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Graduation project

Assessment of Inflammatory and regulatory cytokines against trypanosoma cruzi

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بسم الله الرحمن الرحيم

" قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا ۖ إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيم

"

صدق الله العظيم

سورة البقرة الاية 32

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We would like to thank God for his mercy and grace, and we hope that God will accept this work with good acceptance

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List of abbreviation

abbreviation	Key
T.cruzi	**Trypanosima cruzi**
CCC	**Chronic chagasic cardiomyopathy**
IFN-gamma	**Interferon gamma**
NO	**Nitric oxide**
IL1	**Interleukin1**
IL10	**Interleukin10**
IL17	**Interleukin 17**
TGF-beta	**Transforming growth factor beta**
ROS	**Reactive oxygen metabolite**
TNF	**Tumor necrosis factor**
TH1	**T helper 1**
CD 4	**Cluster of differentiation 4**
CD 8	**Cluster of differentiation 8**
TLRs	**Toll like receptor**
TGF	**Transforming growth factor**
GPI	**Glycosyphosphatidylinositols**
LPS	**Lipopolysaccharide**
TS	**Trans-sialidase**
NO	**Nitric oxide**
EBI3	**Epstein barr virus induced gene 3**

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Abstract :

Trypanosoma cruzi is an intracellular trypanosomatid protozoan that is spread to humans by reduviid bugs, which are part of the insect subfamily Triatominae.The acute period lasts about two months and is asymptomatic in the majority of infected people, though others may experience symptoms such as excessive fever, anorexia, nausea, vomiting, and diarrhea. Cytokines play a critical role in the control of hemopoiesis and lymphopoiesis, as well as the action of all cell types involved in an immune response. Interferon gamma (IFN-gamma) has been widely studied as a lymphokine that protects against T. cruzi. IFN-gamma activates macrophages, causing the release of reactive oxygen metabolites (ROS) and nitric oxide (NO) . T. cruzi infection causes the formation of cytokines, which in turn modulates parasite resistance and, most likely, the progression of chronic Chagas disease . proinflammatory cytokines and microbicidal mediators produced by macrophages and natural killer cells limit the initial increase in parasitemia. Following these innate immune responses, there is polyclonal activation of major lymphocyte subsets and the onset of acquired immunity against the parasite, which is mediated by CD4+T cells, CD8+ T cells, and B cells . T. cruzi infection raises the number of splenic DCs . However, most splenic DCs remain immature, as evidenced by decreased CD86 expression and inability to migrate toward the T-cell zone in response to lipopolysaccharide (LPS) injection. Cruzipain, the primary cysteine proteinase of T. cruzi, induces IL-10 and TGF-secretion as well as arginase production in macrophages, resulting in enhanced intracellular replication of T. cruzi. T. cruzi infection necessitates the elicitation of Th1 cytokines, lytic antibodies, and coordinated activities of phagocytes, T helper cells, and cytotoxic T lymphocytes

Introductions:-

Trypanosoma cruzi is an intracellular trypanosomatid protozoan that is spread to humans by reduviid bugs, which are part of the insect subfamily Triatominae. Other means of dissemination include oral exposure from raw food, congenital transmission, blood transfusions, organ transplants, and lab oratory inoculation by mistake.(1). This reality, together with the lack of required screening for both blood and tissue donors, suggests that the epidemiology of Chagas disease will change in the near future. Chagas disease is determined by the bur den and the lineage of the inoculated parasite, as well as the infection path and the host's immune competence.Following the entry of T. cruzi into the host, the disease progresses through two stages .(2)

The acute period lasts about two months and is asymptomatic in the majority of infected people, though others may experience symptoms such as excessive fever, anorexia, nausea, vomiting, and diarrhea. During this point, there are a lot of parasites in the host's bloodstream and tissues, as well as a lot of cytokines in the plasma and a lot of B and T lymphocyte activation.(3) Additionally, parasite nests inside tissues can be associated with lymphoadenopathy, splenomegaly, and severe inflammatory processes. A small number (5–10%) of affected people will experience a more serious infection, such as myocarditis or meningoencephalitis, from which they will die. (3)

