being synthesized as inactive zymogen, containing one pro-domain and two sub-units, one major (p20) and one minor (p10) (Figure 2). In general, the proteolytic cleavage separates the two subunits and removes the pro-domain [39]. After that, a heterotetrametric structure is formed, active and ready to start the cellular demolition cascade.

The cysteine proteases can be classified according to their functions and thus are considered as pro-apoptotic and pro-inflammatory. Caspases 2, 3, 6, 7, 8, 9 and 10 are known for their intimate and complex relationship with the signaling of cell death, while caspases 1, 4, 5, 11, 12, induce the expression of cytokines in inflammatory processes [40; 41].

The process of cell death induction is complex and requires the participation of family member death receptors of tumor necrosis factor, TNFR1 (DR1, CD120a, p55 and p60), Fas (DR2, APO-1 and CD95), DR3 (APO-3, LARD, TRAMP and WSL1) among others [42].
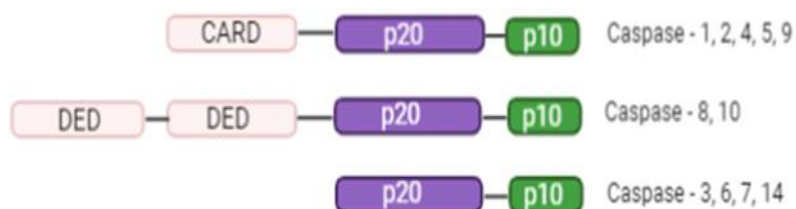

Figure 2 Scheme of caspase organization and their death domains. As shown, initiator caspases possess longer death pro-domains, called caspase recruitment domain (CARD) (caspases 1, 2, 4, 5 and 9) or death effector domain (DED), here represented by caspases 8 and 10. They are capable of auto activating themselves when in complex with proper factors thus starting apoptotic response. Effector caspases have short prodomains and that means they are not capable of self-activation (caspases 3, 6, 7 and 14), in general, activated by initiator caspases. Within the figure, regions p20 and p10 represent larger and smaller subunits, respectively.

3. Regulation of Apoptosis

The regulation of apoptosis is an extremely complex event involving the participation of pro- or anti-apoptotic molecules, members of the Bcl-2 family. In addition to these, proteins such as IAP (endogenous inhibitor of apoptosis) and SMAC/DIABLO (inhibitor of IAP) also act on the balance of the expression of molecules will allow or inhibit cell death [43,44].

Some anti-apoptotic limbs such as Bcl-2 and Bcl-xL act preserving the integrity of the mitochondrial membrane potential, which prevents cytochrome c from leaving the cytosol. In contrast, Bax, Bak and Bim translocate from cytosol to mitochondria, promoting the opening of pores in the membrane with consequent release of cytochrome c [45; 46; 47]. In cytosol, cytochrome c binds and activates a framework protein called APAF-1 (factor 1 apoptotic protease activator), with energy expenditure. (Schimmer, 2004) The activation of APAF-1 exposes its CARD domain and a seven-arm structure is then formed, similar to a weathervane, called apoptosome [21]. In the apoptosome, the activated caspase 9, cleaves to caspase 3, initiating the process of cell death [49,23]. This cascade can be amplified in at least two ways: either by activating other effector caspases or, when active caspases cleave Bid which binds much more efficiently to the mitochondria, thus releasing more apoptogenic products [50;51].

4. Modulation of apoptotic pathways by *Trypanosoma cruzi* in the host

In multicellular organisms, the maintenance of homeostasis is achieved by the balance between proliferation and cell death. [52]. In this sense, apoptosis can be an interesting mechanism of elimination of damaged cells, also acting in infectious diseases [53,54]. Caspases have an important role in pathological processes, since their absence can lead to the development of cancer, autoimmune pathologies, degenerative disorders and also immunodeficiency [40].

During parasitic infections, programmed cell death may be triggered by pathogen antigens or as a result of an intense inflammatory process [55,56].

It is known that modulation of apoptotic pathways is a common strategy among intracellular pathogens such as *Cryptosporidium parvum, Leishmania spp* and *Trypanosoma cruzi* to ensure their survival in the host [52].

T. cruzi, invades and resides in different types of cells, avoiding their direct destruction by the action of mechanisms of immune response evasion and also by manipulation of apoptosis pathways in the host cell. The mechanisms involved in this complex task still remain obscure, and therefore more studies are needed to illustrate the participation of specific molecules.

Vasconcelos et al. (2012) [57] demonstrated that experimental infection with the *T. cruzi* Y strain triggers an immune response that is deficient and delayed, occurring about 20 days after the peak of parasitemia. Thus, it was observed that during infection, these animals had little proliferative CD8^{+} T lymphocytes with a large proapoptotic phenotype, evidenced by increased CD95 and annexin V on their surface. Such response profile was extremely harmful, leading to the death of almost 100% of the animals.

