Chapter

TLR-Mediated Host Immune Response to Parasitic Infectious Diseases

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Abstract

Toll-like receptors (TLRs) are important for the host immune response to a variety of pathogens, including bacteria, viruses, fungi, and parasites. These receptors become activated upon recognizing pathogen-associated molecular patterns (PAMPs) and thus initiate the innate immune response to the corresponding pathogen. A key aspect of TLRs is their activation of signaling that leads to cytokine production and an inflammatory response. Additionally, TLRs act as the bridge between innate and acquired immunity, enhancing phagocytosis and the process of killing parasites. We herein focus on how parasites (protozoans and helminths) and their derived products have the capability of stimulating or evading the host response by triggering or inhibiting TLR activation. Parasites often develop successful survival strategies that imply interference with the host immune response. Accordingly, many of these organisms have molecules that modulate inflammation and other aspects of host immunity. Taking advantage of such mechanisms, there are some anti-inflammatory therapies based on human infection with helminths. Helminths and protozoans influence the activity of various TLRs, especially TLR2, TLR4, and TLR9. A better understanding of the role of TLRs and their parasite-derived ligands should certainly provide new therapeutic tools for combatting various parasitic and inflammatory diseases.

Keywords: TLRs, protozoans, helminths, immune response, disease

1. Introduction

Toll-like receptors (TLRs) have the important function of recognizing a variety of pathogens, including bacteria, viruses, fungi, and parasites. They recognize pathogen-associated molecular patterns (PAMPs) and consequently initiate the innate immune response. The well-known TLRs that are responsible for binding to PAMPs act as a bridge between innate and acquired immunity. Accordingly, they are not only involved in the production of cytokines and chemokines but also enhance phagocytosis and the process of killing parasites.

Parasites are organisms that live on or inside an organism and benefit by deriving nutrients at the expense of the host. We herein review how protozoan and helminth parasites trigger differential activation of TLRs in order to regulate host immune cells. In some cases, the activation of these TLR receptors by PAMPs contributes to an effective control of the infection, while in other cases there is a negative

regulation resulting in the exacerbation of the infection, as shown in distinct in vivo animal models. In patients with diverse clinical manifestations of parasitic infections, TLRs and cytokines play a key role in the host response to the disease. A better understanding of the role of TLRs and their ligands should certainly provide new therapeutic tools and perhaps allow for the development of vaccines to control parasitic infections.

2. Protozoan parasites

Among the many protozoan parasites, those presently examined are *Leishmania* spp., *Trypanosoma* spp., *Naegleria fowleri, Plasmodium* spp., *Toxoplasma gondii*, *Giardia lamblia*, *Entamoeba histolytica*, *Trichomonas vaginalis*, *Blastocystis* spp., and *Acanthamoeba* spp.

2.1 *Leishmania* spp.

Leishmaniasis, an infectious disease endemic in 88 countries representing 5 continents, is caused by parasitic protozoa in the genus *Leishmania* [1]. This parasite gives rise to a variety of disorders, ranging from cutaneous lesions to visceral disease [2]. The latter can be generated by *Leishmania donovani*, *L. infantum*, and *L. chagasi* [3].

Regarding cutaneous lesions, the most pathogenic agents of American cutaneous leishmaniasis in Brazil are *L. (Viannia) braziliensis* and *L.* (L.) *amazonensis*, capable of inducing localized cutaneous leishmaniasis, borderline disseminated cutaneous leishmaniasis, anergic diffuse cutaneous leishmaniasis, and mucosal leishmaniasis [4]. In Mexico, *L. mexicana* evokes a wide spectrum of cutaneous diseases. For instance, localized cutaneous leishmaniasis is characterized by ulcers at the site of parasite inoculation, while parasites in diffuse cutaneous leishmaniasis spread throughout the skin and form disfiguring nodules [5]. In Iran, cutaneous leishmaniasis is endemic in 18 of 31 provinces, and approximately one-fifth of the cases belong to anthroponotic cutaneous leishmaniasis, stemming from *L. tropica* [6]. *L. panamensis*, a member of the *Viannia* subgenus of *Leishmania*, is known to provoke mucosal leishmaniasis. It produces destructive lesions of the nasal, oral, and hypopharyngeal mucosa [1].

2.2 *Trypanosoma* spp.

The causal agent of Chagas disease, *Trypanosoma cruzi*, was first described by the Brazilian physician Carlos Chagas in 1909 [7]. This pathology is endemic in Central and South America, and evidence exists of some cases in the United States, Europe, and Japan due to travel and migration. The parasite is an intracellular protozoan of the Trypanosomatidae family, transmitted to humans by blood-feeding reduviid bugs.

There are two phases of Chagas disease. The acute phase is generally asymptomatic, although some patients present symptoms such as fever, nausea, vomiting, anorexia, and diarrhea [8]. In the chronic phase, infected individuals can remain asymptomatic for decades, although around 30% eventually develop cardiac or gastrointestinal complications characteristic of the disease.

2.3 Other protozoans

Naegleria fowleri is a protozoan that invades the central nervous system and provokes primary amoebic meningoencephalitis. During the process of infection, it induces an important inflammatory response [9].

