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Review Article

Chagas Disease: Navigating the Complexity of Host Immune Responses to *Trypanosoma cruzi*

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Abstract

Trypanosoma cruzi is the source of the persistent systemic infection known as Chagas disease. There are various stages in its life cycle for both host mammals and vector insects. Along with regional variations in morbidity and mortality, distinct clinical presentations of Chagas disease are caused by distinct strains of *Trypanosoma cruzi*. The cytokine interferon-gamma is initially produced by natural killer cells during the early stages of *T.cruzi* infection. Cytokines secreted by phagocytes stimulate other defense-related cells and promote inflammation. In response to *T. cruzi*, B lymphocytes initiate an efficient humoral immune response, whereas dendritic cells, monocytes, and macrophages regulate the adaptive immunological response. This review includes several studies that address the primary immune mechanisms involved in *T.cruzi* infection, pathogen approaches against host cells, inflammasome, and virulence factor processes, and novel approaches to disease prevention, control, and treatment.

Keywords: *Trypanosoma cruzi*, Chagas disease, Cytokines, humoral immune response, novel approaches

1. Introduction

The World Health Organization considers Chagas disease, a persistent and systemic disease caused by *Trypanosoma cruzi* infection, to be a neglected disease. The parasite is mostly spread by blood-sucking reduviid insects, which belong to the subfamily *Triatominae*, via different pathways, such as domesticated, undomesticated, and wild pathways (Little et al. 1966). It can infect humans and various wild and domestic animal species. *T.cruzi* is effectively transmitted to humans via vectors such as *Triatoma infestans, Triatoma dimidiata, and Rhodnius prolixu* (Tibayrenc & Ayala 1988). *T. dimidiata* has been identified in Mexico, whereas *R.prolixus* is located in Central and South America, and *T. infestans* is mostly found in sub-Amazonian areas (Franzén 2012; Ramsey et al. 2000).

Blood transfusion and vertical transmission are two possible human transmission methods (Velascohernandez 1994; Vrielink & Reesink 1998). *T.cruzi* passes through a number of phases in its life cycle in host animals and its vector insects. The parasite in insects can occur in one of two forms, known as replicative epimastigotes or metacyclic trypomastigotes. Non-replicating blood tripomastigotes and replicative intracellular mastigotes are the major types of mastigotes in mammals (Bao et al. 2010). Different *T.cruzi* strains are found in both insect vectors and mammalian hosts. The various clinical symptoms of Chagas disease, along with the variations in mortality and

morbidity that are recorded in different parts of the world, may be attributed to this heterogeneity (Colley & Howard 1991; de Sousa 2000).

Chagas disease typically develops through three stages in humans (Rassi et al. 2010).The first phase is the acute phase, which has a period of two months; the second phase is called the asymptomatic period, also called the intermediate phase; and the third phase is called the chronic phase, which lasts for a long period of time. Patients affected with *T.cruzi* are typically asymptomatic and have very minor signs of disease. However, after an incubation period of 5 to 40 days, between ten and thirty percent of infected people exhibit unspecific symptoms. Abdominal discomfort, anorexia, fever, malaise, lymphadenopathy, and hypertension of the liver, spleen, and lymph nodes, in addition to regional or widespread subcutaneous edema, are some of these symptoms. At the point of entry, *T.cruzi* vector transmission presents two very common signs (Pereira et al. 2009). The first sign is Chagoma, which leads to edema and skin rash that last for many weeks at the injection site (Organization 2002).The second is the Romaa sign, which develops when contaminated feces accidentally deposits in the conjunctival sac as a result of rubbing the eyes (Klotz et al. 2010). The transfer of trypomastigotes to the conjunctiva, often accompanied by lymphadenitis or cellulitis, can result in lymphadenitis or cellulitis (Neva 2007).

