Molecular Regulation of NLRP3 Inflammasome Activation During Parasitic Infection

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Abstract

Parasitic diseases are a serious global health concern, causing many common and severe infections, including Chagas disease, leishmaniasis,and schistosomiasis. The NLRP3 inflammasome belongs to the NLR (nucleotide-binding domain leucine-rich-repeat-containing proteins) family, which are cytosolic proteins playing key roles in the detection of pathogens. NLRP3 inflammasomes are activated in immune responses to *Plasmodium, Leishmania, Toxoplasma gondii*, *Entamoeba histolytica, Trypanosoma cruzi* and other parasites. The role of NLRP3 is not fully understood, but it is a crucial component of the innate immune response to parasitic infections and its functions as a sensor triggering the inflammatory response to the invasive parasites. However, while this response can limit the parasites' growth, it can also result in potentially catastrophic host pathology. This makes it essential to understand how NLRP3 interacts with parasites to initiate the inflammatory response. *Plasmodium* hemozoin, *Leishmania* glycoconjugate lipophosphoglycan (LPG) and *E. histolytica* Gal/GalNAc lectin can stimulate NLRP3 activation, while the dense granule protein 9 (GRA9) of *T. gondii* has been shown to suppress it. Several other parasitic products also have diverse effects on NLRP3 activation. Understanding the mechanism of NLRP3 interaction with these products will help to develop advanced therapeutic approaches to treat parasitic diseases. This review summarizes current knowledge of the NLRP3 inflammasome's action on the immune response to parasitic infections and aims to determine the mechanisms through which parasitic molecules either activate or inhibit its action.

Keywords: NLRP3 Inflammasomes, *Plasmodium, Leishmania, T. gondii, Entamoeba histolytica Trypanosome cruzi, Helminths*

Abbreviations: NLR: Nucleotide-binding domain and leucine-rich repeat; NLRP3: NLR family pyrin domain containing 3; PAMPs: Pathogen associated molecular patterns; DAMPs: Danger associated molecular patterns; PRRs: Pattern recognition receptors; LPG: Leishmania glycoconjugate lipophosphoglycan; TLRs: Toll-like receptors; NLRs: NOD-like receptors; CLRs: C-type lectin receptors; RLRs: Rig-I-like receptor; GRA9: Dense Granule Protein 9; NOD:Nucleotide-binding oligomerization domain; LRR: Leucine-rich repeat; NLRP4: NLR family pyrin domain containing 4; NLRC4: NLR family CARD domain containing 4; AIM2: Absent-in-melanoma 2; PYD: Pyrin domain; NACHT: Nucleoside-triphosphatase; GPIs: Glycosylphosphatidylinositols anchor;Hz: Hemozoin; ASC: Apoptosis-associated speck-like protein containing a CARD; P2X7R: Purinergic Receptor P2X 7; Src: Proto-oncogene tyrosine-protein kinase; Lyn: LYN Proto-Oncogene, Src Family Tyrosine Kinase; gDNA: Genomic DNA; IRF3: Interferon Regulatory Factor 3; SOCS1: Suppressor of cytokine signalling 1; LPG: Lipophosphoglycan; GP63: Glycoprotein-63; GPI: Glycosylphosphatidylinositol; LmSd: Leishmania major Seidman strain; LTB4: Leukotriene B4; LCL: Localized cutaneous leishmaniasis; ATG5: Autophagy related 5; CARD9: Caspase Recruitment Domain Containing Protein 9; STAg: Soluble total Ag; ROP7: Rhoptry protein 7; TgP: Profilin from T. gondii; GRA 9: Dense granule protein 9; Gal/GalNAc: Galactose/ N-acetylgalactosamine; LPPG: Lipopeptidophosphoglycan; MIF: Migration inhibitory factor; EhMIF: Entamoeba. histolytica Migration inhibitory factor; PGE2:

Prostaglandin E2; EhCP-A5 RGD: E. histolytica cysteine 5 proteinases contain an arginine-glycine-aspartate; CP5: Cysteine protease 5; Prx: Peroxiredoxins; Panx1: Pannexin-1; COX: Cyclooxygenase; EP4: Prostaglandin EP4 receptor; GIPLs: Glycophospholipids; TcAg: T. cruzi antigen; SEA: Schistosomal egg antigens; FhCL3: Fasciola hepatica cathepsin L3; FhHDM-1, Fasciola. hepatica Helminth defense molecule-1; MLES: Muscle larvae excretory-secretory.