In contrast, other studies point to the participation of parasite molecules that act by preventing apoptosis in mice cardiomyocytes, favoring the survival of the protozoan in parasitic cells [58;59].

It is possible that this modulation of cell death in host cardiomyocytes occurs as a result of a molecule present in the parasite, called cruzipain. Also known as GP57/51 antigen, cruzipain is the most abundant protease of *T. cruzi* [59], besides its protease activity, it is also considered an important virulence factor that plays a central role in differentiation, nutrition and parasite invasion in host cells [60,61]. This molecule presents similar activity to of caspases in mammals and has been related to the survival of infected cardiomyocytes, thus avoiding cell apoptosis by increasing Bcl-2 expression [62].

Cruzipain is expressed in all stages of development of *T. cruzi* in organelles similar to lysosomes. High concentration of Cz is found in organelles of epimastigotes. In amastigotes most of Cz is in the plasma membrane, while in trypomastigotes it is in the middle [63;64]. Despite some advances in the area, it is still not known what the real participation of this molecule in the progression of chagasic cardiomyopathy.

5. Implication of apoptosis in the development of cardiac complication in Chagas disease

According to Lopes et al. (1995) [65], the induction of apoptosis would be related to one of the possible mechanisms involved in the suppression of the immune response by *T. cruzi*. Thus, according to the study, the death of splenic cells of phenotype T CD4^{+} and T CD8^{+} could contribute to the evolution of parasitic infection.

Other evidence also supports that *T. cruzi* infection potentiates the induction of cell death by Fas route activation, causing apoptosis of CD4^{+} T lymphocytes in infected animals [66]. The same group also observed that the Fas/FasL expression was increased in previously infected mice CD4^{+} T cells and that treatment with in vitro anti-FasL was able to decrease apoptosis, consequently increasing the proliferative response of this group of cells.

In addition to CD4^{+} T lymphocytes, CD8^{+} T lymphocytes are also of paramount importance in promoting an effective parasite elimination response, which occurs primarily through the secretion of cytokines such as IL-2, IFN-γ and IL-10, among others [67]. The cytokine IFN- γ has been described as one of the main activators of the effector functions of macrophages, but also as a promoter of differentiation and activation of CD8^{+} T lymphocytes [68]. Still according to Brener & Gazzinelli (1997) [69] CD8^{+} T lymphocytes have an important role in controlling parasite replication.

A study conducted on specimens of patients with heart failure showed that they had a significantly lower proliferative response after in vitro stimulation with *T. cruzi* antigens, when compared to asymptomatic patients. This response pattern was associated with activation of apoptosis in cardiomyocytes. In addition, the presence of apoptosis in peripheral blood mononuclear cells (PBMCs), and the low proliferative response were associated with Fas/Fas-L expression and high production of TNF-α, widely known to induce programmed cell death. Thus, the authors of the study suggest that apoptosis of PBMCs, probably triggered by the expression of Fas/Fas-L and production of TNF-α, was implicated as an immunoregulatory mechanism during the chronic phase of Chagas' disease [70].

Tostes et al. (2005) [71] evaluated the relationship between the presence of apoptotic heart cells in patients during the chronic phase of Chagas' disease. The data found by the group showed that in chronic heart patients there was a significant increase in the extent of fibrosis, in the number of inflammatory cells and apoptotic cells in the heart tissue when compared to left ventricle specimens obtained from patients without heart failure. Thus, the authors suggest that the loss of myocardial cells by apoptosis and fibrosis contributes to the development of heart failure in the chronic phase of Chagas' disease [71].

Also, according to Savill & Fadok (2000) and Tostes et al., (2005) [28;71], the intensity, persistence and nature of pro-inflammatory mediators, observed in the heart wall, may contribute in some way to the induction of programmed myocardial cell death. In contrast, phagocytosis of apoptotic bodies, a classic event of this type of cellular death, may induce the secretion of TGF-β, which allows the parasite to escape [28;71].

Corroborating data published by other research groups, our group found that mice infected with the *T. cruzi* Y strain had a totally impaired immune response, which was reflected in the expression of poorly proliferated CD8+ T lymphocytes with a pro-apoptotic phenotype evidenced by the increased expression of CD95 and annexin V on their surface. Thus, the immune response of infected animals was not only delayed (about 20 days after the peak of parasitemia), but also not very effective, which led to 100% of the animals to death [57].

6. Conclusion

The reasons why some patients infected with *T. cruzi* will never develop any clinical symptoms of the disease while others will suffer harsh cardiac complications are still unknown. The apoptosis seems to be an essential factor in this scenario therefore it is of paramount importance that we continue investigating and debating this subject deeply and broadly.

Compliance with ethical standards

Acknowledgments

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

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