Most infected people stay asymptomatic (indeterminate form) for years or even decades, but about 30% of patients experience heart or gastrointestinal symptoms, which are hallmarks of the Chagas disease's persistent process. For years, researchers have been debating the pathological cause of chronic chagasic cardiomyopathy (CCC). Immunopathology caused by parasite persistence is

thought to be a major factor in the production of CCC, though autoimmunity can also play a part. During the chronic period (indeterminate or not), there are little or no parasites in the bloodstream, but reactivation may occur due to immunosuppression, especially AIDS, and pregnancy.(4)

The only appropriate and licensed medications for treating the acute phase or reactivation of the disease are nitrofuran (nifurtimox, Lampit) and nitroimidazole (benznidazole, Rochagan), which are not entirely satisfactory due to their reduced effectiveness in the chronic stage and significant adverse side effects. T. cruzi host regulation has been shown to be dependent on both humoral and cell-mediated adaptive responses, as well as innate immune system elements (5)

However, no human vaccine against T. cruzi infection is currently available. Finally, the economic and social costs of Chagas disease's early morbidity and mortality are significant, resulting in significant economic losses.(5)

Role of cytokines in resistance and pathology in Trypanosoma cruzi infection

Cytokines play a critical role in the control of hemopoiesis and lymphopoiesis, as well as the action of all cell types involved in an immune response. Interferon gamma (IFN-gamma) has been widely studied as a lymphokine that protects against T. cruzi. IFN-gamma activates macrophages, causing the release of reactive oxygen metabolites (ROS) and nitric oxide (NO).(6)

Interleukin 4 (IL-4), interleukin 10 (IL-10) and transforming growth factor beta (TGF-beta) will, on the other hand, reduce the intracellular regulation of T. cruzi infection by IFN-gamma-activated macrophages, inhibit NO release, and reduce the function of the TH1 cell subset (IFN-gamma producers). Though TNF-alpha

has been linked to resistance as well as tissue injury, interleukin 6 (IL-6) and interleukin 1 (IL-1) have been linked to a number of changes in endothelial cell activity that may be responsible for the microvascular spasm seen in chagasic myocardiopathy.(6)

Several cytokines, including IFN-gamma, IL-1 alpha, IL-6 and TNF-alpha have been shown to modulate the expression of adhesion molecules which participate in inflammatory process by recruitment of lymphocytes into inflammatory sites, contributing to the progression of the local inflammatory reaction in chagasic cardiomyopathy. Thus, it has been shown that acute infection with different strains of T. cruzi induced enhanced expression of ICAM-1 not only on infiltrating leukocytes but also on sarcolemma of cardiocytes and paralleled the production of proinflammatory cytokines. (7)

T. cruzi infection causes the formation of cytokines, which in turn modulates parasite resistance and, most likely, the progression of chronic Chagas disease. As a result, it is possible that chronic Chagas disease is caused by a change in the quantity and/or content of cytokine synthesis.(7)

Interleukin-17 mediated immunity during infections with Trypanosoma cruzi

A healthy immune response is needed for host resistance during infection with Trypanosoma cruzi and other protozoans. To be effective against these pathogens, both innate and adaptive cell populations must work together, including macrophages, neutrophils, dendritic cells, CD4+ and CD8+ T cells, and B cells, among others.(8)

Indeed, during most protozoan infections, only a balanced synthesis of inflammatory (TH1) and anti-inflammatory (TH2/regulatory) cytokines allows parasite spreading to be regulated without losing host tissue integrity. The discovery of TH17 cells, a novel effector helper T cell lineage that expressed IL-17 as its signature cytokine, sparked a rethinking of the pathways that mediate defense and immunopathology during protozoan infections.(8)

Parasite survival is favored by immune regulation that follows the initial immune responses

Following infection, proinflammatory cytokines and microbicidal mediators produced by macrophages and natural killer cells limit the initial increase in parasitemia. Following these innate immune responses, there is polyclonal activation of major lymphocyte subsets and the onset of acquired immunity against the parasite, which is mediated by CD4+T cells, CD8+ T cells, and B cells . These powerful immune responses work together to reduce, but not remove, the parasite burden.(9)

According to research, both CD4+ and CD8+ T cells are needed for parasitemia control and host survival . Th1 responses mediate defense against T. cruzi infection, while Th2 responses associate with parasite persistence.(10)

Toll-like receptors (TLRs) are a type of pattern recognition receptor that can detect microbial pathogens . TLRs are expressed by phagocytes and other forms of cells that patrol the body, and they cause the release of proinflammatory cytokines .(11)