1. Introduction

Inflammasomes are intracellular multimeric complexes playing key roles in innate immunity against numerous pathogens and physiological stimuli, their action is important in regulating inflammatory response. Since excessive inflammation can be harmful to cells and tissues, whereas inadequate inflammation response can be beneficial for pathogens. Innate immunity response mostly involves the detection of pathogens or danger associated molecular patterns (PAMPs and DAMPs, respectively) by Pattern recognition receptors (PRRs) such as Toll like receptors TLRs, NOD-like receptors (NLRs) , C-type lectin receptors (CLRs) , and RIG-I-like receptors (RLRs) (2 ,1) .This will incuse a signaling cascade resulting in triggering inflammation response in attempting for the agent clearance. Contrasting to the other known PRRs, some members of the NLR family are unique in their capability to form an inflammasome complex to activate caspase-1, an enzyme that cleaves pro-inflammatory Interleukin-1β (IL-1β) and Interleukin-18 (IL-18) leading to inflammation and pyroptosis a form of cell death of the infected cell (3).

Inflammasomes are parts of the innate response that contribute to the inflammatory response by stimulating the caspase-1 inflammatory pathway, resulting in the maturation of Interleukin-1β (IL-1β) and Interleukin-18 (IL-18), and pyroptotic cell death (4). The inflammasome is a signalling platform with three components (sensor, adaptor, and effector) that begin to form when endogenous and/or external threats are detected. Resulting in successive oligomerization of pro-caspase-1 to effecter caspase-1. Inflammasomes are formed from five-member proteins, the nucleotide-binding oligomerization domain (NOD), leucine-rich repeat (LRR)-containing proteins, the NLR family members NLRP1, NLRP3, and NLRC4, the absent-in-melanoma 2 (AIM2), and pyrin (a bipartite adaptor protein) (5, 6).

The NLRP3 inflammasome is critical for host immune defences against several types of infections, including bacterial, fungal, and viral (7-10). It is mostly expressed as a result of inflammatory stimulation in antigen-presenting cells (APCs) such as macrophages, dendritic cells (DC), neutrophils, and monocytes (11). Interestingly, NLRP3 activation has been associated with the pathogenesis of certain inflammatory conditions, including cryopyrin-associated periodic syndromes (CAPS), Alzheimer's disease, diabetes, gout, autoinflammatory disease, and atherosclerosis (12, 13). NLRP3 is a 118 kDa cytosolic protein expressed by a diversity of cells like lymphocytes, osteoblasts and neurons in addition to APCs. Its structure includes a multilateral protein complex containing an amino-terminal pyrin domain (PYD), which recruits proteins for inflammasome complex formation (14, 15). A central nucleotide-binding and oligomerization domain (NOD, NACHT domain), and a C-terminal

leucine-rich repeat (LRR) domain (16). The pyrin domain of NLRP3 interacts with the pyrin domain of ASC to trigger inflammasome formation (17). Similar to other inflammasomes, the NLRP3 inflammasome complex contains a sensor (NLRP3 protein), an adaptor (apoptosis-associated speck-like protein, ASC), and an effector (caspase-1) (18, 19). NLRP3 is unable to bind to stimuli directly, and instead senses the frequent cellular signals triggered by their presence. Current models of the classical or canonical NLRP3 activation divide into two signalling steps, priming (signal 1) and activation (signal 2), in addition to non-canonical activation pathway (20).

1.1 The NLRP3 inflammasome activation mechanism

1.1.1 The priming signal (signal 1) is the first step required for NLRP3 Inflammasome activation, that is responsible for the transcriptional upregulation of NLRP3 and pro-interleukin (IL) -1β and pro-IL-18 (21). It occurs when a cell exposed to priming stimuli like LPS or necrosis factor (TNF) and IL-1β through TLRs, tumor necrosis factor receptor (TNFRs), NOD2, IL-1R respectively. The detection of these inflammatory stimuli causes the activation of proteins and nuclear factors such as myeloid differentiation primary response protein (MyD88), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) to increase NLRP3 and IL-1β transcription, leading to up-regulation of NLRP3 protein and pro-IL-1β (22). However, when these component molecules translocate from nucleus to cytoplasm they are in inactive forms and require a second signal to be activated (23).