Acanthamoeba spp. are free-living amoebae found in lakes, rivers, swimming pools, thermal baths, and tap water [10]. They infect humans and animals as opportunistic pathogens in immunocompromised hosts [11]. These parasites are able to generate severe diseases, including amebic *Acanthamoeba keratitis*, a painful sight-threatening infection of the cornea, and granulomatous amebic encephalitis, a fatal disease of the central nervous system.

Giardia lamblia, the causal agent of giardiasis, colonizes the lumen of the upper small intestine. The parasite adheres to the surface of enterocytes without traversing the enterocyte barrier [12].

P. falciparum, the protozoan parasite responsible for malaria, is transmitted by the bite of mosquitoes. It results in a wide spectrum of clinical manifestations during the vector-parasite-host interaction. The first asexual reproduction process occurs in the human liver [13].

Trichomonas vaginalis is a flagellated protozoan parasite that infects the human genitourinary tract. It is the causative organism of trichomoniasis, one of the most prevalent sexually transmitted diseases in the world [14].

Entamoeba histolytica, the etiologic agent of amebiasis, is a pathogenic enteric protozoan. The manifestations of the disease range from mild diarrhea to severe dysentery, with liver abscesses forming in rare cases [15].

Blastocystis, an enteric parasite, colonizes the colonic epithelia of human and animal hosts [16]. Infection with this parasite gives rise to diarrhea, abdominal pain, flatulence, vomiting, and bloating [17].

3. Helminth parasites

The word helminth is derived from the Greek "helmins," which means parasite worm. Helminth is an umbrella term that includes many species of worms from different genera, having parasitism in common. They are quite frequently found in the population. The immune response depends largely on the type of parasite and its interaction with the host.

These large extracellular organisms have a complicated life cycle. They frequently migrate through blood vessels and tissues until reaching the definitive organ, such as the intestine, lungs, liver, or lymphatic organs. Some invade and colonize various cell types.

Because helminths have developed strategies of evasion of the host immune response, they are often able to survive. Hence, they can weaken the immune response and survive for years in the infected host, establishing chronic infection [18]. When the host immune response is adequately activated, on the other hand, infections are eliminated quickly.

Fasciola hepatica is a trematode worm that mainly affects livestock (e.g., cows, sheep, and goats) and humans. It is transmitted through the ingestion of aquatic plants contaminated with metacercaria. *Ascaris lumbricoides*, a parasitic intestinal worm, is transmitted by ingesting water contaminated with embryonic eggs. *Trichuris trichiura* and *T. suis*, other species of intestinal worms, remain in the large intestine of mammals in the form of adult larvae. The infection occurs after the ingestion of the eggs, which hatch in the small intestine and release the infective larvae [19]. *Schistosoma mansoni* are larvae of worms that live in waters and ponds contaminated by feces. They are able to penetrate the skin of people who bath or swim in contaminated pools. In the adult stage, these parasites produce eggs that are excreted through the stool [20]. *Strongyloides stercoralis* is an infection generated by the larvae. These are acquired through the skin and later lodge themselves in the intestine. *Trichinella spiralis* is a parasitic nematode, which infects the muscle tissue

of practically all mammals. *Toxocara canis*, round worms found in dogs, can infect humans if ingested in the form of the infective eggs of *Toxocara*. The larvae migrate to the intestine, liver, or lungs.

4. Molecules and protozoan and helminth parasites activate through TLRs

4.1 *Leishmania* spp. protozoans

L. major prompts the activation of the IL-1β promoter and mRNA expression in macrophages through the MyD88-pathway [21]. Additionally, *L. major* LPG upregulates the expression of TLR2 in NK cells and the production of cytokines, such as IFN-γ, TNF-α, and nuclear factor NFκB [22].

Amastigotes from *L.* (*V.*) *braziliensis* reduce the expression of TLR4 on the membrane of macrophages. This receptor modulates the production of TNF-α and IL-10 [23]. *L. infantum* promastigotes stimulate the production of IFN-α/β in plasmacytoid dendritic cells and the release of IL-12 by myeloid dendritic cells. Both these cytokines are dependent on TLR9 [24]. The expression of TLR9 and the production of TNF-α and IL-12 were observed in macrophages exposed to DNA from *L. mexicana* promastigotes [25]. Similarly, DNA from *L. major* was identified as the specific ligand that triggered TLR9-dependent activation of dendritic cells [26]. On the other hand, antigens of *L. donovani* stimulated the production of TNF-α, IL-12, and IFN-γ and increased TLR2 gene expression on RAW264.7 macrophages [27].

L. panamensis infection upregulates the expression of TLR1, TLR2, TLR3, and TLR4 and the production of TNF-α in human primary macrophages [28]. In peripheral blood mononuclear cells, *L. mexicana* LPG fostered the production of TNF-α, IL-1β, IL-12 p40, IL-12 p70, and IL-10 and the expression of TLR2 and TLR4. The triggering of the latter TLRs led to phosphorylation of the extracellular regulated kinase (ERK) and p38 mitogen-activated protein kinase (MAPK) [29].

4.2 Other protozoans

Glycosylphosphatidylinositol (GPI), a molecule derived from trypomastigotes, was found to be a potent activator of TLR2 from human and mouse origin [30]. Another family of GPIs, glycoinositolphospholipid (GIPL), has been purified from epimastigotes and triggers NF-κB activation via TLR4 [31].