Proinflammatory responses caused by microorganisms are countered by anti-inflammatory responses induced by IL-10 and transforming growth factor- (TGF-)

to stop life-threatening organ damage . Both IL-10 and TGF- are thought to play important roles in the modulation of host immune responses to T. cruzi .(12)

T. cruzi-induced anti-inflammatory responses are effective enough to prevent the production of experimental autoimmune encephalomyelitis in mice , and they play an important role in parasite survival .(13)

The cost of suppressing proinflammatory cytokine secretion may be parasite persistence. T cells containing both Th1 and Th2 cytokines are found in the inflammatory cell infiltrates in the heart at peak parasitemia . Infiltrating macrophages also express arginase I, a marker of alternative macrophage activation, in a way that is linked to susceptibility to T. cruzi infection .(14)

TLRs are essential in the early stages of innate immune responses to T. cruzi .The cost of suppressing proinflammatory cytokine secretion may be parasite persistence. T cells containing both Th1 and Th2 cytokines are found in the inflammatory cell infiltrates in the heart at peak parasitemia . Infiltrating macrophages also express arginase I, a marker of alternative macrophage activation, in a way that is linked to susceptibility to T. cruzi infection (14). TLRs are essential in the early stages of innate immune responses to T. cruzi .(15)

The acute phase: immunomodulatory molecules are released by the parasite

The survival of the parasite is determined by a number of factors, including the release of molecules that interact with immune responses. When opposed to other pathogens, the onset of antigen-specific cytotoxic T-cell responses to T. cruzi can be impaired as a result of early immuno suppression . This lag in CD8+ T-cell responses may be to blame for the parasite's unregulated dissemination in the host. Surface glycosylphosphatidylinositol (GPI)-anchors have been shown to have both pro- and anti-inflammatory properties in parasites.(16)

GPI-anchored mucins

GPI-mucins are polymorphic proteins with variable and conserved domains. It has been hypothesized that parasite surface heterogeneity is caused by a large array of GPI-mucin genes, resulting in differential tissue adherence and immune evasion. The role of GPI-mucins in inducing host defensive responses is debatable. TLR2 expression is needed for GPI-mucins to work.(17)

The role of GPI-mucins in inducing host defensive responses is debatable. TLR2 expression is required for GPI-mucins to cause cytokine and mediator release .(18)

Furthermore, signaling via TLR2 and TLR9 is responsible for parasitemia regulation . TLR2-deficient animals, however, have shown that GPI-mucins and TLR2 play a regulatory rather than proinflammatory role in vivo . One possible reason for this paradox is that TLR2 needs additional signaling mechanisms to be activated in vivo to exert proinflammatory impact.(11)

This T. cruzi mucin binds to L-selectin on T cells, and L-selectin expression is needed to inhibit T-cell response . The above-mentioned findings are most likely attributed to intact GPI-mucins. (19)

Polymorphic GPI-mucins are necessary for processing and the production of a large number of partially related T-cell epitopes. Simultaneous exposure to several GPI-mucin epitopes decreases each epitope's expression below the threshold expected for CD4+ T cell interferon (IFN) secretion . IL-4 responses, on the other hand, are sustained , meaning that bulk responses to GPI-mucins prompt a shift toward Th2.(20)

GPI-mucins and induction of dysfunctional host dendritic cells

Modulation of the immunogenic properties of dendritic cells is one significant method of evasion (DCs). T. cruzi infection raises the number of splenic DCs . However, most splenic DCs remain immature, as evidenced by decreased CD86 expression and inability to migrate toward the T-cell zone in response to lipopolysaccharide (LPS) injection . (21)

T. cruzi in vitro exposure fails to induce maturation and blocks LPS-induced DC maturation, which coincides with enhanced IL-10 and TGF- secretion . These findings imply that sensitivity to T. cruzi molecules makes DCs ineffective for protective responses. Although the exact existence of T. cruzi molecules that modulate DC differentiation is unknown, GPI-mucins seem to be the most possible candidates.(22)

Induction of defective host dendritic cells by GPI-mucins T. cruzi lacks the ability to synthesize sialic acid. T. cruzi, on the other hand, produces trans-sialidase (TS), an enzyme that converts sialic acid from host glycoproteins to parasite GPI-mucins A virulent T. cruzi strain expresses high TS activity and is capable of suppressing both IL-12 formation through DCs and T-cell activation induction (23).