1.1.2 NLRP3 inflammasome activation (signal 2). The second signal facilitates the oligomerization of the inactive inflammasome complex (NLRP3, ASC, and pro caspase-1), leading to the maturation, and up-regulation of the pro IL-1β and pro IL-18 (24, 25). Conflicting to other PRRs, NLRP3 can be activated by abundance of stimuli such as uric acid crystals, silica, asbestos, extracellular ATP, and toxins, plus to viral, bacterial, fungal, and protozoan molecules (26, 27). In addition, the second signal can be induced by several molecular events such as ionic flux, mitochondrial dysfunction, the production of reactive oxygen species (ROS), and lysosomal damage. (28). Although it is uncertain how NLRP3 is able to identify such different signals, it was proposed that NLRP3 senses a common cellular incident resulted by all stimuli rather than direct binding to them (29). Following the signal 2, the adaptor protein ASC and inactive pro-caspase-1 join together, then subsequently cleaving pro-caspase-1 into active caspase-1, which in turn cleaves pro-IL-1β and pro-IL-18 into their active form, plus activates the membrane pore-forming gasdermin D (GSDMD). GSDMDs N-terminal domain (GSDMD-NT) protein cleaves and oligomerizes to form pores in the cell membrane resulting in pyroptosis and the release of intracellular components, including inflammatory cytokines IL-1 β and IL-18 (30).

1.1.3 NLRP3 inflammasome activation via the noncanonical pathway. NLRP3 inflammasome can be activated indirectly via a noncanonical pathway with the enrolment of caspase-11 in mice or the human analogs caspase-4/5. This noncanonical NLRP3 inflammasome pathway involves the direct senses and binding between these caspases and cytoplasmic LPS through TLR4, that will eventually

result in oligomerization and activation of NLRP3 inflammasome followed by the secretion of IL-1β/IL-18 and pyroptosis (31, 32).

Several studies have found that the NLRP3 inflammasome responds are key in controlling bacterial pathogens (33). Recently further studies have suggested that the NLRP3 inflammasome also plays an important role in the host's response to protozoan infection (34) This review focuses on current advances in research on NLRP3 inflammasome activation and its inflammatory response during different parasitic infections. In addition, it examines the immune evasion mechanisms of parasitic molecules that target the NLRP3 inflammasome response. We also outline novel approaches targeting NLRP3 signaling that could be developed as therapeutic alternatives to current anticancer treatment.

1.2 NLRP3 actions during parasitic infection

1.2.1 *Plasmodium*

Malaria is one of the most common infectious diseases caused by *Plasmodium* species and leads to worldwide human morbidity and mortality (35). According to the World Health Organization (WHO), in 2020, an estimated 241 million new cases of malaria were recorded worldwide, resulting in half a million deaths (36). Malaria infections can be asymptomatic, have only mild symptoms, or be fatal, depending on factors such as parasite virulence and host genetics (37). Malaria symptoms are characterized by periodic paroxysms, severe anaemia and headaches, and can lead to metabolic, renal, and cerebral complications that can be fatal in untreated individuals (20).

Plasmodium is a eukaryotic organism capable of morphological alterations during its complex life cycle which includes both sexual and asexual stages within two different hosts (35). The *Plasmodium* species that infect humans are *P. falciparum, P. vivax, P. malariae, P. ovale, and P. knowlesi,* with *P. falciparum* being the most dangerous to humans (38, 39). *Plasmodium* is transmitted by a female *Anopheles* mosquito when it feeds on the host's blood. Once in the host, the parasite enters the blood stage of its development, which in humans is the stage that causes the pathology of malaria (23, 40). A strong immune response is therefore necessary to control the early infection and reduce disease severity (41).

The parasite produces several immunomodulatory molecules, such as glycosylphosphatidylinositols anchor (GPIs), hemozoin (Hz), and immunostimulatory DNA, that trigger strong innate immune mechanisms, including the production of phagocytic cells, NK cells and the expression of inflammasome-related genes such as MyD88, caspase-1, ASC, P2X7R, and NLRP3 (42, 43). The innate immune response to malaria infection is crucial to the development of the adaptive immunity needed to regulate parasite pathogenesis (44). This adaptive immunity includes promoting Th1 responses to produce pro-inflammatory cytokines, such as IL-1β, IL-18, IL-12, tumour necrosis factor (TNF-α), and interferon (IFN)-γ to effectively clear the infection. However, under some conditions, the immune system may fail, resulting in a proinflammatory storm of cytokines such as IL-1β, IL-18, TNF-

α and IFN-γ that associated with increased disease severity and poorer clinical outcomes (45, 46). However, many details of the immune response against intracellular parasites, including Malaria, are not fully understood.