Whether the recognition of different GPI anchors is mediated by TLR2 or TLR4 depends on their variable lipid moiety composition. There is one report of RA (high virulence) and K 98 (low virulence) strains of *T. cruzi* used to obtain total lipid extracts. The authors demonstrated that the total lipids from both strains promote the formation of lipid bodies and the release of pro-inflammatory molecules, including cyclooxygenase-2, TNF-α, and nitric oxide (NO) in macrophages as well as HEK cells, all through a TLR2/6-dependent pathway [32].

P. falciparum GPI contributes to the pathology of malaria by inducing the release of cytokines. In vitro studies have shown that this molecule is recognized by TLR2 and to a lesser degree by TLR4 [33]. Hemozoin stimulates the production of IFN-γ and inflammatory cytokines by triggering TLR9 in dendritic cells [34].

T. vaginalis parasites enhance TLR2 gene expression and activate NOD-like receptor type 3 (NLRP3) inflammasome in mouse macrophages and THP-1 human macrophages, respectively [35]. They also regulate the production of pro-inflammatory cytokines by the activation of MAPK and NFκB p65 and prompt pyroptotic cell

death through the release of IL-1β [36]. TLR4 upregulation has been reported in a prostate stromal cell line exposed to *T. vaginalis* [37].

Profiling like molecules and heat shock protein 70 from *T. gondii* activates dendritic cells through TLR4 and TLR11 [38]. The lipopeptidophosphoglycan (LPPG) and DNA from *E. histolytica* are recognized by TLR2, TLR4, and TLR9, triggering the release of IL-10, IL-12p40, TNF-α cytokines, and IL-8 from human monocytes [39, 40]. Unmethylated CpG oligodeoxynucleotides (CpG ODN) from the same parasite generate MMP-9 expression via the TLR9-dependent activation of ERK and p38 MAPK followed by the activation of NFκB [41]. *G. lamblia* trophozoites trigger TLR2, resulting in the activation of ERK and MAPK and the production of pro-inflammatory cytokines in peritoneal macrophages of wild-type mice [42]. Live *Blastocystis* spp. parasites and whole cell lysate alone cannot trigger TLRs in THP-1 human monocytes. ST4WR1 parasites inhibit LPS-mediated activation of NF-κB in these same cells [43]. *N. fowleri* elicits the expression and production of pro-inflammatory cytokines and β-defensing-2, mainly through the canonical TLR4 pathway in a time-dependent manner [44].

4.3 Helminths

The host immune response mounted against helminth infections is activated through TLRs, which are triggered by the glycoproteins, secretion/excretion products, and various other molecules of the parasites [45].

Cathepsin cysteine protease (FheCL1) of *F. hepatica* inhibits the secretion of various inflammatory mediators, such as TNF-α, IL-12, and NO [46]. This is carried out by the null activation of macrophages through the degradation of TLR3 in the endosome in a TRIF-dependent pathway independent of MyD88 [47]. Glucans promote the production of high levels of IL-10 and IL-4, thus favoring a Th2 response. These glycoconjugates modulate the function and maturation of dendritic cells. In the process of phase changes, *Fasciola* employs an immunosuppressive mechanism that favors a Th2 response [48], in part by releasing various excretory/secretory substances that inhibit the maturation of dendritic cells and trigger TLRs via the MyD88-dependent signaling pathway.

The excretory/secretory molecules of *Taenia crassiceps* initiate the phosphorylation of cRAF by means of MGL (lectins) and TLR2, as well as decreasing the maturation of dendritic cells and the production of IL-12 and TNF-α. These molecules also modulate the signaling pathways of NFκB p65 and p38 MAPK activated by LPS through TLR4 [49, 50] and regulate the type C receptor of lectin [51]. The carbohydrates of the parasite induce the production of IL-6 through TLRs [52].

On the other hand, the lysophosphatidylserine and lipopolysaccharide of *A. lumbricoides* generate signaling through TLR2, modulate a Th2 response (leading to the secretion of IL-10), and promote phosphorylation of ERK 1/2. This helminth binds to hyaluronic acid (the main constituent of connective tissue), activates dendritic cells by TLR4 (in adult parasites, or in larvae depending on the larval concentration), and provokes inflammatory processes. Through its antioxidant properties, it can eliminate free radicals and act as a barrier to tissue degradation.

Trichuris trichiura inhibits the function of TLR4 by suppressing LPS-induced production and the secretion of TNF-α [53]. Similarly, cathepsin B1 secreted by *S. mansoni* inhibits the recognition of TLR3 and TLR4 by inactivating the MyD88-independent pathway and modulating Th2 responses. Phospholipids and glycophosphatidylserine trigger TLR2 in dendritic cells, eliciting a Th2 response and the stimulation of Tregs to secrete IL-10.

The soluble antigen of *S. mansoni* suppresses the production of cytokines IL-1β, IL-6, IL-12, and TNF-α as well as increases the secretion of TGF-β and the production of IL-10 in dendritic cells. Activated B cells augment the production of IL-10.

Both processes are dependent on the triggering of TLR2 and activation of MyD88, the latter of which promotes ERK1/2 phosphorylation [20]. dsRNA from *S. mansoni* binds to TLR3 in dendritic cells, thus positively regulating the expression of IFN type 1 genes [54–56].

Although the TLR4 receptor is not essential for killing the larva of *S. stercoralis* during the innate immune response, it is indeed crucial for killing the parasites during the adaptive immune response [57].