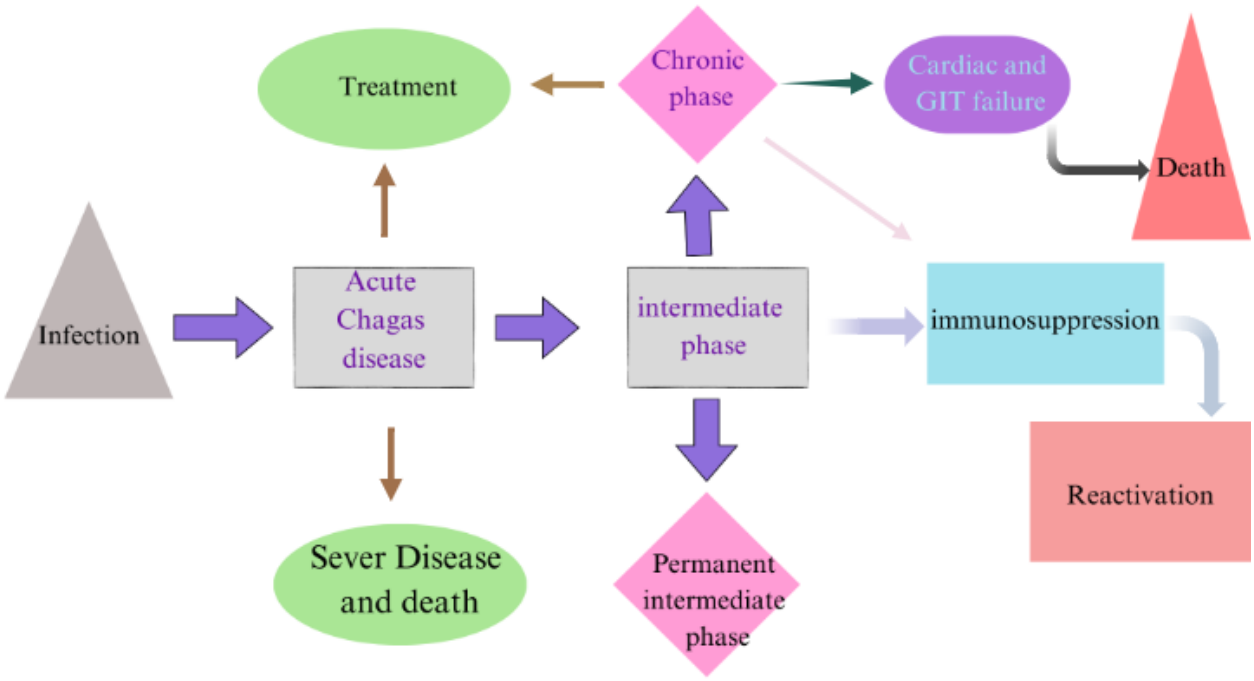
Some individuals may also experience a generalized morbilliform rash in the weeks following the bite (Blumberg & Frean 2005; Schwartz & Medicine 2003). Patients who develop this disease via oral contact may experience stomach discomfort, gastrointestinal bleeding, diarrhea, nausea, and vomiting (Prata 1994; Shikanai-Yasuda & Carvalho 2012). In 5–10% of patients, myocarditis, encephalitis, or meningoencephalitis can develop in the acute phase of the disease; most fatalities often affect young people (Córdova et al. 2010). Complications in the acute phase of infection during pregnancy include fever, premature birth, a fetus with low weight, hepatomegaly, splenomegaly, and anemia (Altcheh 2019; de Noya & Ruiz-Guevara 2020). Abortion and placentitis are other conditions linked to intrauterine infections in the intermediate phase of illness, which may persist for several generations or an entire life in an asymptomatic state and is identified by serological positivity to T. cruzi (Carlier et al. 2020). The chronic phase of Chagas disease, characterized by organ dysfunctions that may cause congestive heart failure, gastrointestinal complications, or death, can occur in almost 30–40% of healthy individuals (Echavarria et al. 2021; Saraiva et al. 2021). HIV-positive patients or transplant recipients who have impaired immune systems may reactivate the infection (Bern 2012; Clark et al. 2024) (Figure 1).

The mechanism of Chagas disease is not fully known. Both direct and indirect harm might result from prolonged parasite persistence. The cellular and neural damage caused by *T.cruzi* constitutes direct injury, whereas the immune system's reaction and autoantigens are responsible for indirect injury (Bonney & Engman 2015; Teixeira et al. 2011).

The balance that develops between the immune system's reaction, inflammation of the host tissue response, and the ability of parasites to reproduce determines the course of the disease (Junqueira et al. 2010; Vitelli-Avelar et al. 2006). As a result, a weak immune response will increase the parasite load and increase persistence, which will trigger an inflammatory reaction that is too strong and damages tissue. There is less tissue damage when there is an efficient immune response, which also reduces the parasite burden and inflammatory implications (Machado et al. 2012).

The aim of this work is to provide a description of the current immunological pathways activated by *T.cruzi* as well as the techniques used by this pathogen to invade host cells. This knowledge will aid in the development and enhancement of novel

Figure 1: In patients with Chagas disease, impaired immune systems may reactivate the infection.



approaches for the prevention, management, and treatment of disease.

