Chapter

Plasticity in Interferon Responses Modulates T-Cell Immunity in Parasitic Infections: Periphery to Thymus

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Abstract

Parasitic infections are the major threat prevalent in tropical and subtropical regions throughout the world. Different parasitic infections take a huge toll on mortality and morbidity at global level. Different parasites invade the host system, multiply inside host cells of their choice and sabotage defense mechanisms to overpower the host. T-cell immunity is majorly affected in different parasitic diseases such that the peripheral T-cell immune response is altered along with lesser explored thymic changes. Direct and/or indirect effect of parasitic infection leads to alterations in T-cell development, differentiation and activation resulting in deregulated T-cell immune mechanisms. Cytokines of interferon family play a significant role in determining the disease outcome and severity. Therefore, in this chapter, we here provide a detailed overview of the functional role played by IFNs during parasitic diseases in terms of their influence on peripheral T-cell activation and tolerance along with lesser explored impact on developing T cells in the thymus with altered microenvironmental niches.

Keywords: parasitic diseases, periphery, IFN, T cells, thymus, immunomodulation, disease outcome

1. Introduction

Parasitism is a relationship among species, in which one organism, the parasite, sustains on the host organism. Parasitic diseases can affect almost all living organisms. Parasites are dependent on the host organisms for their own survival. Not all parasites are harmful but some cause severe pathology to the host, such as *Leishmania*, *Plasmodium*, *Trypanosoma*, etc. Parasites known to affect humans are divided into three classes: protozoans, helminths and ectoparasites [1]. Parasite invasion triggers the innate, inflammatory and adaptive immune responses inside the mammalian host. Innate immunity recognizes the non-self and activates the T-cell–mediated adaptive immune system in order to eliminate the invader. Removal or recruitment of parasite is dependent on the production of distinct pattern of cytokines from specific T cells. T cells are formed through an intricate

developmental process with the dynamic stage-specific changes in the developing lymphocytes. T-cell development takes place in multiple steps originating from bone marrow to progenitors of T cell maturation in the thymus. It has been known that "thymus" plays the main role in the production of a self-tolerant adaptive immune response that is critical against the pathogen's threat [2]. A variety of infectious agents like protozoans mainly *Trypanosoma* spp., *Plasmodium* spp. and *Leishmania* spp. alter thymic structure and function. Thymic atrophy reflected by lymphocyte depletion is considered as a common feature in response to pathogens but the consequences on thymic function may differ significantly in different infections. Together with structural and functional changes induced by the parasite in the thymic microenvironmental niches, the development of thymocytes and thus the altered thymic output have direct implications in peripheral T-cell response. T-cell–based immune responses are further modulated via different types of cytokines viz. interferons (IFNs), tumor necrosis factors (TNFs) and interleukins (ILs) with implication in the disease outcome and progression. Different cytokines work independently or in collaboration as determinants of the disease establishment and progression. Host peripheral immune response influencing the disease outcome during parasitic infection is substantially studied *in vitro* on cell lines, *in vivo* in experimental models and in human subjects. A heterogeneous T-cell response marks the disease pathogenesis. A Th1 cell-mediated immune response is predominated by pro-inflammatory cytokines such as IFN-γ and TNF-α and plays a key role in arresting the disease by limiting the parasite replication. Contrary to this, a shift toward Th2 immune response, represented by increased expression of the anti-inflammatory IL-4, is associated with exacerbation of infection and uncontrolled parasite replication. This response is due to suppressive effects of Th2 cytokines on Th1 immunity. The important role played by the crucial IFN family of cytokines during parasitic diseases is emphasized in peripheral circulation as well as with regard to thymus-centric modulation of T-cell–based immunity in different infectious diseases.

2. A brief note on interferons

IFNs are the key soluble immune molecules belonging to the IFN family with specific structural and functional characteristics. They are divided into three main groups based on the structural details and functional contribution toward modulating the immune response during parasitic infections: IFN-I, II and III. The IFN-I family includes IFN-α and IFN-β. IFN-I signaling is mediated through a common cell surface receptor, (IFNAR). IFN-I production by a wide variety of cells mediates autocrine and paracrine signaling pathways upon viral infections. The IFN-II family represents IFN-γ. Its response is mediated by IFN-γ receptor (IFNGR). IFN-II plays a role in defense against intracellular pathogens by modulating diverse cellular functions. The third IFN-III family, or IFN-λ, comprises four different subtypes: IFN-λ1, λ2, λ3 and λ4. IFN-III is not well studied but has a role similar to IFN-I. The expression of IFN-λR receptor is mainly restricted to cells of epithelial origin [3].

3. Protozoan diseases

Protozoan parasitic infections are among the most common life-threatening infectious diseases. They can enter into the human body generally by a bite from an insect vector or through fecal-oral route. Protozoan parasites are responsible for serious infections. *Plasmodium falciparum* (*P. falciparum*), *Toxoplasma gondii*

(T. gondii), *Leishmania donovani (L. donovani)*, *Trypanosoma cruzi* (*T. cruzi)*, *Trypanosoma brucei* (*T. brucei*) and *Giardia intestinalis* (*G. intestinalis*) are among the most common protozoan pathogenic parasites and cause malaria, toxoplasmosis, leishmaniasis, Chagas disease, sleeping sickness and giardiasis, respectively. The three pathogenic parasitic diseases viz. Chagas disease caused by *T. cruzi*, malaria caused by *Plasmodium* spp. and leishmaniasis caused by *Leishmania spp*. will be discussed in length in relation to discrete T-cell–associated quantitative and qualitative alterations, reported in all these protozoan diseases. Disease-specific cytokine milieu with distinct role of IFNs is implicated in modulating disease progression and outcome. A snapshot of IFN-associated T-cell immune modulation in context to each of the protozoan disease is discussed in subsequent sections.

4. Chagas disease caused by *T. cruzi*

Chagas disease or American trypanosomiasis is caused by the parasite *T. cruzi*, transmitted to mammals by insect vectors. It is a hemoflagellate protozoan belonging to the kingdom Protista, phylum euglenozoa and class Zoomastigophora. It is a multi-host parasite transmitted by insect triatomines and is also called as "assassin bug" or "kissing bug." It is common in parts of Mexico and Central and South America [4].

4.1 Disease transmission

Transmission of *T. cruzi* parasite to humans is understood based on the route of infection as primary or secondary. The primary route is the most frequent route, and infection occurs through insect bite, blood transfusion or congenital and oral route. The secondary route is less frequent such as accidental infection during animal handling or infected organ transplant. The most common transmission route in the Brazilian region is oral transmission and the second is through contaminated food/beverage, whereas in Argentina, Bolivia, Colombia, Ecuador, French Guinea and Venezuela, contaminated food consumption is the main reason of infection [4].

4.2 Disease severity and diagnosis

Chagas disease has two phases of infection: an acute and a chronic phase. Acute form is mild. The parasite remains in the blood circulation for a long time (few weeks to months). Acute phase is followed by prolonged asymptomatic "chronic phase," marked by very few or negligible parasite in blood. Chronic Chagas disease symptoms include dilated colon or esophagus and different heart rhythm abnormalities. Diagnosis of acute phase of infection is marked by the presence of parasite in peripheral blood circulation and can be observed by microscopic examination of stained blood smear. Diagnosis of chronic Chagas disease is generally made by testing blood for parasite-specific antibodies [4].

4.3 Peripheral T-cell response in *T. cruzi* infection

Chagas disease is associated with several immunological alterations due to change in the expression pattern of cytokines that play a fundamental role in regulating the functionality of almost all cell types. *T. cruzi* infection triggers the nitric oxide (NO) production and may exert protective or toxic effects on the host immune system. NO can induce oxidative stress via damaging the host tissues. Inducible NO synthase pathway gets activated upon parasitic invasion,

produces NO and is highly responsible for macrophage-mediated intracellular *T. cruzi* elimination within infected cells. The cytokines such as IFN-γ, TNF-α and chemokines are produced in large amounts during *T. cruzi* acute infection and are potent inducers of NOS [5]. Along with NO synthase (NOS), several potent effector mechanisms such as T-cell–mediated immunity involving both $CD4^+$ and $CD8^+$ T-cell compartments are essentially involved in defense against *T. cruzi* invasion and replication in mammalian host. Relevance of T cells is well documented in experimental *T. cruzi* mice model where mice lacking T-cell subsets develop disease with high parasite load in tissues and periphery [6, 7]. These findings support the important role of T-cell populations in dealing with acute and chronic phase of *T. cruzi* infection in humans. Double positive (DP) $CD4^+CD8^+$ T-cell population was found to be increased in number with increased expression of activation markers (CD38 and HLA-DR) during chronic Chagas disease demonstrating that these DP T cells contribute to immune response against *T. cruzi* infection [8]. Cardiac inflammatory infiltrate of DP T cells in patients who have undergone cardiac transplant suggests that their performance in controlling the cardiac disease in humans is worth considering [8].