Phosphorylcholine is a glycoprotein released by *T. spiralis* that binds to TLR4 [58]. Several TLRs decrease the susceptibility of effector T cells to Treg-mediated suppression [59]. During the first week of infection, the gene expression of TLR1,

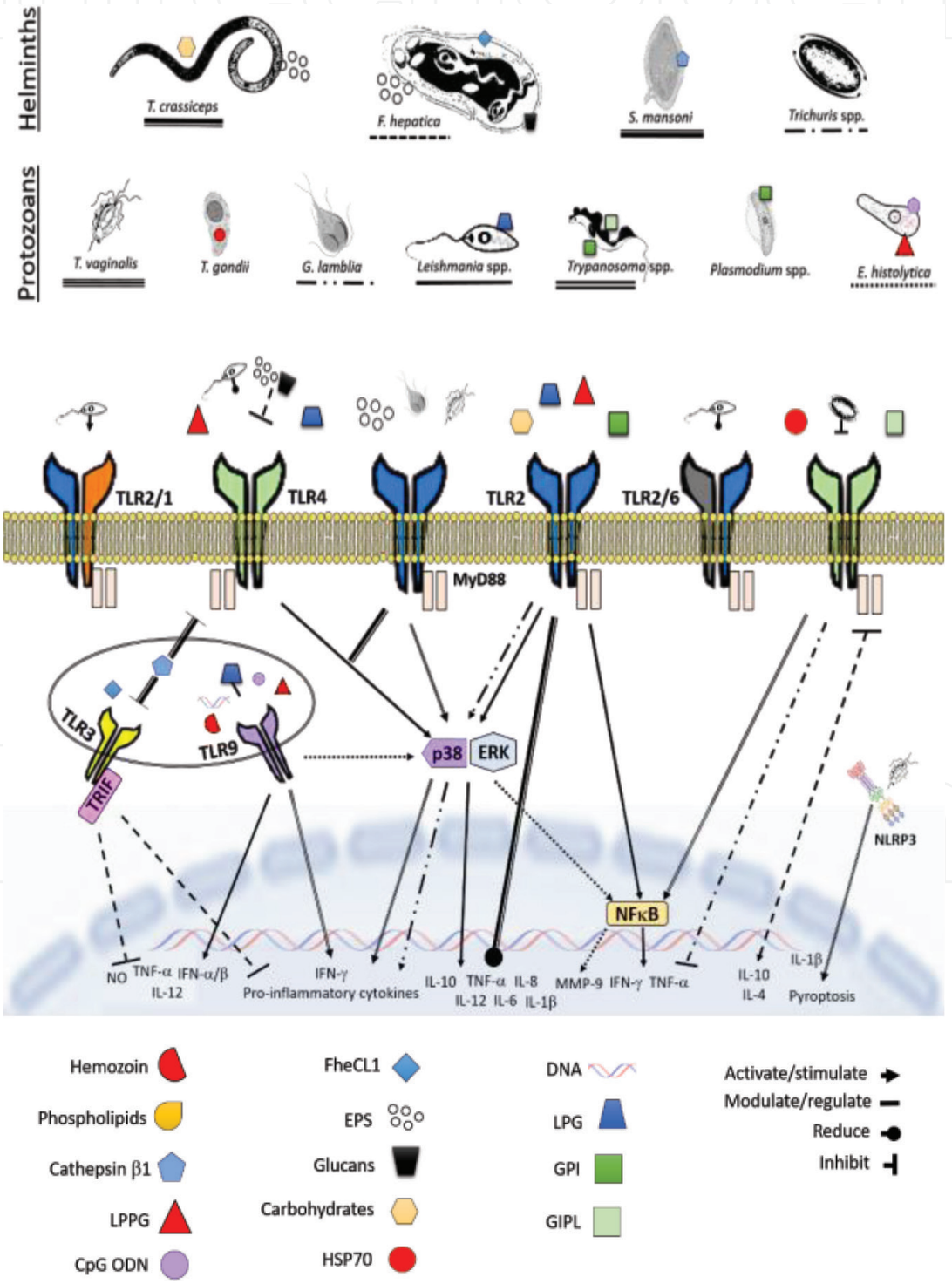

Figure 1.
TLR activation by PAMPs of parasites.

TLR2, TLR4, TLR5, and TLR9 increases significantly in the intestinal and muscular phases [60]. Infection also activates dendritic cells through TLR2 and TLR4 [61]. Since the TLR4/MyD88/NF-κB signaling pathway affects the secretion of inflammatory cytokines in macrophages, it may participate in immune suppression. In this sense, *T. spiralis* infection modulates the expression of TLR2 and TLR4 during different stages. Cytokine levels are regulated via TLR4-mediated signaling pathway, suggesting that TLR4 modulates host immunosuppression during infection [62].

Infection with *Toxocara* prompts cells to produce cytokines such as IL-4, IL-5, and IL-13, which generate increased IgE levels [63]. *Toxocara canis* inhibits the response of TLR2 to the activation of dendritic cells and competes with the host for lectins, thus blocking host immunity [64]. The activation of TLRs pathways by PAMPs of protozoans and helminths is summarized in **Figure 1**.

5. TLRs contribute to the effective control of parasite infection

5.1 *Leishmania* spp.