2. The Innate Immune Response to *Trypanosoma cruzi*

Phagocytes, a part of the host's innate immune system, attack *T.cruzi* as it encounters infecting host cells (Cardoso et al. 2016; Kaufman et al. 2023). They use membrane receptors such as Toll-like receptors (TLRs) and pattern recognition receptors (PRRs) to recognize different pattern receptors, such as damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs) (Sato et al. 2009). TLRs are able to recognize T.cruzi, and cytokine production induced by these receptors plays a very essential role in the response generated by the host. *T.cruzi* can also be detected by nucleotide-binding oligomerization domain-like receptors (NLRs); they are capable of identifying PAMPs, which can enter cells through openings and can be phagocytosed (Lopez et al. 2018; Olive 2012). In addition, phagocytes release cytokines that stimulate other immune cells to stimulate inflammation at the site of infection. Antigens produced by *T.cruzi* act as signals for identifying and controlling the release of proinflammatory cytokines, including interleukin-1 (IL-1), IL-10, IL-12, and tumor necrosis factor α (TNF-α), by macrophages. After *T.cruzi* infection, interferon- γ (IFN-γ) and effector T cells, along with proinflammatory cytokines, impact the activation state of macrophages (Machado et al. 2012). IFN-γ in conjunction with TNF-α, can also stimulate macrophages via a traditional mechanism. Reactive oxygen species and microbicidal intermediates produced by activated macrophages can eliminate *T.cruzi* and cause a type I polarized response (López-Muñoz et al. 2018). Rather than following the conventional pathway, the cytokines IL-4 and IL-13 produced during other types of reactions, cause the

mobilization of macrophages in another manner (Teixeira et al. 2011). A number of parasite antigens cause macrophages to become classically activated, which increases Nitric oxide (NO) production. For example, glycophosphatidylinositol-anchored mucin-like glycoproteins (GPIs) can cause NO production from IFN-γ-stimulated macrophages (Ropert et al. 2002). TLR2, a GPI receptor, functions by inducing the synthesis of IL-12, TNF-α, NO, IL-12, and TNF-α (Debierre-Grockiego et al. 2007). In addition to the antigen itself, dendritic cells (DCs), neutrophils, and macrophages release costimulatory molecules when PAMPs and DAMPs are also present, which permits T-cell activation (Padilla et al. 2009a). Professional antigen-presenting cells, such as macrophages and DCs, are essential for the development of immunity. Based on the *T.cruzi* strain, the profile of the cytokines generated may vary (Van Oers 2006).

T.cruzi secretes substances that might promote tolerance by blocking the synthesis of TNF-α and IL-12, which in turn affects the activities of dendritic cells (Gil-Jaramillo et al. 2016). Because they generate TNF-α and IFN-γ, natural killer (NK) cells are essential for the innate response. By stimulating macrophage activation during the first stages of *T.cruzi* infection, these cytokines control the removal of parasites. During the acute stage of the illness, children were shown to have increased levels of activated NK cells (CD16+ and CD56–) (Vitelli-Avelar et al. 2006).

Extracellular parasites are efficiently removed when NK cells establish cell–pathogen interactions, which decrease pathogen movement and enhance cell membrane damage. IL-12 triggers this NK-mediated killing response, which damages the *T.cruzi* cell membrane and induces the exocytosis of cytotoxic granules (Belizário et al. 2018).

The complement system, which has plasma proteins as its activating component, is a crucial part of both humoral and innate immunity. These proteins initiate successive protease-based events corresponding to the contact, fibrinolytic, and coagulation pathway (Feys 2006). The infectious agent is killed by drawing phagocytic cells to the infected site. It can be activated by a variety of routes, including lectin, alternative, and classical routes, which converge to cause C3 to split into C3a, which has a pro-inflammatory effect, and C3b, which increases the efficiency of phagocytosis by binding to complement receptor 1 (CR1), which is found on the outer membrane of phagocytes. Additionally, the proinflammatory factor C5a is produced as a result of C3b (Cestari et al. 2013; Würzner 2003).

The multimeric protein complex known as the inflammasome is formed in the host in response to different kinds of stress signals or when microorganisms are present (Salminen et al. 2008; von Moltke et al. 2013). The inflammasome directs the host cell toward pyroptosis and results in the production and secretion of cytokines. Inflammatory caspases (caspase-1), a key adaptor molecule (ASC), and NLRP (a sensor protein) are the three main inflammasome components. These processes promote the pathogen uproot of diseased tissues, activate acquired immunity, and help to restore equilibrium in tissues. NLRP3 begins to function in response to *T.cruzi* infection. TLRs, which become activated when they come into contact with infectious molecules and then trigger NOD1, are the mechanism by which *T.cruzi* is recognized. The final step in this process is the formation and action of the inflammasome complex, which regulates caspase-1 breakdown as well as the release of pro-IL-1β into the cytokine.

3. The Adaptive Immune Response to *Trypanosoma cruzi*

An increase in the flow of B lymphocytes, which generate and release antibodies, plays a vital part in the adaptive humoral immune response to the development of adaptive immunity in the host. According to previous studies, the immune system's humoral reaction is essential for limiting

T.cruzi infection; mice (deficient in antibodies) are unable to inhibit *T.cruzi* development and collapse at the early stages of infection (Kumar & Tarleton 1998).