4.4 IFN-I–associated immune changes in *T. cruzi* infection

IFN-I has an important role in inhibiting the parasite multiplication. Induction/production of IFN-I in response to *T. cruzi* is stage specific [9] and primarily dependent on the dose/amount of parasite, and route of infection. Exogenous IFN-I treatment in *T. cruzi*–infected mice showed that mice develop increased resistance to infection by stimulating natural killer and T-cell activities [10]. Protective action of IFN-I associated with LRG-47 [IFN-inducible p47GTPase] is well documented in experimental mice models. LRG-47 regulates host resistance against intracellular pathogens in a comparative study with wild-type (WT) and knockout (KO) mice, where LRG-47 KO mice exhibit severe anemia, thrombocytopenia and atrophy of thymus, in contrast to WT counterparts. Similar to *in vivo* model, IFN-I–induced *in vitro* stimulation of LRG-47 KO macrophages also display a defect in intracellular killing of amastigotes [11]. IFN-I is reported to play a dual role during disease: protection from disease and establishing pathology. Disease-exacerbating role of IFN-I has been reported in WT and $IFNAR^{-/-}$ mice model such that $IFNAR^{-/-}$ mice were able to restrict the parasite growth and survive, while the WT mice failed to resist the infection [12]. It suggests that under the conditions of increased parasite load, IFN-I contributes to the pathogenesis of infection [12].

4.5 IFN-II (IFN-γ)–associated immune changes in *T. cruzi* infection

IFN-γ has a central role in Chagas disease cardiomyopathy. The disease is characterized by increased production of IFN-γ in the periphery [13]. Several cytokines, including IFN-γ, IL-1-α, IL-6 and TNF-α, modulate the expression of immune cells and contribute to the inflammatory process by recruiting the T cells into the inflammatory sites. Conversely, IL-4, TGF-β and IL-10 negatively regulate NO production and downregulate the intracellular control of *T. cruzi* infection by IFN-γ–activated macrophages [14]. In humans, IFN-γ was detected as a predominant cytokine in circulation during *T. cruzi* infection [15]. IFN-γ regulates the expression of several genes, transcription factors, inflammatory cytokines such as TNF-α, chemokines, and other pathogen-resistance genes including inducible nitric oxide synthase 2 (iNOS or NOS2) [16]. Higher amount of IFN-γ along with TNF-α leads to an efficient parasite killing and enhanced function of memory T cells [17]. It is evident from the fact that mice deficient in IL-12 that is necessary for IFN-γ production

exhibit severe tissue and systemic parasitism suggesting the importance of the IFN-γ in controlling intracellular parasitism [18]. It is known that T-cell–mediated control of the disease is dependent on the duration of infection and tissue damage. The detection of IFN-γ and TNF-α during early phase of chronic *T. cruzi* infection is associated with IL-10 production by $CD4^+$ T cells [17]. IFN-γ production is higher in chronic Chagas cardiomyopathy compared to asymptomatic patients, wherein IL-10 is reported to be highly expressed [16]. IL-10 has a counter effect on Th1 responses via downmodulating IFN-γ response, which, if sustained for long time, may have harmful effects on the host [19]. Altered cytokine profile either quantitatively or qualitatively can be a major cause of chronic Chagas disease. IFN-γ is well known as a protective lymphokine against *T. cruzi*, but there are many reports stating the dual role (antiparasitic, protective and pathogenic) of IFN-γ in Chagas disease [13, 14]. Several reports suggest myocarditis and heart failure in patients with Chagas disease, possibly due to continuous production of IFN-γ by T cells [16]. IFN-γ controls infection through NOS production and activating ROS through induction of NADP oxidases, while resistance of *T. cruzi* to ROS induces serious alterations in heart function. The detrimental role of overexpression of IFN-γ has been proven in experiment with transgenic mice, where it results in TNF-α–dependent murine myocarditis and cardiomyopathy [20].

4.6 IFN-III–associated immune changes in *T. cruzi* infection

Type III IFNs serve as regulatory cytokines by reducing the damage caused by pro-inflammatory cytokines or by retaining the more potent IFN-I for times when immune responses are inadequate [21]. This subtype is poorly recognized and has not been studied in Chagas disease.

4.7 Thymic alterations in *T. cruzi* infection

Circulation of T cells in response to parasitic infection is securely controlled as various cytokines and chemokines influence the disease outcome. It has been reported that in *T. cruzi* infection, both Th1 and Th2 cytokines are associated with resistance and susceptibility to disease, respectively. Influence of cytokines on thymus function is not much studied. Pérez et al. [22] made a detailed evaluation of the effect of pro-inflammatory (IFN-γ, IL-12 and iNOS) and anti-inflammatory cytokines (IL-4 and IL-10) on thymus in a study with experimental C57BL/6 murine model. Uninfected knock out mice for both pro-inflammatory and anti-inflammatory cytokines showed thymocyte cellularity similar to wild-type mice, although apoptotic loss of DP thymocytes was seen in infected mice group, showing that thymic atrophy is independent of IFN-γ or iNOS [22]. However, in another study, it is shown that upon *T. cruzi* infection in C57BL/6 mice, IL-10 and IFN-γ play a role in controlling thymic T-cell activation via altering the thymic cell function, but the extent of immunological disturbances was not clearly described [23]. In experimental Chagas disease, it was observed that the increased level of extracellular matrix (ECM) in thymus favors the export of immature thymocytes from thymus. Increased migration of thymocytes in response to fibronectin leads to a high number of DP T cells' migration from the thymus to peripheral lymphoid organs. The frequency of peripheral $CD4^+CD8^+$ DP T cells is increased in acute *T. cruzi* infection up to 16 times in subcutaneous lymph nodes [24, 25]. Thymic atrophy is an acute phenomenon observed in the infected mice, accompanied by alteration in the thymic structure. The mechanism is understood in terms of hormonal dysregulation induced under infection condition. The production of pro-inflammatory cytokines, IL-1, IL-6 and TNF-α increases during the infection and

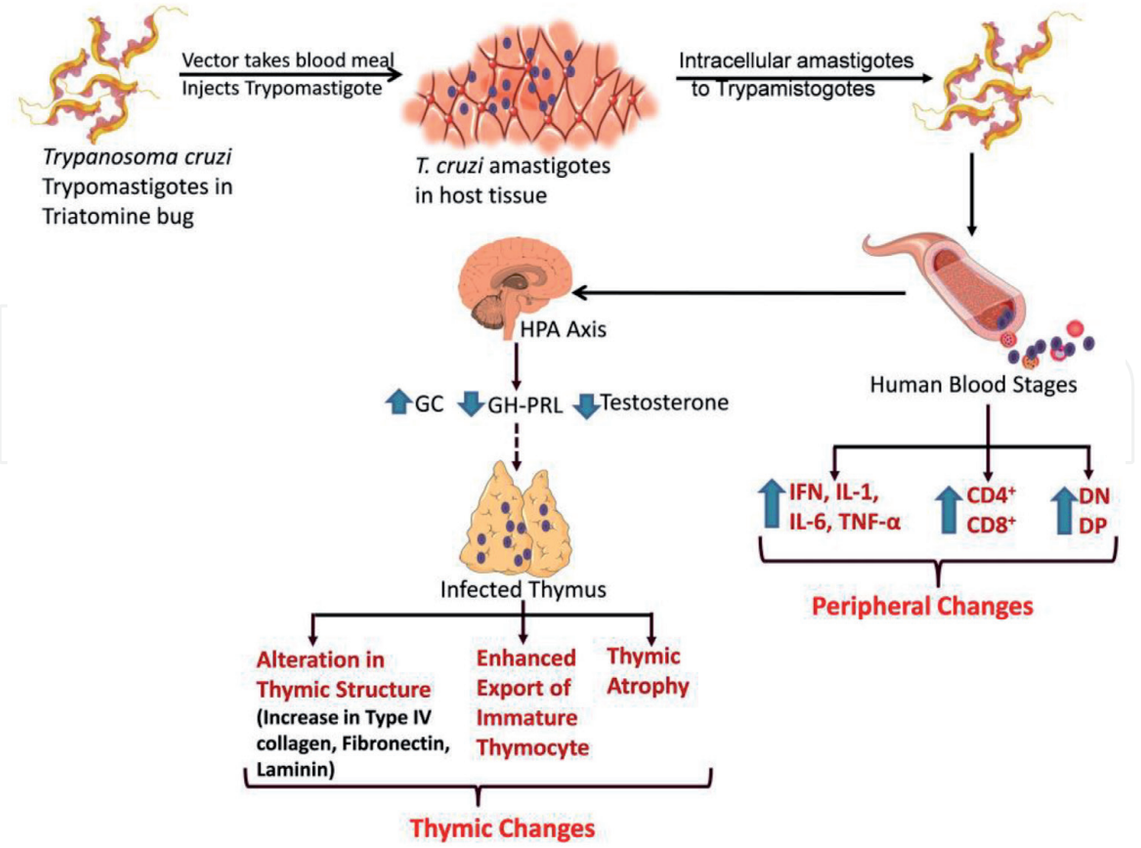

Figure 1.
Peripheral and thymic changes induced in host organism (mice and/or human) upon T. cruzi *infection.*