Infection by *L. major* in TLR4-deficient mice proliferates and correlates with a higher activity of arginase. In contrast, the same infection in TLR4-competent mice leads to low parasite replication that correlates with higher levels of inducible nitric oxide synthase. Hence, TLR4 competence could resolve cutaneous lesions and control parasite growth [65, 66].

TLR2 is unique among TLRs because it forms heterodimers with TLR1 or TLR6 and modulates downstream signaling pathways. In peritoneal macrophages from BALB/c mice infected with *L. major* promastigotes, the expression of TLR1 and TLR2 increases but not that of TLR6. TLR2-TLR2 association increases but TLR2-TLR6 association diminishes. The disparity in the formation of heterodimers between TLR2 and TLR1 or TLR2 and TLR6 brings about a TLR2 functional duality [67]. Additionally, LPG from *L. major* participates in the modulation of TLR9 expression and function.

The question arises as to whether the functional duality of TLR2 contributes to such modulation. The co-administration of CpG and the anti-TLR2 antibody reduces infection in susceptible BALB/c mice, establishing a relationship between LPG, TLR2, and TLR9 during an *L. major* infection [27]. In C57BL/6 mice deficient in either TLR2, 4, or 9, only the TLR9-deficient animals are more susceptible to infection with *L. major*. The deficiency of TLR9 inhibits the development of the curative Th1 response [26]. This effect has also been observed in TLR9-deficient mice infected with *L. infantum*. Therefore, TLR9 appears to play a key role in infections by *L. major* or *L. infantum* [2]. On the other hand, TLR11 and TLR12 play an important role in an *L. major* infection, evidenced by the fact that the silencing of these receptors reduces the parasite burden, enhances the level of IFN-γ, and diminishes the production of IL-4 [68].

Bone marrow-derived dendritic cells of MyD88$^{-/-}$ and TLR2$^{-/-}$ mice were infected with *L. braziliensis.* Compared to control mice, the infected dendritic cells from MyD88$^{-/-}$ mice showed low levels of activation and decreased production of cytokine IL-12p40, leading to greater infection by *L. braziliensis* and a limited expansion of CD4^{+} T cells that produce IFN-γ and IL-17 during infection. In contrast, TLR2$^{-/-}$ mice were more resistant to infection than control mice, which was associated with increased production of IFN-γ. Thus, MyD88 seems to be essential for the recognition of *L. braziliensis*, and TLR2 apparently has a regulatory role in modulating the immune response to the same parasite [69]. In peripheral blood mononuclear cells from cutaneous leishmaniasis patients infected with *L. braziliensis*, an evaluation was made of the expression of co-stimulatory molecules CD80,

CD86, and TLR9. Monocytes decreased the expression of these co-stimulatory molecules, but their expression was higher when the cells were exposed to soluble *Leishmania* antigen [70].

In the spleen of mice infected with *L. chagasi*, possible correlations were analyzed between TLR2 and TLR4 mRNA expression and the production of cytokines and nitric oxide (NO). Whereas the mRNA expression of TLR2, TLR4, IL-17, TNF-α, and TGF-β increases in the early stage of infection, it decreases during the late stage, which correlates with parasite load. The mRNA expression of IFN-γ and IL-12 declines at the peak of infection [3].

On the other hand, mice with the A20 protein silenced (a protein that inhibits NFκB activation by modulating the function of IKK, TRAF6, and NEMO) were infected with *L. donovan*, finding enhancement of the activation of NFκB and the host-protective pro-inflammatory response (compared to normal mice), which correlates with effective parasite clearance [71].

5.2 Other protozoans

Single-nucleotide polymorphisms have a role in innate immune responses and in susceptibility/resistance to infection. However, the genetic association of TLR4 and TNF-α polymorphisms is a determinant factor for the development of disease in Chagas disease. It has been reported the greatest parasite load exists in individuals with the C/C-Asp/Asp-Thr/Thr combination of homozygous genotypes at the TNF-α promoter 1031 and on the TLR4 polypeptide. The former homozygous genotype is represented by C/C. The latter homozygous genotypes encode Asp/Asp and Thr/Thr at codons 299 and 399, respectively [72]. Two TLR1 polymorphisms, rs4833095 (Asn248Ser) and rs5743618 (Ser602Ile), were assessed among 302 primiparous Ghanaian women for their association with *P. falciparum* infection and placental malaria and susceptibility to placental malaria [73]. It was found that TLR4-Asp299Gly and the TLR4-Thr399Ile variants confer greater risk of severe malaria in Ghanaian children [74]. The single-nucleotide polymorphism of TLR6 S249P may be a risk factor for the development of malaria [75]. Regarding TLR5, the single-nucleotide polymorphism of the TIR domain (TIRAP) S180 L provides protection against malaria, while the single-nucleotide polymorphism of R392 stop codon increases susceptibility to the same disease [76]. An rs4986790 polymorphism of the TLR4 gene can modulate the susceptibility toward a *P. vivax* infection. The AA genotype proved to be protective against the development of this parasite in a local population of Pakistan [77]. Additionally, TLR4 A299G, TLR6 S249, and TLR 9-1486C/T influence the levels of circulating cytokines IL-6, IFN-γ, IL-12, IL-10, and IL-4 during a *P. vivax* infection [78].