Trans-sialidase (TS)

The TS protein family is a member of the GPI-mucin superfamily TS is released and interacts directly with host lymphocytes through desialylation and resialylation of acceptor glycoproteins, in addition to its effects on GPI-mucins .(24)

TS induces CD4+ T-cell proliferation and cytokine formation by binding to the lymphocyte mucin CD43 . TS, on the other hand, causes lymphocyte apoptosis via target cell resialylation .(25)

The mechanisms of virulence are unclear, but a recent study indicates that FLY, a conserved cell-bind ing peptide from TS family proteins, increases parasitemia, mortality and the number of CD4+CD25+FoxP3+ regulatory T cells in the hearts of infected animals .(26)

Reduced sialylation plays an important role in increasing cellular contacts and increasing CD8+ T cell reactivity to their cognate peptide-MHC class I ligands . CD8+ T cells tend to be an effective subject of T. cruzi TS. In vitro and in vivo, TS treatment resialylates CD8+ T cells, including CD43 resialylation .(27)

Increased resialylation impairs activated CD8+ T cells' ability to destroy targets containing T. cruzi epitopes . These findings indicate that T. cruzi manipulates host T-cell sialylation during the acute process to evade immune responses.(28)

Glycoinositolphospholipids

Glycoinositolphospholipids (GIPLs) are free GPI anchors derived from various stages of T. cruzi life that modulate host immune responses .GIPLs suppress CD4+ T-lymphocyte activation in vitro and in vivo through the ceramide domain .(29)

Furthermore, GIPLs inhibit IL-2 but not IL-4 secretion, implying that GIPLs move the immune response toward a Th2 profile. GIPLs cause B-cell Ig secretion polyclonally, and the activity is located in the glycan moiety .(30)

Other modulatory molecules from T. cruzi

Cruzipain, the primary cysteine proteinase of T. cruzi, induces IL-10 and TGF- secretion as well as arginase production in macrophages, resulting in enhanced intracellular replication of T. cruzi . (31)

When paired with traditional adjuvants, Tc52, a T. cruzi glutathione thioltrans ferase, stimulates the immune system through TLR2 and elicits defensive immune responses.Tc52 enhances IL-10 mRNA in macrophages in the absence of adjuvants. Furthermore, it appears that Tc52 expression is necessary for optimal T. cruzi replication in the host .(28)

The chronic phase:an equilibrium between parasite killing and replication

Chronic infection in mice results in long-term activation of parasite-specific CD8+ T cells, while chronic infection in humans results in a decrease in the capacity to react to T. cruzi antigens . The distinction is attributed to the differing time frames of illness, which are two years in mice and several decades in humans .(16)

Chronic infection with T. cruzi, despite causing strong immune responses in mice and humans, cannot be destroyed by the immune system. The number of parasites is drastically decreased in chronic infection.As a result, it is unlikely that molecules released by the parasite play a significant role in evasion mechanisms. The collection of escaped parasites due to the differential display of antigenic epitopes by infected cells is one potential method of evasion (17).

The mucin-associated surface protein (MASP) family is a novel multigene family of T. cruzi surface proteins . Immunofluorescence experiments with a MASP-derived peptide have shown that the expression of a specific MASP is restricted to a subset of the parasite population.(32)

This result lends credence to the likelihood of parasite selection based on resistance to CD8+ T-cell responses directed toward variable epitopes. Despite the fact that thousands of proteins are expressed, the CD8+ T-cell reaction to T. cruzi is heavily based on a few peptides encoded by the TS gene family . The cause of this immunodominance is unknown. Indeed, it has been proposed that

immunodominance is a method for reducing the frequencies of the most efficient anti-parasite lymphocyte clones .(33)

The mechanisms are not well known, but indirect mechanisms are involved, and cells expressing MHC class II molecules are needed . During the chronic process, the number of parasites destroyed by the immune response should be nearly equal to the number of new parasites formed by replication. As seen in Figure 1, one possible mechanism of escape is based on the positive association between T. cruzi replication and TGF- generation by the host. TGF- production is a typical function of cell death, phagocytosis of apoptotic cells in response to immune responses or tissue turnover, and anti-inflammatory immune responses. TGF- signals are necessary for T. cruzi replication in epithelial cells.(34)