activates the HPA axis causing the release of glucocorticoids (GCs) [26]. GCs are steroidal hormones that lead to thymus atrophy with depletion of immature cortical thymocytes. Thymocyte depletion is seen to be directly proportional to increased TNF-α levels. However, this depletion is attributed to TNF-induced glucocorticoids rather than TNF-α directly such that it is not the cytokines, but the downstream molecules induced by them that lead to the observed thymic changes [27, 28]. It has been reported that during *T. cruzi* infection, prolactin (PRL) has a significant role in homeostatic balance of thymic corticosterone [29, 30]. Under stressful conditions, PRL balances the negative effects of GC by increasing thymocytes and thymic epithelial cell (TEC) proliferation. PRL rescues these cells from apoptosis in opposition to GC, which inhibits thymocyte growth. Recent reports show that PRL secretion is also altered along with GC secretion with decreased level of PRL paralleling increased GC levels during acute *T. cruzi* infection, causing an imbalanced cross talk that may correlate with the thymic involution [31, 32]. Thus, it can be said that GC and PRL are responsible for the loss of thymocytes, which leads to thymic atrophy. Thus, a dysregulated immuno-endocrine axis leads to profound effects on the thymus and disease outcome during *T. cruzi* infection. Peripheral and thymic changes associated with *Trypanosoma* infection are depicted in **Figure 1**.

5. Malaria caused by *Plasmodium* spp.

Malaria is a deadly disease caused by *Plasmodium* parasite belonging to the family Plasmodiidae. The disease is transmitted to humans by the bite of infected female Anopheles mosquito. Parasite species *P. falciparum* and *P. vivax* cause malaria in humans. Based on WHO reports, malaria is prevalent in 87 countries

throughout the world, with estimated 219 million cases and 435,000 estimated death reports [33]. An estimated 91% of all deaths due to malaria occur in Africa.

5.1 Disease transmission

Malaria is generally transmitted through the bite of *Anopheles* mosquitoes with high activity between dusk and dawn. Disease transmission is dependent on factors such as climatic conditions comprising rainfall patterns, temperature and humidity, host immunity, parasite species and the vector involved [33].

5.2 Disease severity and diagnosis

Malaria can be fatal if not treated. Disease outcome is determined by the parasite species and host immunity. Complications may arise in the form of cerebral malaria (CM) wherein the parasite infects the brain and leads to serious damage including seizures and coma accompanied with breathing problems, organ failure and low blood sugar. Early detection and disease treatment are important to reduce the risk of disease severity. Staining-based microscopic parasite diagnosis methods or malaria rapid diagnostic tests (RDTs) are widely used for preliminary diagnosis of the disease. RDTs detect specific antigen produced by malaria parasite in human blood using a dye-labeled capture antibodies providing an evidence of malaria infection [33].

5.3 Peripheral T-cell immune response associated with *Plasmodium* infection

Host immune response against *Plasmodium* parasites *in vitro* and *in vivo* is well studied in murine models (*P. yoelii*, *P. vinckei*, *P. chabaudi* and *P. berghei*) and humans (*P. malariae*, *P. vivax*, *P. falciparum*, *P. ovale* and *P. knowlesi*) [3]. The parasite stimulates multifaceted immune responses, including antibodies, NK and NKT cells, $CD4^+$ and $CD8^+$ T cells [34]. T cells play a major role in protection against *Plasmodium*. Both Th1 and Th2 subsets of $CD4^+$ T cells are the major players to control the systemic infections [35]. $CD4^+$ T cells stimulate $CD8^+$ T-cell cytotoxic activity, inhibit the development of liver stages and prevent the infection of red blood cells [36]. Thus, a balance between the cytokines and other immune molecules produced by different cell types is critical in determining the outcome of the infection.

5.4 IFN-I–associated immune changes in *Plasmodium* infection

IFN-γ is the most widely studied in malaria and has a versatile effect on the host. It may exert a protective or destructive effect, depending on the stage of the infection or the species of *Plasmodium* involved. Disease-protective phenomenon was observed in mice infected with *P. berghei*, where post-IFN-β treatment survival of mice is prolonged compared to non-treated counterparts [37]. Protection to disease is driven by a sensory mechanism against *Plasmodium* in the liver that mediates a functional antiparasite response driven by type I IFN. IFN-I is known to be active during the late phase of the liver stage infection. It is evident by the fact that treatment of *P. yoelii–infected* mice with recombinant IFN-α does not alter the hepatic parasite burden. This results in partially limiting the parasite growth in the liver and influences the commencement of erythrocytic stage infection. Leukocytes are recruited around the liver-stage of the parasite leading to reduced parasitemia [38]. Blood transcriptional profile of mild and severe malaria infection cases revealed that a specific set of genes was significantly associated with a mild

form as compared to their expression pattern in severe form of malaria. Studies on malaria-infected individuals from Malawi region revealed that genes responsible for IFN-I signaling pathway have an important role in the development of protective immune response against malaria. This is proved by molecular studies wherein mutations within IFN-α receptor (IFN-αR) lead to disease susceptibility and severe disease in Malawian population [39]. In contrast to the protective effects discussed above, a pathogenic role for IFN-I in *Plasmodium* infections has also been described. This has been reported in murine models, where the absence of IFN-I signaling in *P. berghei*–infected mice led to reduced parasite load and resistance against CM. The development of CM occurs as a result of detrimental brain injuries due to damaging inflammatory host immune response [40, 41]. Expression analysis of $CD4^+$ T cells from *P. berghei* ANKA (PbA)-infected mice revealed that $CD4^+$ cells showed dominance of IFN-I and IFN-γ signaling pathway-related genes. Mice deficient in IFN-I signaling had reduced parasite burden and displayed no CM-related symptoms. IFN-I suppressed IFN-γ production *via* inhibiting $CD4^+$ $T\text{-}bet^+$ T-cell derived IFN-γ production and hampered protective Th1-mediated control of parasitemia in *P. chabaudi*–infected mice [41]. Progression to CM can be modified by host genetic factors. A robust association between IFNAR1 and CM protection is well documented in experimental CM in $IFNAR1^{-/-}$ mice infected with *P. berghei* [40]. It is reported that splenic $CD8^+$ T cells from $IFNAR1^{-/-}$ mice got activated functionally but were unable to mediate any damage to brain tissue and cause CM development. This proves that IFNAR1 signaling promotes $CD8^+$ effector activity, which is mandatory for CM, in both humans and mouse [40]. There are controversial reports stating the IFN-I–mediated suppression on IFN-γ activity. During early stage of *P. chabaudi* infection, IFN-I induced by the infection plays a disease-exacerbating role by suppressing IFN-γ producing $CD4^+$ T cells in C57BL/6 mice [41]; however, in 129 Sv/Ev mice, IFN-I has minor roles in controlling the disease pathology [42]. Similar instance is observed in humans where polymorphism in human gene encoding for IFNARI strongly supports protection from the disease [43]. These controversial reports suggest that duration of activity and levels of IFN-I are important in regulating immune response against parasite growth [3].