Mice deficient in the adaptor protein MyD88 and IL-18 were more susceptible to *P. yoelii* infection and increased parasitemia during the early phase of the infection. Greater lethality was observed in the MyD88-deficient animals. In mice deficient in IL-1R, parasitemia showed a slight increment during infection with *P. yoelii* [79]. During infection with the same parasite, there was a high level of parasite burden in $TLR2^{-/-}$ mice, which was closely associated with a reduction in pro-inflammatory cytokines in the liver [80].

On the other hand, the TLR9 variant −1237C/C correlates with acute parasitemia during a *P. vivax* infection [81]. An endoplasmic reticulum resident protein, UNC93B1, is critical for host resistance to *T. cruzi* and *T. gondii* [80, 82]. Its function is the translocation of nucleotide-sensing TLRs from the endoplasmic reticulum to endolysosomes. Interestingly, $TLR2^{-/-}$ and AKT-blocked mice that are infected with *Giardia* display a decreased parasite burden, an increased weight gain rate, and a shorter parasite persistence compared to normal mice infected with the same protozoan [42].

5.3 Helminths

The immune responses against helminth infections do not efficiently protect the host. The immune system is unable to eliminate chronic infection, and the immune memory fails to protect against reinfection, even after a cure mediated by pharmacological treatment [83].

For an *S. mansoni* or *A. lumbricoides* infection, dendritic cells are fundamental in the protection and activation of host defensive responses. When TLR receptors on the surface of dendritic cells bind to the ligands of the *S. mansoni*, *A. lumbricoides*, and *T. trichiura* parasites, there is an increased expression of co-stimulatory molecules (CD40, CD80, and CD86) and synthesis of pro-inflammatory mediators, such as IL-12 and TNF-α, that evoke a Th1 lymphocyte response [84, 85]. For intestinal parasites, the mRNA expression of TLR1, TLR2, TLR3, TLR4, and TLR9 is regulated in the early stages of infection. During the adult stage of an infection with *T. spiralis*, TLR1 and TLR4 activate the signaling pathway dependent on MyD88. Once newborn larvae appear, however, all expression of TLRs is inhibited, except for TLR2. The expression of TLR2/4 in the small intestine and muscle tissue during infection could be closely associated with immune responses mediated by Treg cells and greater expression of cytokines such as IL-10 and TGF-β [86, 87]. During an infection by *S. mansoni*, TLR2 and TLR4 limit the activation of the intestinal immune response. Consequently, the deficiency of these TLRs promotes the expulsion of adult worms. In the course of the first weeks of infection of animals with such deficiency, the expression of several TLRs increases, an active state that begins to decrease as of the fourth week. This favors an early activation of the immune response in the host and the prompt expulsion of the parasites [88].

6. Negative regulation by TLRs

6.1 *Leishmania donovani, Giardia lamblia*, and *Schistosoma mansoni*

L. donovani infection results in a suppression of TLR2- and TLR4-stimulated production of IL-12p40 and an increase in IL-10 production. Additionally, the parasites modulated the MAPK pathway by suppressing TLR2-dependent MAPK and ERK phosphorylation [89].

An in vitro infection with *G. lamblia* leads to a decreased production of pro-inflammatory cytokines by activating the AKT signaling pathway via TLR2.

Helminths are regulated by the expression of soluble antigens, which in parasites such as *S. mansoni* and *F. hepatica* exert an inhibitory effect on the maturation of dendritic cells induced by TLR ligands. The dendritic cells activated by soluble antigens produce a smaller amount of IL-12 than those activated only with the ligand for TLR4 [90–92]. The soluble antigen extracts of *S. mansoni* inhibit the ability of CpG, poly I:C, hyaluronic acid, and LPS to stimulate the production of IL-12 or increase the surface expression of CD80, CD86, and MHC class II in dendritic cells. Therefore, these extracts decrease the production of IL-12, IL-6, and TNF-α and the expression of co-stimulatory CD80/86 molecules, which in turn suppresses the Th1 response and favors the Th2 response elicited by LPS [93, 94]. The negative regulation of TLRs can reduce the production of pro-inflammatory cytokines, which could protect the host from autoimmune pathogenesis. The mechanism of this action is the regulation of the Th1/2 cell balance and the modulation of TLR4 expression [92, 95, 96].

In order to adapt to the immune system, some helminth parasites activate and/or downregulate TLRs, as well as interfere with the expression of several genes related to the transduction pathway [86]. Several studies suggest that continuous exposure

to helminth antigens may negatively regulate the response of cells to PAMPs derived from these parasites, implying a weakened immune response in individuals infected with helminths.

6.2 Highly virulent parasites downregulate TLR expression

According to an in vitro assay, *T. cruzi* strains with low virulence cause relatively high expression of TLR4 and high levels of pro-inflammatory cytokines such as IL-12 and TNF-α. Contrarily, virulent *T. cruzi* strains maintain a low expression of TLR4 and reduced production of TNF-α [97]. Likewise, at 2 days post-infection with *Acanthamoeba* strains Ac55 and Ac43, the mRNA expression of TLR2 and TLR4 was elevated in the brain of mice (versus control animals) [98]. Compared to uninfected mice, moreover, *Acanthamoeba* sp. generated a high expression of the mRNA and high levels of *TLR2* in the brain of animals at 2, 4, 8, 16, and 30 days post-infection [99].