B lymphocytes induce an efficient immune response in the earliest phases of *T.cruzi* infection (Frosch et al. 1997; Padilla et al. 2009b). The virus primarily generated contrary to *T.cruzi* antigens on the exterior may not eradicate the infection, allowing the parasite to infect the host indefinitely (Gürtler 2015). During *T.cruzi* infection, cytokines play an effective role in coordinating humoral and cellular immune responses (Letterio & Roberts 1998).

B cells function as a link between innate and adaptive immunity in addition to producing antibodies, releasing cytokines, such as IL-10 and IL-17, and transporting antigens to immune cells (Stockwin et al. 2000).

B lymphocytes play a critical role by triggering Th1 cells, which aid in the monitoring of parasite development (Denkers & Gazzinelli 1998). It has been observed that the spleen supernatants of mice devoid of mature B cells have lower levels of proinflammatory cytokines (Young et al. 1997). Due to the lack of developed B cells, the body's defense system cannot detect effector CD8+ T cells or provide a message to Th1 cells of cytokines (Harty et al. 2000).

Adaptive immune reactions depend primarily on T cells and alter the expression of molecules and generation of cytokines, ultimately producing T lymphocytes with different functions. Their response begins with signals via their receptors at the exterior of antigen-presenting cells, leading to the identical generation of immature T cells (Bonilla & Oettgen 2010). Effector and memory T cells, which are capable of long-term survival and regeneration, are generated by the activation of T cells. IL-12, which is released by natural killer (NK) and dendritic cells (DCs), stimulates the development and proliferation of both CD8+ and CD4+ T cells, which are attracted to IFN-γ. This leads to the activation of the toxic effects of CD8+ T cells on cells and the activation of effector cells (macrophages). The expansion of B cells and the generation of antibodies can recognize the breakdown of trypomastigotes, which are induced by CD4+ lymphocytes. Furthermore, T cells migrate to regions where IFN-γ increases the production of chemokines during the early stages of the illness. To prevent injury to tissues and regulate the parasitic load in the body, it is necessary to establish an appropriate equilibrium between cytokines to generate a sufficient response from cells (Redpath et al. 2014) (Figure 2).

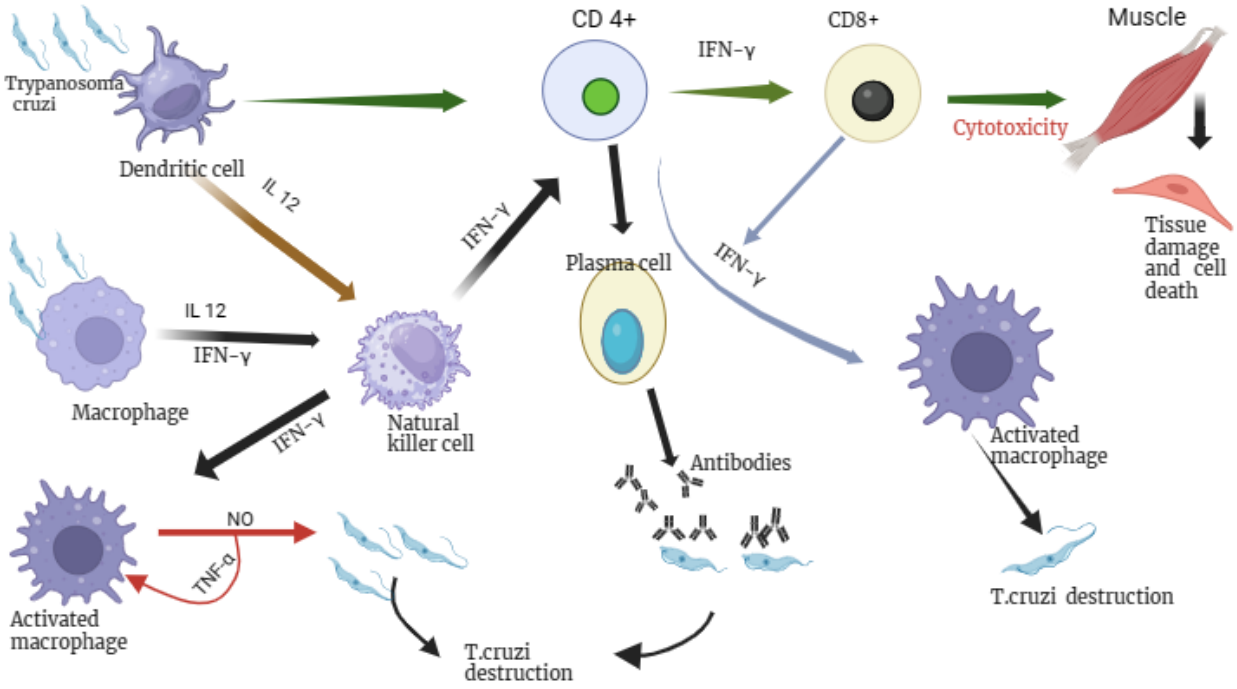
The increase in T cells that have become activated and the consequent release of cytokines that are both pro- and anti-inflammatory are linked to Chagas disease. Most patients with chronic Chagas disease do not exhibit any symptoms (Prata 1994). In contrast with TNF-α and IFN-γ (inflammatory cytokines), the chronic form is linked to a greater generation of regulatory cytokines (IL-10) (Asadullah et al. 2003; Ouyang et al. 2011). As a result, the ability to manufacture IL-10 after the initial stage may be necessary for controlling the response as well as enabling progression to chronic conditions.