5.5 IFN-II (IFN-γ)–associated immune changes in *Plasmodium* infection

IFN-γ regulates various components of the host immune system such as defense against intracellular pathogens by antigen presentation, antimicrobial mechanism, leukocyte development and immune cell trafficking. The protective role of IFN-γ is evident from the *in vitro* and *in vivo* studies, where inhibitory effect of IFN-γ on parasite multiplication was observed in *P. berghei* sporozoites-infected murine hepatocytes and/or human hepatic HEPG2 cells upon treatment with human recombinant IFN-γ [44–47]. IFN-γ helps in controlling the parasitism by activating macrophages and promoting phagocytosis of circulating parasites and plays a crucial protective role during blood-stage infection. *P. chabaudi* AS-infected mice treated with monoclonal antibody against IFN-γ had less control of parasite multiplication, suggesting that IFN-γ is essential for limiting parasite multiplication. Similar effects were evident in *P. chabaudi* AS-infected mice that were lacking IFN-γ receptor. These mice had lower survival rates as compared to the WT controls [48]. This suggests that IFN-γ production at different stages during infection could alter parasite survival and hence disease outcome. In *P. berghei* infection, IFN-γ along with TNF-α also plays a protective role by parasite removal activity [49]. The natural resistance to *Plasmodium* infection is reported in humans from tribes in Mali where resistance was correlated with increased

levels of IFN-γ [50], suggesting a protective role for IFN-γ against malaria. IFN-γ is essential in both protective immunity and pathogenesis of the diseases. During malaria infection, elevated levels of IgE antibodies are also observed. IgE containing immune complexes are pathogenic and not protective as they are involved in overproduction of TNF-α. TNF-α acts as a major pathogenic factor in malaria and poses an increased risk of severe disease or death due to *P. falciparum* infection [35]. IFN-γ promotes migration of leukocytes and pathogenic $CD8^+$ T cells to the brain during infection induced by *P. berghei* ANKA in WT 129P2Sv/Ev mice compared to IFN-γ R1-deficient mice. The production of elevated levels of IFN-γ during parasite blood-stage is associated with susceptibility to severe CM malaria [51]. Its protective or harmful effect on the host depends on the stage of infection and target organ [44].

5.6 IFN-III–associated immune changes in *Plasmodium* infection

Type I and type II interferons [IFNs] are critical to govern the disease outcome; however, reports on the involvement of recently identified IFN-III humans during malaria infection are scarce [3].

5.7 Thymic alterations in *Plasmodium* infection

Malarial infection results in increased levels of IFN-γ and TNF-α in human serum. Both these cytokines have been shown to be involved in double positive T-cell death [52–54]. However, neutralizing the effect of IFN-γ and TNF-α did not alter the apoptosis-inducing capacity of the serum [28]. Conversely, TNF-α neutralization helps in the reduction of DP T-cell count due to increased apoptosis, stating that TNF-α exerts a protective rather than a destructive role in malaria-induced thymic atrophy [28]. Studies done on BALB/c mice model show a high level of apoptosis and premature migration of thymocytes in mice upon *Plasmodium* infection along with overexpression of TNF-α associated with thymic atrophy [55, 56]. Reports on direct effect of IFN family on thymic changes during malaria

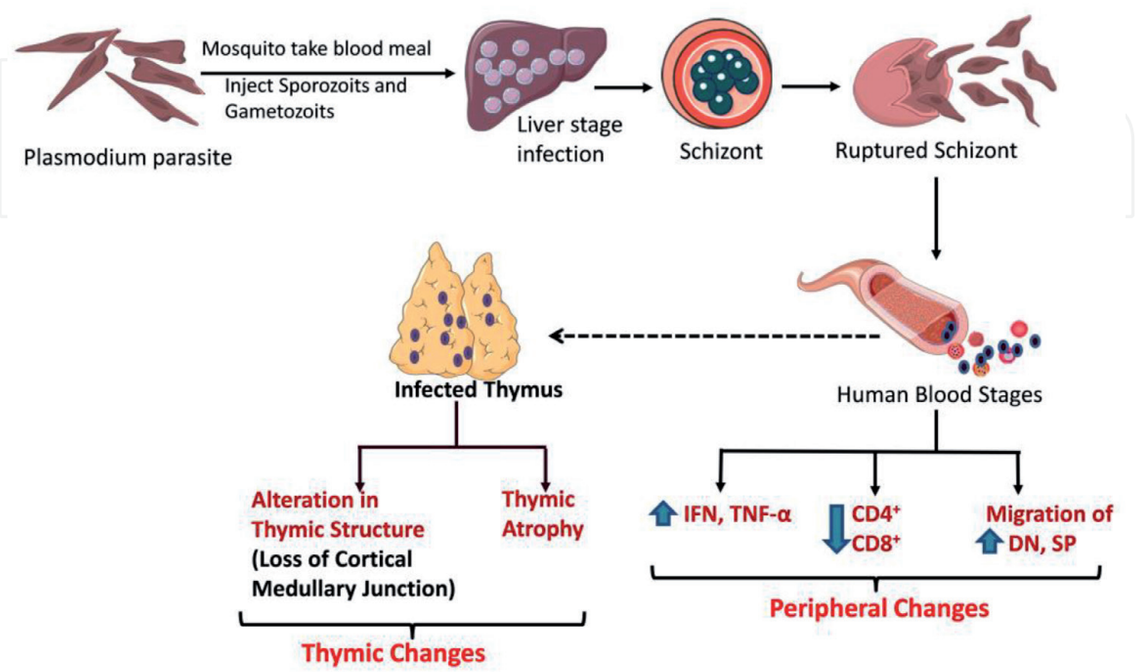

Figure 2.
Peripheral and thymic changes induced in host organism (mice or human) upon Plasmodium *infection.*

infection are scarce. In *P. berghei*–infected mice, changes in the thymic microenvironment alter the thymocytes' migration pattern with the direct implication in the export of immature cells to the periphery [57]. It is modeled that in *Plasmodium*-infected mice, the number of $CD4^+$ T cells decreases due to the destruction or reduced production of $CD4^+$ T cells and the number of $CD8^+$ T cells increases due to peripheral expansion or redistribution of preexisting cytotoxic T cells or due to an increase in thymic output [58]. Thymus atrophy seen in *Plasmodium* infection is accompanied by alterations in thymus architecture with loss of cortical-medullar delimitation [56]. The atrophy of thymus starts with an early stage of infection and the thymus weight is reduced markedly [59]. *Plasmodium* infection interferes with the positive and negative selection process of thymocytes resulting in apoptosis of thymocytes and thymic atrophy. This is evident from *P. chabaudi* non-lethal malaria model where thymic atrophy is reported to occur due to depletion of single positive $CD4^+$ and $CD8^+$ T cells [56, 60]. Changes in the thymic microenvironment, altered expression of the ECM proteins and chemokines observed in *P. berghei*–infected mice result in an altered intrathymic thymocyte migration pattern and defective thymocyte development [57]. Thus, a dysregulation in thymic immune cross talk comprising IFNs results in thymic structural and functional changes as depicted in **Figure 2**.

6. Leishmaniasis caused by *Leishmania* spp.

Leishmania is a tropical protozoan parasite belonging to the family Trypanosomatidae. The parasite is transmitted by the bite of the female phlebotomine fly species in old world countries and by Lutzomyia species in new world countries. More than 20 *Leishmania* species are known to circulate in endemic foci in Africa, Asia, the Middle East, the Mediterranean region, Central-South America, and southern Europe. The *L. donovani* and *L. infantum/chagasi* complex is responsible for VL; the *L. major, L. tropica*, *L. aethiopica* and *L. mexicana* complex causes CL; and the subgenus *L. Viannia* complex causes CL and MCL as per the classical association of specific parasite species with distinct clinical outcomes. The disease has a wide geographical occurrence covering 97 countries and territories with endemic foci for each of the different clinical manifestations [61].

6.1 Disease transmission

Female phlebotomine sandflies transmit the Leishmania parasite during blood meal. Disease transmission is dependent on the parasite or sandfly species, environmental conditions, host immunity and animal reservoir [62].

6.2 Disease severity and diagnosis

There are three main clinical forms of leishmaniasis: Visceral leishmaniasis (VL) or kala-azar is characterized by hepatosplenomegaly, fever and anemia. Cutaneous leishmaniasis (CL) is the most common form of the disease, characterized by skin lesions on exposed body parts, scars on the body and societal stigma. Mucocutaneous leishmaniasis (MCL) manifestation involves mucous membranes of the nose, mouth and throat. Diagnosis is generally based on microscopic examinations of *Leishmania* amastigotes in skin lesions in case of CL and rapid diagnostic recombinant K39 tests in case of VL with recent complementation with parasite-specific molecular diagnostics [62–64].

6.3 Peripheral T-cell response associated with *Leishmania* infection

There is a mixed Th1 and Th2 immune response during *Leishmania* infection with discrete quantitative and qualitative changes in T-cell subsets and the associated cytokines. Numerous reports explain counter-regulatory effects of T-cell subset–specific cytokines both at transcriptional and at translational levels. *Leishmania*-infected host exhibits a dynamic peripheral Th1/Th2 immune environment such that Th1 immune-activation is associated with IL-2, IFN-γ and TNF-α, which leads to macrophage activation and disease resolution, while Th2 response is associated with IL-4, IL-5 and IL-13 that supports disease progression [65]. Treg cells that produce IL-4 and IL-10 cytokines are also involved in regulating Th2/Th1 balance toward disease outcome. In mice infected with *L. donovani*, $CD4^+$ T cells are activated on the first day of infection and proliferate several folds resulting in splenomegaly [66]. $CD8^+$ T cells also produce cytokines and chemokines, which enhance immunity to pathogens [67]. So along with $CD4^+$ T-cell response, $CD8^+$ T cells also provide a level of control through production of IFN-γ and contribute to disease outcome. In contrast to protection mechanism, $CD8^+$ T cells induce cytotoxicity in *L. braziliensis* infection [68]. Thus, in the acute phase of *Leishmania* infection, $CD8^+$ T cells are protective because they produce IFN-γ, while in the chronic phase, they promote pathology because of cytotoxicity. In *L. infantum*-infected murine model, alterations in the number of peripheral $CD4^+$ and $CD8^+$ T cells are observed, wherein increase in peripheral $CD8^+$ T cells is responsible for the control of *L. infantum* infection with a slight decrease in the number of $CD4^+$ T cells [69].