7. TLRs in the outcome of disease

7.1 Leishmaniasis

Splenic biopsies and peripheral blood samples (to obtain mononuclear cell isolates) were taken from patients with visceral leishmaniasis in India, detecting the mRNA expression of TLR2 and TLR4 but not TLR9. The mRNA expression of IL-10 and IFN-γ was greater in pre-treatment versus posttreatment splenic biopsies. Additionally, the levels of IFN-γ and IL-10 mRNA were higher in peripheral blood mononuclear cells for pre-treatment versus posttreatment patients and healthy controls [100].

In Brazil, the peripheral blood mononuclear cells and lymphocytes ($CD14^+$ and $CD3^+$) were analyzed in 13 patients diagnosed with visceral leishmaniasis before and after treatment. Compared to healthy controls, there was a greater expression before treatment of TLR2 and TLR4 in lymphocytes and monocytes. Additionally, the levels of TNF-α, IL-10, and TGF-β increased, and those of IFN-γ, IL-17, and NO decreased. Although the expression of these two receptors did not change in lymphocytes after treatment, in monocytes they were found to be lower for TNF-α and IL-10 and higher for TGF-β, IFN-γ, IL-17, and NO [101].

Samples of whole blood taken from patients in Eastern Sudan with visceral leishmaniasis were stimulated with live *L. donovani* promastigotes. The expression of TLR2, TLR4, and TLR9 was found, which correlated with the production of IFN-γ, TNF-α, and IL-10 [102]. Similarly, the expression of TLR2 and TLR4 was measured in peripheral blood mononuclear cells of patients with cutaneous leishmaniasis, both those with healing and non-healing wounds, observing a significantly greater level in the macrophages of individuals with the healing versus non-healing lesions. This suggests a possible role of TLR2 and TLR4 in the outcome of cutaneous leishmaniasis lesions [103].

It is known that *L.* (*V.*) *braziliensis* and *L.* (L.) *amazonensis* interact with these same TLRs to promote a differential T-cell immune response and cytokine expression in mucosal leishmaniasis and anthroponotic cutaneous leishmaniasis. Biopsies taken from skin and mucosal lesions of infected patients were examined by immunohistochemistry, finding an important expression of TLR2, TLR4, and TLR9. Whereas tissues associated with *L.* (*V.*) *braziliensis* exhibited strong expression of TLR2 and TLR4, those tissues linked to *L.* (L.) *amazonensis* displayed similar results in relation to TLR9. The greatest expression of $CD4^+$ T cells was encountered in mucosal leishmaniasis and the lowest in anergic diffuse cutaneous leishmaniasis. Similarly, $CD8^+$ T cells showed their lowest expression in the latter disorder compared to the

other forms of the disease. There was greater expression of TNF-α in anergic diffuse cutaneous leishmaniasis versus mucosal leishmaniasis and of IL-10 and TGF-β in mucosal leishmaniasis versus anergic diffuse cutaneous leishmaniasis [4].

Although NK cells are detected in individuals infected with *L. mexicana* and suffering from localized cutaneous leishmaniasis as well as diffuse cutaneous leishmaniasis, the number of cells and the effector mechanisms differed drastically between the two groups. The number of NK cells, production of IFN-γ and TNF-α, and expression of TLR2, TLR1, and TLR6 were all lower than normal in patients with diffuse cutaneous leishmaniasis while being normal in those with localized cutaneous leishmaniasis. The altered protein expression found in NK cells of the former group correlated with the downregulation of IFN-γ gene expression in LPG-stimulated and non-stimulated cells. In conclusion, the lower number of NK cells and their limited activity in individuals with diffuse cutaneous leishmaniasis, evidenced by reduced TLR expression and cytokine production, are likely involved in the severity of the disease [5].

Peripheral blood was taken from patients with anthroponotic cutaneous leishmaniasis, including those responsive and unresponsive to treatment as well as healthy controls. Some mononuclear cells from these blood samples were exposed to *L. tropica* and others unexposed. An evaluation was made of the gene expression of TLR2, TLR4, TLR9, and TNF-α and the activity of iNOS and arginase in monocytes from patients unresponsive and responsive to Glucantime treatment. Upon comparing the monocytes exposed and unexposed to *L. tropica*, the former exhibited greater expression of all three TLRs and TNF-α and lower expression of iNOS in both groups of patients (responsive and unresponsive to treatment). Additionally, there was a significant downregulation of TLR2 and TNF-α expression and upregulation of TLR9 expression in isolates from unresponsive versus responsive individuals. Isolates from the former group also showed a significant increase in the level of arginase in monocytes stimulated with *L. tropica* and cultured promastigotes [6].

7.2 Chronic chagasic cardiomyopathy (CCC)

Chagas disease has different clinical forms: indeterminate, digestive, and cardiodigestive. CCC is associated with greater expression of TLR2, IL-12, and TNF-α. However, the expression of MyD88 mRNA is greater in cardiodigestive than indeterminate and cardiac patients. Serum mRNA expression of IL-12 and TNF-α transcripts was found in cardiac patients, who showed a higher production of TLR-induced inflammatory cytokines (TNF-α and IL-12) than indeterminate patients and uninfected individuals. Digestive and cardiodigestive clinical cases are correlated with elevated mRNA expression of TLR8 and IFN-β [104].