4. Toll-like receptors (TLRs)

Innate immunity-related cells, such as macrophages, share a family of pattern-recognition receptors called TLRs. With the detection of various microorganism structures, TLRs participate in the early phases of the immune system reaction (Delgado et al. 2009; Leulier & Lemaitre 2008) and induce particular biological responses through adaptor molecules that include the Toll/interleukin-1 (IL-1) receptor (TIR) domain, such as MyD88, TRAM, and TRIF (Narayanan & Park 2015).

The elimination of *T.cruzi* and the activation of phagocytes towards the region of infection are both facilitated by these receptors (Campo et al. 2016; Cardoso et al. 2016). The development of a pathological state, however, could be linked to the

Figure 2: The intricate interaction between the cells and cytokines in Chagas disease.



improper triggering of these specific receptors (Kawai & Akira 2010; Schöneberg et al. 2004). Various studies on the activity of TLRs have indicated that TLR2 mediates the immune system response to *T.cruzi* infection (Rodrigues et al. 2012). Since then, many other kinds of proofs have been presented. Several TLR ligands capable of eliciting an immune response from the innate system as well as an adaptive immunological response are present in *T.cruzi* and are connected to immunity to infection as well as pathogenesis (Zamboni & Lima-Junior 2015). TLR2 has been shown to activate Rab-5, a small guanine phosphonucleotide-binding protein that causes macrophages to ingest *T.cruzi* (Maganto-Garcia et al. 2008). Furthermore, TLR2 can increase the lifespan of diseased mice if it becomes active just before exposure (Johnson et al. 2005). The cell membrane of the trypomastigote of *T.cruzi* contains glycoinositolphospholipids (GIPLs) along with glycosylphosphatidylinositol (GPI) anchors, which are detected by the TLR2 and TLR4 receptors and activate the NF-κB and MAPK pathways (Almeida & Gazzinelli 2001). As an outcome, a Th1-type response is triggered, and proinflammatory cytokines and NO are generated (Chen et al. 2000). Additionally, TLR-9 binds to unmethylated CpG patterns found in *T.cruzi* DNA (Johnson 2013) and, through the activation of Th1 response, causes professional antigen-presenting cells to produce cytokines (Murtaugh et al. 2002).

A greater incidence of infestation and mortality in mice with a threefold deficiency in TLRs, e.g., TLR3, TLR7, and TLR9, indicated that the mice were at greater risk of disease with *T.cruzi* (Khardori 2011). *T.cruzi* stimulates neutrophils, especially to produce fibrous nets; these are traps of DNA involved in pathogen killing and include granules and histones (Sousa-Rocha et al. 2015). Antibodies directed against TLR2 and TLR4 have been shown to decrease the release of Neutrophil Extracellular Traps (NET).

Compared to healthy cells, macrophages infected with *T.cruzi* can generate more extracellular

vesicles called EVs. Through their relationship with TLR2, these vesicles cause NF-κB to relocate to the nucleus, triggering the generation of proinflammatory factors such as TNF-α, IL-6, and IL-1β that can sustain an inflammatory reaction (Morris et al. 2016).

In orally transmitted Chagas disease, the presence of certain TLR4 variations (TLR4 Asp/Gly-Thr/Ile genotype, 299/399 TLR4 haplotype, and 299/399 TLR4 haplotype) has been correlated with an increased risk of longevity and intensity due to cardiovascular involvement (Boscardin et al. 2010). Although TLR4 agonists were able to lower parasitic loads in the hearts of BALB/c mice infected with T. cruzi, they were unable to prevent damage to the heart (Ponce et al. 2012).