6.4 IFN-I–associated immune changes in *Leishmania* infection

IFN-II is considered the main player in cell-mediated immune responses against infections, but recently, IFN-I is also being reported to play a role in Leishmaniasis pathology outcome. Activated macrophages initiate the parasite elimination via the production of iNOS. Deficiency of this enzyme in mice promotes susceptibility to *L. major* infection [70]. The protective role of IFN-I was studied in vivo where neutralizing IFN-I in mice experimentally infected with *L. major* made them more vulnerable to infection and increase in parasite load due to enhanced parasite multiplication. Blocking IFN-I function led to dissolution of iNOS activity and reduced cytotoxicity at early stages of infection [71]. The stage of parasitic infection and the dose of IFN-I play a significant role in predicting the consequences of the disease [12, 37]. Mattner et al. [72] revealed that IFN-I acts in a dose-dependent manner, where a low dose against a high dose of IFN-I protected the *L. major*-infected BALB/c mice from progressive leishmaniasis. IFN-I treatment aids in IFN-γ production via STAT4-dependent pathway [72].

6.5 IFN-II (IFN-γ)-associated immune changes in *Leishmania* infection

Leishmania immunity is mostly mediated by T lymphocytes and immune response is shown to be dependent on host genotype. This is evident from the fact that some inbred strains of mouse are susceptible, while others are resistant to *Leishmania* infection. In the human body, IFN-γ is not secreted alone, but other cytokines mainly IL-12, IL-10 and IL-4 influence the IFN-γ both at the level of induction and at the level of effector function. This further determines the course of infection [73, 74]. Several *in vivo* and *in vitro* experiments have shown that IFN-γ hinders the activation/expansion of $CD4^+$ Th2 cells, resulting

in the preferential expression of Th1 immune response and Th1 immunity. IFN-γ expression pattern is well documented for correlation with protection against the parasitic diseases in old and new world *Leishmania* infection model. The absence of IFN-γ or IFN-γ receptor leads to expansion of Th2-type cellular response in C57BL/6 mice making the host highly vulnerable to *L. major* or *L. amazonensis* infection [75]. IFN-γ-mediated immune protection against *Leishmania* infection is also evident where CXCL10-treated, *L. donovani*-infected BALB/c mice display generation of perforins and granzyme B via $CD8^+$ T-cell–dependent strong host-protective Th1 response, accompanied by significant downregulation in Th2- and Treg-associated cytokines [76, 77]. Pretreatment of macrophages obtained from BALB/c, C57BL/6 and C3H/HeJ mice significantly reduces *L. amazonensis* load via an NO-mediated mechanism of IFN-γ production in the presence of recombinant CXCL10 [78]. Immune response against leishmaniasis is also dependent on *Leishmania* spp. involved in infection. Several comparative studies conducted with crude antigen extracts of *L. braziliensis* and *L. amazonensis* reported that the extracts of *L. braziliensis* are more potent over *L. amazonensis* in stimulating CXCL10 production correlating to IFN-γ-positivity and multi-functional $CD4^+$ T cells in CL patients. Therefore, in agreement with the findings from murine infection models, CXCR3 and CXCL10 chemokines are also involved in protection and disease pathogenesis in leishmaniasis [79, 80]. Human VL is generally known to be predominated by Th2-type response. Anti-leishmanial drug treatments induce a significant Th1-type response in cured patients marked by the production of IFN-γ and IL-4 in the viscera. Contrary to VL, CL patients show a disease-healing response dominated by IFN-γ. IL-4, a Th2 cytokine, is rarely detected in CL cases [81]. In patients with active VL, the expression of IFN-γ is increased in the periphery, but it may be possible that the effect is not enough to overcome the parasite multiplication or there is unresponsiveness to *L. donovani* antigen. This may be due to elevated level of immunosuppressive Th2-specific cytokines in active VL patients [82].

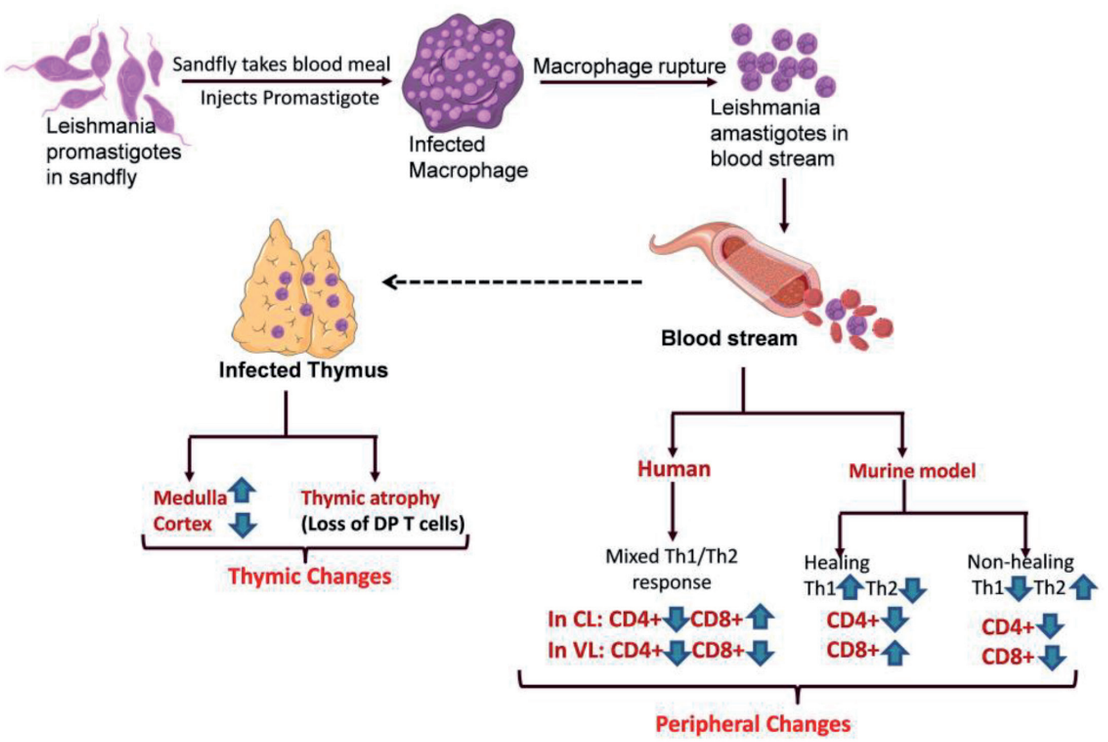

Figure 3.
Peripheral and thymic changes induced in host organism (mice or human) upon Leishmania *infection.*

6.6 Thymic alterations in *Leishmania* infection

Thymus is the least studied in context of *Leishmania* infection. A recent report demonstrates a decrease in thymic cellularity and concomitant thymic atrophy with severely compromised thymic microenvironment in a murine model co-conditioned with protein malnutrition and *L. infantum* infection. These mice exhibited a significant reduction of the thymic corticomedullary ratio [83]. Similar studies done in our laboratory with *L. donovani* infected VL murine model demonstrate that the parasite homes to thymus and lead to expansion of medullary regions when compared to control uninfected mice (unpublished data). *L. infantum* infection in protein-malnourished mice causes thymic atrophy due to a decrease of DP thymocytes and alters thymic chemotactic factors by diminishing CCL5, IGF1, CXCL9, 10 and 12 with significantly increased levels of IL-1α and IL-10 [83, 84]. It has been observed that due to *Leishmania* infection in mice, positively selected $CD8^+$ or $CD4^+$ T cells upregulate CCR7 and migrate to the medulla in response to CCL19/CCL21. CCR7 knockout mice were associated with cortical accumulation of SP thymocytes and decreased medullary $CD4^+$SP and $CD8^+$SP T cells. The migration of T cell is decreased in protein-malnourished infected mice as the components of extracellular matrix and adhesion molecules are altered that compromise the migratory capabilities necessary for adequate lymphocyte proliferation, intrathymic maturation and extrathymic activation [85]. Peripheral and thymic changes associated with *Leishmania* infection are depicted in **Figure 3**.