7.3 Helminth infections

During the interaction of *S. mansoni* with the host immune system, TLR4 activation provides a protective role against infection, while TLR2 activation is favorable for the parasite. The gene expression of the TLRs 1, 3, 7, and 8 is suppressed after infection. The antigens of *A. lumbricoides* promote the expression of TLR2 and thus engender a Th2 response [105–108].

8. Role of TLRs in strategies for the control of parasitic infections

TLRs have potential as therapeutic targets, either alone or in combination with conventional immunotherapy and pharmacotherapy. In recent years, antagonists

of TLRs or agonists of their negative regulators have been investigated as vaccine adjuvants to enhance an effective immune response against tumors, allergies, and infectious diseases [109].

The vaccine adjuvant properties of TLR7 and/or TLR8 agonists imiquimod and R848 were tested in the model of infection by *L. major*, determining the immune response before and after infection. Protective immunity was generated following subcutaneous but not intramuscular vaccination [110]. In another study, the effect of *L. major* polyclonal anti-murine TLR2 and TLR4 antibodies was assessed on cutaneous leishmaniasis and inflammatory arthritis. Both antibodies suppressed the development of clinical parameters, accompanied by reduced pro-inflammatory cytokine production. Hence, anti-TLR2 and TLR4 antibodies possibly have a synergistic therapeutic effect on inflammatory disease [111].

In visceral leishmaniasis, evaluation was made of anti-*Leishmania* immune responses. The protective efficacy of the glycosphingophospholipid (GSPL) antigen of *L. donovani* parasites when acting as a ligand for β-(1-4)-galactose terminal NKT cells suggests an important role of TLR4. This receptor may function as an upstream sensor by GSPL and induce the intracellular inflammatory signaling necessary for killing parasites. Treatment with GSPL was able to cause a highly effective T-cell response that contributed to good control of infection. Therefore, the synergism of TLR4 and NKT cells prompted GSPL to evoke a host-protective immunological response in experimental visceral leishmaniasis [112].

The collateral effects of a malaria infection during pregnancy correlate with immune activation in placental tissue. Since TLR4 plays a key role in this process, its blockage could be a potential strategy for therapeutic intervention to reduce the incidence of malaria-induced pathology both in the mother and the fetus [113].

Skin scarification with the *P. falciparum* peptide vaccine in combination with a TLR agonist produces systemic neutralizing antibodies with the potential of blocking parasite egress from the skin (and thus avoiding the invasion of liver cells) [114].

A good adjuvant formulation is crucial in the development of a successful vaccine. In the amebiasis model, the nanoliposome adjuvant containing synergistic TLR4 and TLR7/8 agonists successfully elicited balanced systemic humoral and cellular immune responses. The immunization protected against infection with up to 55% efficacy [115].

Since helminths use various molecules to regulate the host immune response, some of these may have potential therapeutic action against allergies and other inflammatory diseases. This anti-inflammatory strategy has been successful in treating diseases in animal models. Helminth-derived molecules are potent immunomodulators that could possibly be used for the design of new anti-inflammatory drugs. For example, the nematodes *T. suis* and *T. spiralis* induce a significant suppression of symptoms in autoimmune encephalomyelitis, an animal model validated for multiple sclerosis. Therefore, infection with live nematodes is not a prerequisite for the suppression of inflammation [53, 116].

Crohn's disease and ulcerative colitis are closely related to inflammatory processes. Some therapies, such as the ingestion of eggs of *T. suis*, promote a TLR2- and TLR4-regulated decline in inflammatory activity as well as a reduction in adverse effects of inflammation. This is based on the ability of helminths to polarize the response of helper T cells to a Th2 type, which inhibits inflammation. *F. hepatica*, on the other hand, exerts influence on dendritic cells activated by CpG, thus fostering the development of Tregs and a decrease in the severity and incidence of the disease [117]. *S. mansoni* alleviates allergies and reduces inflammation in airways. *A. suum* diminishes ocular allergic disease and *T. spiralis*, by evoking a Th2 response, and inhibits the production of IFN-γ to relieve colitis. These results are interesting because they demonstrate the interference of a helminth infection with the

establishment of an inflammatory immune response that favors other pathologies [118]. The aforementioned evidence of helminths regulating the immune response through TLRs could be instrumental in the development of therapeutic targets as well as in the inhibition or stimulation of their expression. Further research is needed to clarify the potential role of helminths in the modulation of the inflammatory response.

9. Conclusion

TLRs, one of the best characterized families of receptors, have a critical role in the host defense against infection. Additionally, they play a key role in the capacity of different protozoans and helminths to be able to generate a continuous activation of the host immune system by modulating the elements of the innate and/or adaptive response. Such intervention by these parasites in the immune response is aimed at promoting their survival inside the host. Single-nucleotide polymorphisms in TLRs participate importantly in increasing parasitemia in the host. However, agonists of TLRs can have a dual role. Whereas they may serve as adjuvants or vaccines to promote the maturation of dendritic cells and thus induce an adaptive immune response, they are also capable of triggering inflammatory cytokine production that has a pathogenic role in many diseases. Consequently, antibodies to TLRs and inhibitors of TLR signaling pathways have considerable potential as therapeutic agents. It is still necessary to clarify their mechanisms for modulating the response of these receptors to be able to design and develop innovative therapeutic targets.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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