Lower levels of TLRs, such as TLR2, TLR4, TLR9, TRIF, and Myd88, were associated with infections in mice with more pathogenic *T.cruzi* strains. As a result, these mice had decreased IL-12 levels, which increased parasitic activity, cardiac failure, and death (Rodrigues et al. 2012). Another research investigation examined the relationship between clinical symptoms in individuals with various chronic conditions of Chagas disease and cytokines, adaptor molecules, and innate immune receptors. Researchers have shown that patients with digestive cardiac conditions express more TLR2 and TLR8 receptors as well as TNF-α, IFN-β, and IL-12 (Pereira et al. 2018). TLR2 suppression increases the pathological harm caused by parasitic organisms, decreases the release of IL-6 and IL-10, and enhances the expression of markers associated with cell death and proliferation (Machado et al. 2012). Galactosidases from vacuole membrane debris are used by certain intravacuolar infections to evade the host's phagolysosomal pathway. These pathogens can bind to epithelial cells and macrophages via galectin-3, a β-galactoside-binding lectin that is involved in multiple biological functions (Zúñiga et al. 2001). In mice with pathogen-infected hearts, it can promote collagen deposition, cardiac fibrosis, and cellular infiltration. The immune response in deficient animals may be reduced in vivo by inappropriate TLR expression on APCs (Wan 2021).

5. Virulence Factor

T.cruzi can affect numerous host cells through a variety of infectious processes, including cell invasion, humoral immune response, and defense against damage caused by oxidation (Machado et al. 2012). During the various stages of its life cycle, several factors are sequentially related to its virulence. When an infection occurs, metacyclic trypomastigotes primarily target the tissues at the site of infection, along with fibroblasts (Solís-Oviedo et al. 2018). The antioxidant systems of T. cruzi are essential for deactivating oxygen and nitrogen, which are reactive, and host cells are generated in the initial phases of the disease (Machado-Silva et al. 2016). Numerous enzymes, including peroxidases, which function on various compounds generated by the cellular oxidative cycle, are produced by the parasite. Exogenous hydroperoxides are deactivated by the glutathione peroxidase TcGPXI, which is found in the cytosol, and lipid hydroperoxides are inactivated by TcGPXII, which is found in the endoplasmic reticulum (Patel 2007). Peroxidase (TcCPX) and mitochondrial TcMPX, ascorbate-dependent heme peroxidase (TcAPX), prevent hydroxyl ions from interacting with oxygen. Iron superoxide dismutases (FeSOD) is another protein found in *T.cruzi* that detoxifies reactive oxygen that is produced in the cell cytoplasm and organelles (Piacenza et al. 2009). The life cycle of *T.cruzi* is linked to the expression of enzymes in its antioxidant network; these enzymes may also withstand the destructive impacts of the complement system as well as the humoral immune reaction after being modified into trypomastigotes in the blood (Machado et al. 2012). The trypomastigote surface is composed of glycoproteins that limit the activation of both kinds of complement pathways (classical and

alternative) and facilitate escape (Cestari et al. 2009).

A glycoprotein on the surface of trypomastigotes called decay-accelerating factor (T-DAF) prevents the assembly of both pathways by C3 convertase (Cestari et al. 2009). Only trypomastigotes produce the glycoprotein known as complement regulation protein (CRP), which is surface-anchored and blocks the complement system's activation pathway (Ramírez-Toloza & Ferreira 2017). The glycoproteins T-DAF and CRP are members of the *T.cruzi* trans-sialidase superfamily and resemble trans-sialidase (Frasch 2000). Both proteins interact with C3b and C4b to hinder the production of C3b (Cestari & Ramirez 2010). A surface molecule called calcreticulin (TcCRT) binds to C1q to prevent the classical pathway from being activated (Ferreira et al. 2004). Through cleavage of the common C2 factor, the C2 receptor inhibitor trepanning (TcCRIT) factor hinders the initiation of complement cascades via both paths. It also hinders the production of the C3 convertase enzyme through its linkage with C4 (Hui 2005). The intracellular and secreted proline racemases (PRs) TcPRACB and TcPRACA, respectively, are enzymes (Chamond et al. 2005). TcPRACA is a B-cell mitogen that is essential for *T.cruzi* escape (Coutinho et al. 2009) and stability, which is caused by the overexpression of TcPRAC isoforms that results in invasion of cells (H Lopes et al. 2010).

Tc52 (a protein), which is released, inhibits the growth of T cells (Ouaissi et al. 2002) and can also control the activity of inducible Nitric Oxide Synthase (iNOS) and macrophage cytokines and the generation of nitric oxide (NO) (Cummings & Tarleton 2004). The parasites need compounds that enable efficient cellular invasion that promotes attachment to cells and the stimulation of signaling pathways because after they have distinguished into extracellular amastigotes (EAs), they begin a new cycle of infection and penetrate new hosts. These amastigotes secrete the proteins P21 and *T.cruzi* mevalonate kinase (TcMVK) to facilitate attack by host cells. Actin polymerization and phagocytosis are triggered by the rearrangement of P21 in the host cell's actin filaments (de Castro Neto et al. 2021). HeLa cells are exposed to TcMVK, which attaches to their membrane and promotes parasite intake (Estrada-Reyes et al.). Mucins, mucin-associated surface glycoproteins, transialidases, and phospholipases are surface proteins that *T.cruzi* produces via association with carbohydrates, enabling the adherence of extracellular amastigotes and metacyclic trypomastigotes to host cells (Zacks 2007). The metacyclic phase of *T.cruzi* has a surface protein called Gp82, which attaches to the host and initiates the Ca2+ signaling process, which ultimately results in parasite absorption (Campo et al. 2016).