7. Conclusions

In conclusion, the role of IFN family in both immune-protective and immune-pathogenic processes in parasitic infections makes it a key set of molecules to be studied in depth (**Figure 4**). Modulatory effect of IFNs on T cells and downstream

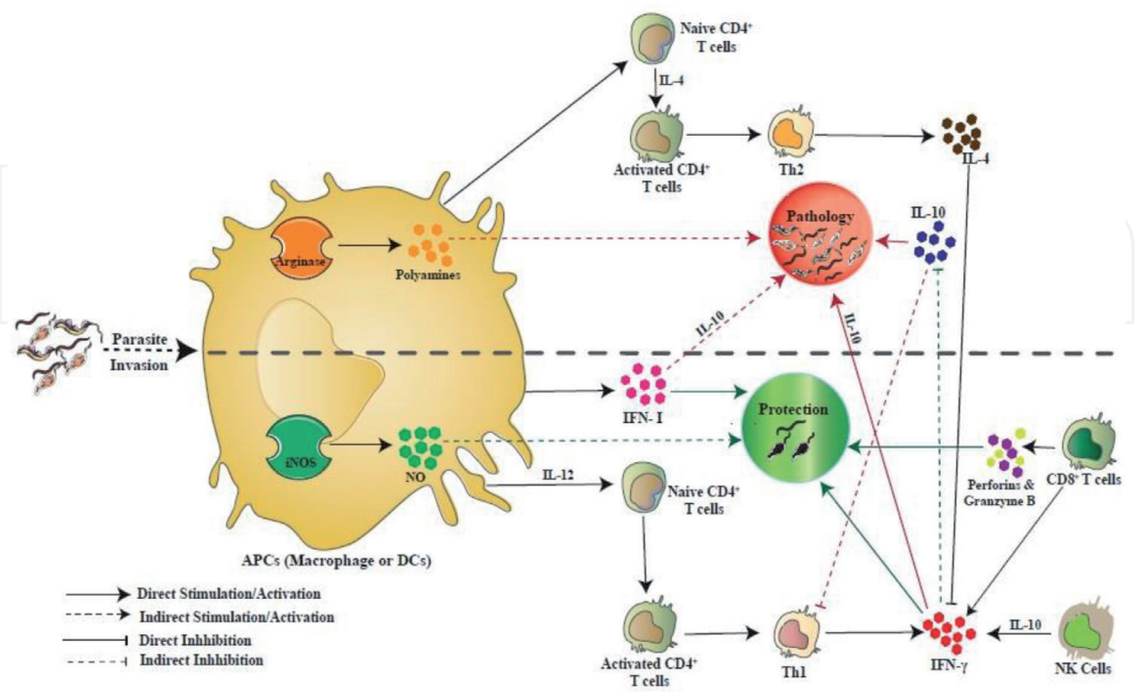

Figure 4.
Mechanistic of IFNs (IFN-I and IFN-γ) upon parasitic invasion in host organism. IFNs play a dual role in disease progression and/or protection, depending on the type and expression levels in relation to other secretory molecules and cytokines (Th1/Th2/Treg) and cause-effect cross talk between different IFN producers and effector immune cells.

effector function of T cells along with their complex cross-network functionality in other circulating blood and tissue-resident immune cells warrant further understanding on their role in disease manifestation and outcome. IFNs as the modulators of thymic structure and function are an interesting dimension of the immune-regulatory capabilities of these soluble immune molecules in infectious diseases. IFNs work as double-edged sword to modulate immune effector mechanisms determined by parasite and host components. This family of important cytokines can be tailored to be used as immunomodulators and/or immunotherapeutic molecules.

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References

[1] Centers for Disease Control and Prevention. Parasites. 2016. Available from: https://www.cdc.gov/parasites/about.html

[2] Savino W, de Moraes MC, Barbosa SD, Da Fonseca EC, De Almeida VC, Hontebeyrie-Joscowicz M. Is the thymus a target organ in infectious diseases? Memórias do Instituto Oswaldo Cruz. 1992;**87**(Suppl 5):73-78

[3] Silva-Barrios S, Stäger S. Protozoan parasites and type I IFNs. Frontiers in Immunology. 2017;**8**:14

[4] Centers for Disease Control and Prevention. Parasites—American Trypanosomiasis (also Known as Chagas Disease). 2019. Available from: https://www.cdc.gov/parasites/chagas/

[5] Gutierrez FR, Mineo TW, Pavanelli WR, Guedes PM, Silva JS. The effects of nitric oxide on the immune system during *T. cruzi* infection. Memórias do Instituto Oswaldo Cruz. 2009;**104**:236-245

[6] Tarleton RL, Sun J, Zhang L, Postan M. Depletion of T-cell subpopulations results in exacerbation of myocarditis and parasitism in experimental Chagas' disease. Infection and Immunity. 1994;**62**(5):1820-1829

[7] Rottenberg M, Bakhiet M, Olsson T, Kristensson K, Mak T, Wigzell H, et al. Differential susceptibilities of mice genomically deleted of CD4 and CD8 to infections with *T. cruzi* or Trypanosoma brucei. Infection and Immunity. 1993;**61**(12):5129-5133

[8] Giraldo NA, Bolaños NI, Cuellar A, Guzman F, Uribe AM, Bedoya A, et al. Increased CD4+/CD8+ double-positive T cells in chronic Chagasic patients. PLoS Neglected Tropical Diseases. 2011;**5**(8):e1294

[9] Chessler A-DC, Ferreira LR, Chang T-H, Fitzgerald KA, Burleigh BA. A novel IFN regulatory factor 3-dependent pathway activated by trypanosomes triggers IFN-β in macrophages and fibroblasts. The Journal of Immunology. 2008;**181**(11):7917-7924

[10] Rottenberg M, Cardoni R, Andersson R, Segura E, Örn A. Role of T helper/inducer cells as well as natural killer cells in resistance to *T. cruzi* infection. Scandinavian Journal of Immunology. 1988;**28**(5):573-582

[11] Santiago HC, Feng CG, Bafica A, Roffe E, Arantes RM, Cheever A, et al. Mice deficient in LRG-47 display enhanced susceptibility to *T. cruzi* infection associated with defective hemopoiesis and intracellular control of parasite growth. The Journal of Immunology. 2005;**175**(12):8165-8172

[12] Chessler A-DC, Caradonna KL, Da'dara A, Burleigh BA. Type I interferons increase host susceptibility to *T. cruzi* infection. Infection and Immunity. 2011;**79**(5):2112-2119

[13] Ferreira LRP, Frade AF, Baron MA, Navarro IC, Kalil J, Chevillard C, et al. Interferon-γ and other inflammatory mediators in cardiomyocyte signaling during Chagas disease cardiomyopathy. World Journal of Cardiology. 2014;**6**(8):782-790

[14] Laucella S, Rottenberg M. Role of cytokines in resistance and pathology in *T. cruzi* infection. Revista Argentina de microbiologia. 1996;**28**(2):99-109

[15] Albareda MC, Laucella SA. Modulation of *T. cruzi*-specific T-cell responses after chemotherapy for chronic Chagas disease. Memórias do Instituto Oswaldo Cruz. 2015;**110**(3):414-421

[16] Chevillard C, Nunes JPS, Frade AF, Almeida RR, Pandey RP,

Nascimento MS, et al. Disease tolerance and pathogen resistance genes may underlie *T. cruzi* evasion, parasite persistence and differential progression to Chagas disease cardiomyopathy. Frontiers in Immunology. 2018;**9**:2791

[17] Sathler-Avelar R, Vitelli-Avelar D, Massara R, Borges J, Lana M, Teixeira-Carvalho A, et al. Benznidazole treatment during early-indeterminate Chagas' disease shifted the cytokine expression by innate and adaptive immunity cells toward a type 1-modulated immune profile. Scandinavian Journal of Immunology. 2006;**64**(5):554-563

[18] Michailowsky V, Silva NM, Rocha CD, Vieira LQ, Lannes-Vieira J, Gazzinelli RT. Pivotal role of interleukin-12 and interferon-γ axis in controlling tissue parasitism and inflammation in the heart and central nervous system during *T. cruzi* infection. The American Journal of Pathology. 2001;**159**(5):1723-1733

[19] Dutra WO, Gollob KJ. Current concepts in immunoregulation and pathology of human Chagas disease. Current Opinion in Infectious Diseases. 2008;**21**(3):287

[20] Torzewski M, Wenzel P, Kleinert H, Becker C, El-Masri J, Wiese E, et al. Chronic inflammatory cardiomyopathy of interferon γ–overexpressing transgenic mice is mediated by tumor necrosis factor-α. The American Journal of Pathology. 2012;**180**(1):73-81