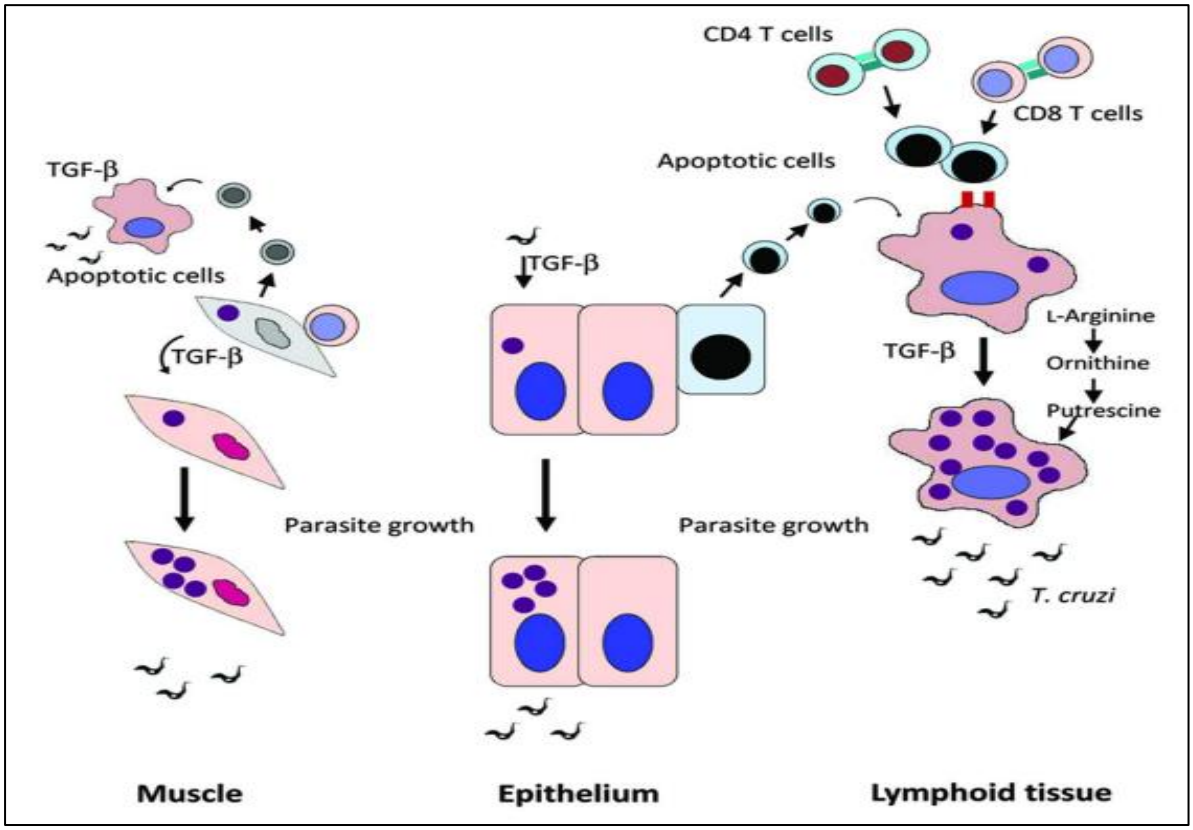

Figure 1. Role of the transforming growth factor-β (TGF-β) signaling pathway in the replication of Trypanosoma cruzi.

T. cruzi causes apoptosis in cardiomyocytes and macrophages and undergoes apoptosis itself . Apoptosis of leukocytes, cardiomyocytes, and viruses is a typical characteristic of T. cruzi-infected dogs' cardiac inflammatory infiltrates .(35)

Trypomastigote variants of T. cruzi, including apoptotic cells, express phosphatidylserine on the surface and engage TGF-signaling pathways in macrophages. The macrophage receptor involved, however, has yet to be identified. This research indicates that exposing macrophages to phosphatidylserine induces apoptosis and aids parasite reproduction .(36)

Furthermore, T. cruzi infection causes T and B lymphocyte apoptosis, and lymphocyte apoptosis has immunoregulatory consequences for host immune responses .(37) The increased parasitemia elicited by injection of a modified T. cruzi recombinant protein that induces apoptosis demonstrates the correlation between apoptosis induction and T. cruzi development .(38)

Phagocytosis of apoptotic cells causes a regulatory phenotype in macrophages, which is conducive to intracellular pathogen replication under inflammatory conditions .(39)

Immunity to Trypanosoma cruzi infection

The innate immune system is the first to effectively elicit a protective function, followed by a cascade of adaptive immune response components. In a nutshell, innate immunity is made up of an anatomic barrier, a physiologic border, phagocytic cells, and inflammatory components.