The pathogenicity of *T.cruzi* is dependent on transialidase enzymes (TS) because they enable the virus to obtain sialic acid from target cells and alter trypomastigote surface proteins, which enables the pathogen to cause both cell lysis and paralysis (MUHAMMAD 2018). Because transialidases are eliminated in vast amounts, the host finds it difficult to initiate a neutralizing response. The enhanced pathogenicity of these pathogens is linked to their capacity for large-scale ejection (MUHAMMAD 2018). Other surface glycoproteins of the TS superfamily involved in cell invasion include Gp85, which is found on the surface of BTs. This activity is carried out through the conserved FLY domain, which has a preference for endothelial cells and has the ability to trigger the host's extracellular signal-regulated kinases (Mattos et al. 2014) and promote the invasion of parasites.

On the surface of the pathogen, mucins are glycoconjugates that can access sialic acid residues from a host donor through trans-sialidase (TS). TSs are separated into two groups: TSs provide sialic acid to the TS for attachment, immune system regulation, and complement avoidance,

while TcMUC protects against the immune system in mammals (De Pablos & Osuna 2012; Herreros-Cabello et al. 2020).Conversely, TcSMUG protects against *T.cruzi* by digesting proteases found in the insect vector (Watanabe Costa et al. 2020). MTs and BTs are the primary hosts of infectious pathogens and include a collection of proteins called mucin-associated surface proteins (MASPs), which facilitate intracellular amastigote attack, survival, and replication (de Castro Neto et al. 2021). MTs are internalized into host cells through calcium-mediated processes, which are induced by the mucin-like protein complex Gp35/50 (Onofre et al. 2022). The cysteine endopeptidase cruzipain is involved in intracellular growth, cellular invasion, host tissue destruction, and immune response evasion (Gea et al. 2006). It may be found on the membrane of amastigotes and in lysosome-associated organelles during the primary phases of the *T.cruzi* life cycle (Alba Soto & González Cappa 2019). The membrane attachment and metalloprotease activities of gp63-I are known (Cuevas et al. 2003).

6. Autophagy

The mechanisms of *T.cruzi* differentiation as well as the communication between the pathogen and the host require autophagy. To develop and sustain disease in the host, pathogenic organisms such as *T.cruzi* can either utilize their autophagy processes or the autophagy systems of the host cells. The internalization processes of *T.cruzi* were explained by two primary theories. First, exocytosis is the basis of the model; second, endocytosis. Recent findings indicate that both processes occur sequentially during host cell invasion (Horta et al. 2020; Kumari et al. 2010; Yoshida & Cortez 2008).

The first model states that after initiating lysosomal exocytosis and causing a series of Ca2+ signals in host cells, parasites invade as well as form the parasitophorous vacuole (TcPV), which has lysosomal properties (Romano et al. 2009; Romano et al. 2012b). Along with microtubules and kinesin, which are critical for the transportation of lysosomes toward the cell membrane, this route includes the peripheral pool of lysosomes that are present in the host cell (Gupta et al. 2020; Singh & Haka 2016). By the second concept, absorption takes place via invasion of the host cell membrane, producing a TcPV that is enriched in phosphoinositides of the membrane of the cells (not lysosomes) (Romano et al. 2012a). Research has shown that if this combination is prevented, the internalized parasites cannot exit the host cell and enter the extracellular space (Mann 2020; Villalta et al. 2008). The parasite becomes immobile once the lysosome fuses with the TcPV, which triggers the development of trypomastigotes into immobile amastigotes (Moore-Lai 2002; Won 2023). The acidic pH of the vacuole causes this process to occur (VanDerHeyden 2000). A crucial step in the parasite's life cycle, as well as its ability to remain inside its host cell, is vacuole development.