[21] Lazear HM, Schoggins JW, Diamond MS. Shared and distinct functions of type I and type III interferons. Immunity. 2019;**50**(4):907-923

[22] Pérez AR, Silva-Barbosa SD, Roggero E, Calmon-Hamaty F, Villar SR, Gutierrez FR, et al. Immunoendocrinology of the thymus in Chagas disease. Neuroimmunomodulation. 2011;**18**(5):328-338

[23] raes d M, ML, Minoprio P, Dy M, Dardenne M, Savino W, Hontebeyrie-Joskowicz M. Endogenous IL-10 and IFN-γ production controls thymic cell proliferation in mice acutely infected by *T. cruzi*. Scandinavian Journal of Immunology. 1994;**39**(1):51-58

[24] Cotta-de-Almeida V, Bonomo A, Mendes-da-Cruz DA, Riederer I, is DM, J, Lima-Quaresma KR, et al. *T. cruzi* infection modulates intrathymic contents of extracellular matrix ligands and receptors and alters thymocyte migration. European Journal of Immunology. 2003;**33**(9):2439-2448

[25] Mendes-da-Cruz DA, eis d M, J, Cotta-de-Almeida V, Savino W. Experimental *T. cruzi* infection alters the shaping of the central and peripheral T-cell repertoire. Microbes and Infection. 2003;**5**(10):825-832

[26] Roggero E, Perez AR, Tamae-Kakazu M, Piazzon I, Nepomnaschy I, Besedovsky HO, et al. Endogenous glucocorticoids cause thymus atrophy but are protective during acute *T. cruzi* infection. Journal of Endocrinology. 2006;**190**(2):495-503

[27] Cohen O, Kfir-Erenfeld S, Spokoini R, Zilberman Y, Yefenof E, Sionov RV. Nitric oxide cooperates with glucocorticoids in thymic epithelial cell-mediated apoptosis of double positive thymocytes. International Immunology. 2009;**21**(10):1113-1123

[28] Khanam S, Sharma S, Pathak S. Lethal and nonlethal murine malarial infections differentially affect apoptosis, proliferation, and CD 8 expression on thymic T cells. Parasite Immunology. 2015;**37**(7):349-361

[29] Lepletier A, de Carvalho VF, Rodrigues e Silva PM, Villar S, Perez AR,

Savino W, et al. *T. cruzi* disrupts thymic homeostasis by altering intrathymic and systemic stress-related endocrine circuitries. PLoS Neglected Tropical Diseases. 2013;**7**(11):e2470

[30] Lepletier A, de Almeida L, Santos L, da Silva Sampaio L, Paredes B, Gonzalez FB, et al. Early double-negative thymocyte export in *T. cruzi* infection is restricted by sphingosine receptors and associated with human Chagas disease. PLoS Neglected Tropical Diseases. 2014;**8**(10):e3203

[31] Lepletier A, de Frias Carvalho V, Morrot A, Savino W. Thymic atrophy in acute experimental Chagas disease is associated with an imbalance of stress hormones. Annals of the New York Academy of Sciences. 2012;**1262**:45-50

[32] Pérez AR, Morrot A, Carvalho VF, de Meis J, Savino W. Role of hormonal circuitry upon T cell development in Chagas disease: Possible implications on T cell dysfunctions. Frontiers in Endocrinology. 2018;**9**:334

[33] World Health Organization. Fact Sheets, Malaria. 2019. Available from: https://www.who.int/news-room/fact-sheets/detail/malaria

[34] Tsuji M, Zavala F. T cells as mediators of protective immunity against liver stages of *Plasmodium*. Trends in Parasitology. 2003;**19**(2):88-93

[35] Perlmann P, Troye-Blomberg M. Malaria blood-stage infection and its control by the immune system. Folia Biologica. 2000;**46**(6):210-218

[36] Morrot A, Zavala F. Regulation of the CD8+ T cell responses against *Plasmodium* liver stages in mice. International Journal of Parasitology. 2004;**34**(13-14):1529-1534

[37] Morrell CN, Srivastava K, Swaim AM, Lee MT, Chen J, Nagineni C, et al. Beta interferon suppresses the development of experimental cerebral malaria. Infection and Immunity. 2011;**79**(4):1750-1758

[38] Liehl P, Zuzarte-Luís V, Chan J, Zillinger T, Baptista F, Carapau D, et al. Host-cell sensors for *Plasmodium* activate innate immunity against liver-stage infection. Nature Medicine. 2014;**20**(1):47-53

[39] Krupka M, Seydel K, Feintuch CM, Yee K, Kim R, Lin C-Y, et al. Mild *P. falciparum* malaria following an episode of severe malaria is associated with induction of the interferon pathway in Malawian children, Infection and Immunity. 2012;**80**(3):1150-1155

[40] Ball EA, Sambo MR, Martins M, Trovoada MJ, Benchimol C, Costa J, et al. IFNAR1 controls progression to cerebral malaria in children and CD8+ T cell brain pathology in *Plasmodium* berghei-infected mice. Journal of Immunology. 2013;**190**(10):5118-5127

[41] Haque A, Best SE, Ammerdorffer A, Desbarrieres L, de Oca MM, Amante FH, et al. Type I interferons suppress $CD4^+$ T-cell-dependent parasite control during blood-stage *Plasmodium* infection. European Journal of Immunology. 2011;**41**(9):2688-2698

[42] Voisine C, Mastelic B, Sponaas AM, Langhorne J. Classical CD11c+ dendritic cells, not plasmacytoid dendritic cells, induce T cell responses to *Plasmodium* chabaudi malaria. International Journal of Parasitology. 2010;**40**(6):711-719

[43] Aucan C, Walley AJ, Hennig BJ, Fitness J, Frodsham A, Zhang L, et al. Interferon-alpha receptor-1 (IFNAR1) variants are associated with protection against cerebral malaria in the Gambia. Genes and Immunity. 2003;**4**(4):275-282

[44] Gun SY, Claser C, Tan KSW, Rénia L. Interferons and interferon

regulatory factors in Malaria. Mediators of Inflammation. 2014;**2014**:243713

[45] Schofield L, Villaquiran J, Ferreira A, Schellekens H, Nussenzweig R, Nussenzweig V. Gamma interferon, CD8+ T cells and antibodies required for immunity to malaria sporozoites. Nature. 1987;**330**(6149):664-666

[46] Mellouk S, Green SJ, Nacy CA, Hoffman SL. IFN-gamma inhibits development of *Plasmodium* berghei exoerythrocytic stages in hepatocytes by an L-arginine-dependent effector mechanism. Journal of Immunology. 1991;**146**(11):3971-3976

[47] Doolan DL, Sedegah M, Hedstrom RC, Hobart P, Charoenvit Y, Hoffman SL. Circumventing genetic restriction of protection against malaria with multigene DNA immunization: CD8+ cell-, interferon gamma-, and nitric oxide-dependent immunity. Journal of Experimental Medicine. 1996;**183**(4):1739-1746

[48] Zhong S, Stevenson MM. Central role of endogenous gamma interferon in protective immunity against blood-stage *Plasmodium* chabaudi AS infection. Infection and Immunity. 2000;**68**(8):4399-4406

[49] Taylor E, Onditi F, Maina N, Ozwara H. Immunization of mice with soluble lysate of interferon gamma expressing *Plasmodium berghei* ANKA induces high IFN-γ production, Tropical Diseases, Travel Medicine and Vaccines. 2017;**3**(1):11

[50] McCall MBB, Hopman J, Daou M, Maiga B, Dara V, Ploemen I, et al. Early interferon-γ response against *P. falciparum* correlates with interethnic differences in susceptibility to Parasitemia between sympatric Fulani and Dogon in Mali. The Journal of Infectious Diseases. 2010;**201**(1):142-152

[51] Belnoue E, Potter SM, Rosa DS, Mauduit M, Grüner AC, Kayibanda M, et al. Control of pathogenic CD8+ T cell migration to the brain by IFN-gamma during experimental cerebral malaria. Parasite Immunology. 2008;**30**(10):544-553

[52] Guevara Patiño JA, Marino MW, Ivanov VN, Nicolić-Žugić J. Sex steroids induce apoptosis of CD8+ CD4+ double-positive thymocytes via TNF-α. European Journal of Immunology. 2000;**30**(9):2586-2592

[53] Pérez AR, Roggero E, Nicora A, Palazzi J, Besedovsky HO, del Rey A, et al. Thymus atrophy during *T. cruzi* infection is caused by an immuno-endocrine imbalance. Brain, Behavior, and Immunity. 2007;**21**(7):890-900