Skin and mucosal surfaces act as anatomic walls to avoid external materials from entering the body, while the complement system acts as a physiologic protection. The key phagocytic cells that eat pathogenic species are macrophages and

neutrophils. Dendritic cells can phagocytose and act as antigen-presenting cells during an adaptive immune response.

Basophils and mast cells are in charge of inducing an inflammatory response in the event of injury or infection. Eosinophils have both inflammatory and phagocytic properties that aid in parasite elimination. Natural killer cells play a significant role in the killing of infected cells and in the production of the cytokine interferon-gamma (IFN-). Antigen-specific T cells and B cells are used in the adaptive immune response. Antigen-presenting cells signal B cells to differentiate into plasma cells, which produce antibodies. Antigens can be presented to CD4+ and CD8+ T cells by dendritic cells, macrophages, and B cells.(9)

CD8+ T cells kill infected cells directly, while CD4+ T cells help to amplify the immune response by releasing cytokines. Cytokines and chemokines secreted by innate and adaptive immune cells are responsible for cell recruitment, cell-to-cell coordination, and pathogen clearance through a variety of mechanisms. T. cruzi associates complexly with various arms of the host immune defense and is capable of creating a chronic infection. Recent reviews have addressed how the parasite avoids the classical, lectin, and alternate complement pathways in order to infect the host. (9)

T. cruzi surface glycoproteins (mucins) and/or glycophospholipids (GIPLs) are recognized by innate immune cells and stimulate the production of multiple cytokines, including IFN-, TNF-, IL-1, and IL-6; and chemokines, including MCP-1, RANTES/CCL5, and IP-10 (interferon gamma-induced protein 10) in macrophages. These cytokines/chemokines stimulate macrophage synthesis of superoxide (O2•) and nitric oxide (NO), all of which are essential free radicals for direct T. cruzi killing. However, these free radicals are formed in small quantities

that are insufficient to clear the infection. Others discovered the IFN- encouraged T. cruzi Colombian strain infection of astrocytes.(9)

In mice acutely infected with T. cruzi Y strain, IFN- of NK origin and IL-12 of macrophage origin aided parasite-specific type 1 T cell-mediated adaptive immunity. T. cruzi is regulated by parasite-specific CD4+ T cells that secrete Th1 cytokines (IFN-, IL-2). phagocytic activity of macrophages is increased, B cell proliferation and antibody synthesis is stimulated, and CD8+ T cells are differentiated and activated.(16)

A good lytic antibody response by activated B cells improves parasite opsonization, phagocytosis, and complement-dependent killing. B cells, on the other hand, regulate the inflammatory/anti-inflammatory balance. Humans infected with T. cruzi have antibodies that attack the parasite's -Gal epitope, which contains the Gal1,3-Gal1,4-GlcNAc. This epitope is found on the surface of T. cruzi trypomastigotes in the majority of mucin glycoproteins. Antibodies that attack the -Gal epitope are highly expressed during acute infection, detectable during the chronic period, and capable of killing the parasite in the absence of complement or immune cells (16)

T. cruzi antigen-specific CD8+ T cells have been found in infected mice and humans and have been linked to T. cruzi regulation through cytolysis of infected cells and secretion of Th1 cytokines (IFN-) that induce trypanocidal activity ,Some evidence suggests that immune fatigue of CD4+ and CD8+ T cells contributes to decreased cytokine synthesis and parasite survival in chronically infected mice and patients.

Others, on the other hand, used mice chronically infected with Brazil, TCC, or Colombian strains of T. cruzi to show that CD8+ T cells in infected organs were

immunologically non-exhaustive; and concluded that CD8+ T cell exhaustion was the unlikely culprit for long-term parasite persistence.

To summarize, these studies show that an effective protective response to T. cruzi infection necessitates the elicitation of Th1 cytokines, lytic antibodies, and coordinated activities of phagocytes, T helper cells, and cytotoxic T lymphocytes; and the absence of all of these components results in parasite persistence and pathologic events that contribute to cardiomyopathy and heart failure in humans.

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