To prevent *T.cruzi* from surviving in the host, antiparasitic medications can regulate the autophagy mechanism (Casassa et al. 2019; Veiga-Santos et al. 2014) because the interconversion of different stages of the life cycle (epimastigotes, trypomastigotes, and amastigotes) requires parasitic autophagy. Trypanocidal medications find parasitic autophagy to be a prime target because of these modifications, which enable the parasite to adapt to host alterations throughout its life cycle. A group of genes known as "genes related to autophagy" (genes Atg) regulates autophagy genetically and functions successively during the different stages of autophagosome formation and maturation. Autophagy is implicated in both innate and adaptive immunity proven that a parasite may cause autophagy in primary mammalian cells through two distinct processes: by making it more frequent or by making more LC3, a protein that helps construct autophagosomes and autolysosomes (Hussey et

al. 2009). The primary cysteine proteinase of the parasite Cruzaina (Cz), which is expressed in all embryonic stages and in lysosome-like organelles, is another protein implicated in autophagy. Reservosomes, which are pre-lysosomal organelles present in epimastigotes, contain the highest concentration of Cz. According to a study, reservosomes mature into lysosomes as a result of increased Cz deposition caused by autophagy activation (Vidal et al. 2017).

7. Therapeutic Approaches

Nifurtimox (NFX) is currently the primary medication used to treat this illness. Although this medication has been shown to have anti-parasitic properties, it also has mutagenic and tumorigenic properties (Vermelho et al. 2022). According to Bruno *et al*. (2021), chemicals that are stilbenic and terphenyl, such as Nifurtimox, can cause both apoptosis and caspase-1 inflammasome activation in parasite cells. According to Bruno *et al*. (2021), ST18 is the stilbene molecule with the strongest antiparasitic properties. The mechanism of action of ST18 involves stimulating infected macrophages to produce caspase-1, an enzyme involved in the regulation of parasitemia. This stilbene compound was found to be a good contender against *T.cruzi* because of its anti-proliferative and pro-apoptotic effects as well as its anti-inflammatory, gastroprotective, and hepatoprotective effects (Bruno *et al*. 2021).

Benznidazole (BZN) is another medication that is now utilized in the treatment of Chagas disease (Pandey *et al*. 2022). In certain situations, both BZ N and NFX can effectively treat acute cases by significantly lowering parasitic infection; however, in other situations, they are inefficient, maintaining a high parasite load and increasing the possibility that the infection will progress to the chronic period (Pandey et al. 2022; Pérez-Molina et al. 2009) . However, for various reasons, mostly due to severe contraindications, negative effects on the gastrointestinal tract and skin (NFX and BZN), and genotoxic effects during pregnancy, their usage is restricted (Olivera *et al*. 2015; Ramos *et al*. 2024; Urbina 2009).

Furthermore, these medications are costly or unregulated in specific countries, which limits patient access. Several antiparasitic medications, such as Tak-187, K777, amiodarone, albaconazole, posaconazole, and amiodarone, have shown encouraging in vitro results (Figueiredo Sadok Menna-Barreto & Lisboa de Castro 2017; Junior *et al*. 2017). However, this needs to be clarified. The management of chronic patients is the main emphasis of their care in specialized clinical facilities, which are costly and frequently beyond the patients' reach. To fully understand the processes leading to disease progression, how the immune system plays a part in parasite reactivation, and the severity of the ensuing damage, additional studies are needed (Cazorla *et al*. 2009; Nagajyothi *et al*. 2012). These factors make it unlikely that the illness will have an ideal course of treatment. Future research should concentrate on immune therapy against parasite persistence, along with other medications to prevent severe harm, as controlling the parasite load alone is insufficient to stop the progression of chronic disease. Many experimental vaccines that aim to induce type 1 T-cell-adaptive responses have been developed in the last few decades as a result of research efforts (Farani *et al*. 2024; Pinazo *et al*. 2024).

8. Conclusions

Most endemic regions in Latin America are linked to Chagas disease. The disease is spreading around the world and is becoming a public health concern because of increased migration flow and population mobility in no endemic nations. In this review, we emphasized several ways in which *T.cruzi* subverts or evades the host response and creates a very dynamic and intricate situation. This study concentrated on several immune response components that control infection as well as the primary processes that cause the disease to advance or enter a latent period and cause the host

to experience either severe or protective side effects.

To obtain a better understanding of the involvement of TLR signaling, macrophage activation, IFN-γ release, and the inflammasome pathway in immunity against the parasite, a more thorough analysis of these processes is needed. Many questions remain after recent attempts to elucidate the function of the immune system during *T.cruzi* infection. To evaluate potential novel immunotherapies for this infectious disease, more research is required to consider the complexity of the disease and our current understanding of parasite-host interactions.

Conflict of Interest:

The authors declare that they have no competing interests.

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AN conceptualized the study and wrote the final manuscript, KN, IH, AND KT critically reviewed and edited the manuscript, KN critically reviewed the manuscript and made editing and language improvements. KT AND IH helped in the analysis and figures, supervision, critical review, and finalized manuscript writing.

Data Availability

Data are available upon reasonable request from the corresponding author.

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