[54] Fayad R, Sennello JA, Kim SH, Pini M, Dinarello CA, Fantuzzi G. Induction of thymocyte apoptosis by systemic administration of concanavalin A in mice: Role of TNF-α, IFN-γ and glucocorticoids. European Journal of Immunology. 2005;**35**(8):2304-2312

[55] Liepinsh DJ, Kruglov AA, Galimov AR, Shakhov AN, Shebzukhov YV, Kuchmiy AA, et al. Accelerated thymic atrophy as a result of elevated homeostatic expression of the genes encoded by the TNF/lymphotoxin cytokine locus. European Journal of Immunology. 2009;**39**(10):2906-2915

[56] Francelin C, Paulino LC, Gameiro J, Verinaud L. Effects of *Plasmodium* berghei on thymus: High levels of apoptosis and premature egress of CD4(+)CD8(+) thymocytes in experimentally infected mice. Immunobiology. 2011;**216**(10):1148-1154

[57] Gameiro J, Nagib PR, Andrade CF, Villa-Verde DM, Silva-Barbosa SD, Savino W, et al. Changes in cell migration-related molecules expressed by thymic microenvironment

during experimental *Plasmodium* berghei infection: Consequences on thymocyte development. Immunology. 2010;**129**(2):248-256

[58] Gravely SM, Hamburger J, Kreier JP. T and B cell population changes in young and in adult rats infected with *Plasmodium* berghei. Infection and Immunity. 1976;**14**(1):178-183

[59] Andrade CF, Gameiro J, Nagib PR, Carvalho BO, Talaisys RL, Costa FT, et al. Thymic alterations in *Plasmodium* berghei-infected mice. Cellular Immunology. 2008;**253**(1-2):1-4

[60] Seixas E, Ostler D. *Plasmodium* chabaudi chabaudi (AS): Differential cellular responses to infection in resistant and susceptible mice. Experimental Parasitology. 2005;**110**(4):394-405

[61] Thakur L, Singh KK, Shanker V, Negi A, Jain A, Matlashewski G, et al. Atypical leishmaniasis: A global perspective with emphasis on the Indian subcontinent. PLoS Neglected Tropical Diseases. 2018;**12**(9):e0006659

[62] World Health Organization. Fact sheets, Details, Leishmaniasis. 2019. Available from: https://www.who.int/news-room/fact-sheets/detail/Leishmaniasis

[63] Salotra P, Sreenivas G, Pogue GP, Lee N, Nakhasi HL, Ramesh V, et al. Development of a species-specific PCR assay for detection of *Leishmania donovani* in clinical samples from patients with kala-azar and post-kala-azar dermal leishmaniasis. Journal of Clinical Microbiology. 2001;**39**(3):849-854

[64] El Tai N, Osman O, El Fari M, Presber W, Schönian G. Genetic heterogeneity of ribosomal internal transcribed spacer in clinical samples of *Leishmania donovani* spotted on filter paper as revealed by single-strand conformation polymorphisms and sequencing. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2000;**94**(5):575-579

[65] Loeuillet C, Banuls AL, Hide M. Study of *Leishmania* pathogenesis in mice: Experimental considerations. Parasites and Vectors. 2016;**9**:144

[66] Sarween N, Chodos A, Raykundalia C, Khan M, Abbas AK, Walker LS. CD4+CD25+ cells controlling a pathogenic CD4 response inhibit cytokine differentiation, CXCR-3 expression, and tissue invasion. Journal of Immunology. 2004;**173**(5):2942-2951

[67] Novais FO, Scott P. CD8+ T cells in cutaneous Leishmaniasis: The good, the bad, and the ugly. Seminars in Immunopathology. 2015;**37**(3):251-259

[68] Bousoffara T, Louzir H, Ben Salah A, Dellagi K. Analysis of granzyme B activity as a surrogate marker of *Leishmania*-specific cell-mediated cytotoxicity in zoonotic cutaneous Leishmaniasis. Journal of Infectious Diseases. 2004;**189**(7):1265-1273

[69] Daoudaki M, Diakou A, Frydas S, Fouzas I, Karagouni E, Vavatsi N, et al. Vaccination with *Trichinella spirallis* antigens increases CD8+ peripheral T cells and enhances the Th2 immune response in *Leishmania infantum* challenged mice. International Journal of Immunopathology and Pharmacology. 2009;**22**(1):169-174

[70] Olekhnovitch R, Ryffel B, Müller AJ, Bousso P. Collective nitric oxide production provides tissue-wide immunity during *Leishmania* infection. The Journal of Clinical Investigation. 2014;**124**(4):1711-1722

[71] Diefenbach A, Schindler H, Donhauser N, Lorenz E, Laskay T, MacMicking J, et al. Type 1 interferon

(IFNα/β) and type 2 nitric oxide synthase regulate the innate immune response to a protozoan parasite. Immunity. 1998;**8**(1):77-87

[72] Mattner J, Wandersee-Steinhäuser A, Pahl A, Röllinghoff M, Majeau GR, Hochman PS, et al. Protection against progressive Leishmaniasis by IFN-β. The Journal of Immunology. 2004;**172**(12):7574-7582

[73] Alexander J, Brombacher F. T helper1/t helper2 cells and resistance/susceptibility to *Leishmania* infection: Is this paradigm still relevant? Frontiers in Immunology. 2012;**3**:80

[74] Kumar R, Singh N, Gautam S, Singh OP, Gidwani K, Rai M, et al. *Leishmania* specific CD4 T cells release IFNγ that limits parasite replication in patients with visceral leishmaniasis. PLoS Neglected Tropical Diseases. 2014;**8**(10)

[75] Pinheiro RO, Rossi-Bergmann B. Interferon-gamma is required for the late but not early control of *Leishmania amazonensis* infection in C57Bl/6 mice. Memórias do Instituto Oswaldo Cruz. 2007;**102**(1):79-82

[76] Gupta G, Majumdar S, Adhikari A, Bhattacharya P, Mukherjee AK, Majumdar SB, et al. Treatment with IP-10 induces host-protective immune response by regulating the T regulatory cell functioning in *Leishmania donovani*-infected mice. Medical Microbiology and Immunology. 2011;**200**(4):241-253

[77] Majumder S, Bhattacharjee S, Chowdhury BP, Majumdar S. CXCL10 is critical for the generation of protective CD8 T cell response induced by antigen pulsed CpG-ODN activated dendritic cells. PLoS One. 2012;**7**(11):e48727

[78] Vasquez RE, Xin L, Soong L. Effects of CXCL10 on dendritic cell and CD4+ T-cell functions during *Leishmania amazonensis* infection. Infection and Immunity. 2008;**76**(1):161-169

[79] Macedo ABB, Sánchez-Arcila JC, Schubach AO, Mendonça SCF, Marins-Dos-Santos A, de Fatima Madeira M, et al. Multifunctional CD4+T cells in patients with American cutaneous Leishmaniasis. Clinical and Experimental Immunology. 2012;**167**(3):505-513

[80] Schnorr D, Muniz AC, Passos S, Guimaraes LH, Lago EL, Bacellar O, et al. IFN-γ production to *Leishmania* antigen supplements the *Leishmania* skin test in identifying exposure to *L. braziliensis* infection. PLoS Neglected Tropical Diseases. 2012;**6**(12):e1947

[81] Dayakar A, Chandrasekaran S, Kuchipudi SV, Kalangi SK. Cytokines: Key determinants of resistance or disease progression in visceral Leishmaniasis: Opportunities for novel diagnostics and immunotherapy. Frontiers in Immunology. 2019;**10**:670

[82] Andargie TE, Ejara ED. Pro- and anti-inflammatory cytokines in visceral Leishmaniasis. Journal of Cell Science & Therapy. 2015;**6**(3):1

[83] Losada-Barragán M, Umaña-Pérez A, Durães J, Cuervo-Escobar SA, Rodríguez-Vega A, Ribeiro-Gomes FL, et al. Thymic microenvironment is modified by malnutrition and *Leishmania infantum* infection. Frontiers in Cellular and Infection Microbiology. 2019;**9**:252

[84] Losada-Barragan M, Umana-Perez A, Cuervo-Escobar S, Berbert LR, Porrozzi R, Morgado FN, et al. Protein malnutrition promotes dysregulation of molecules involved in T cell migration in the thymus of mice infected with *Leishmania* infantum. Scientific Reports. 2017;**7**:45991

[85] Cuervo-Escobar S, Losada-Barragan M, Umana-Perez A, Porrozzi R, Saboia-Vahia L, Miranda LH, et al. T-cell populations and cytokine expression are impaired in thymus and spleen of protein malnourished BALB/c mice infected with *Leishmania* infantum. PLoS One. 2014;**9**(12):e114584