NLRP3 inflammasome is a critical part of innate immune response and its activation is an important antimalarial mechanism. During infection NLRP3 inflammasome can be activated by erythrocyte *Plasmodium* molecules (47, 48). However, it is still unknown whether the inflammasome activation has a beneficial or harmful impact on host immunity and mortality during lethal malaria infection. Therefore, several studies have been conducted to understand the interaction of NLRP3 inflammasome with *Plasmodium* during infection. The parasite feeds on the haemoglobin of red blood cells and generates a metabolic waste called hemozoin (Hz). A study using IL-1β deficient mice showed that Hz can induce IL-1β production via NLRP3 activation. The underlying signalling mechanism by which Hz triggers NLRP3 pathway activation and IL-1β production involves the Src kinase Lyn and the tyrosine kinase Syk **(Table 1)** (49) Moreover, Hz-dependent activation of NLRP3 can be enhanced by uric acid released during malaria infection and suppressed by allopurinol (an inhibitor of uric acid synthesis) (50) **(Figure1).** Velagapudi et al. found that incubating BV-2 microglia with HZ increases NLRP3 expression and caspase-1 activity (51) . Accordingly, these findings indicate that the ability of plasmodium product HZ to induce inflammasome action.

NLRP3 activation may also induce neuroinflammation during cerebral malaria (CM), a type of malaria with high mortality and affecting approximately 3 million individuals each year (52). High concentrations of proinflammatory cytokines and chemokines, such as TNF-α, IL-6, IL1β, IFN-γ, and CXCL10, are often correlated with the progression of CM (53). One study found that decreasing NLRP3 activation by injecting mice with IL-33 cytokines in combination with antimalarial drugs, significantly reduced the progression of CM. Consistent with this, inhibiting the NLRP3 inflammasome directly by MCC950 phenocopied inhibitor, promotes the protective role of IL33 towards CM (54). This suggests that the level of NLRP3 activation during *Plasmodium* infection influences CM progression, and that targeting the NLRP3 inflammasome to reduce its activation could be an excellent pharmacological strategy for treating CM.

It has been mentioned that malarial pigment Hz can active the NLRP3 inflammasome, however, this activation has negative influence in conventional CD8α+ type 1 dendritic cell (cDC1) abundance, phagocytosis and T cell activation *in vivo* (55). Eventually, this will advantage the parasite by reducing the effectiveness of the anti-*Plasmodium* humoral response. Hz has previously been found to carry plasmodial DNA into a subcellular compartment reachable by Toll-like receptor 9 (TLR9), resulting in inflammatory signals. An *in vitro* study applying synthetic Hz coated with plasmodial genomic DNA (gDNA), or CpG-oligonucleotides, found that DNA-complexed Hz prompted TLR9 translocation resulting in activation of the NLRP3 and AIM2 inflammasomes. These findings suggest that Hz and DNA collaborate to induce systemic inflammation during malaria (56) **(Figure2)**.

Many studies aim to comprehend the effects of NLRP3 deficiency during malaria. For instants, a study in NLRP3-deficient mice infected with lethal *Plasmodium yoelii YM*, found increased IFN-I cytokine production and a high survival rate in parallel with reduced IL-1β production. Other findings show that NLRP3 and IL-1β knockout mice do not experience increased body temperature during the acute phase of *P. chabaudi Adami* infection, and only exhibit mild symptoms (49). Mice deficient in inflammasome sensors AIM2, NLRP3, or adaptor caspase-1, and infected with *Plasmodium yoelii YM,* show increased production of IFN-I cytokines and IL-1β production, and increases IFN-I production. Since inflammasome activation involves the induction of IL-1β-mediated MyD88-TRAF3-IRF3 signalling and upregulation of suppressor of cytokine signalling 1 (SOCS1). A study found that inhibition of MyD88-IRF7-mediated-IFN-I signalling by SOCS1 reduces the cytokine production in plasmacytoid dendritic cells. In addition, the lack of inflammasome components decreases SOCS1 stimulation, causing inhibition of MyD88-IRF7-dependent-IFN-I signalling, resulting in increased IFN-α/β secretion and host survival. These effects indicate some of the negative aspects of inflammasome activation in the regulation of IFN-I pathways (57). However, IFN-I pathways show conflicting roles during *Plasmodium* infections, due to the organism's complex life cycle **(Figure2)**.

NLRP3 has been targeted in many malaria vaccines, such as QS-21, a soluble saponin adjuvant that induces IL-1β/IL-18 production and promotes Th1 responses in macrophages and dendritic cells (58). Developing vaccination candidates against malaria that target the NLRP3 pathway, may lead to better infection control. However, examining the role of NLRP3 activation in vaccine development for malaria is beyond the scope of this review.

It may conclude that plasmodium molecules such as Hz and (gDNA), and CpG-oligonucleotides have an immunostimulatory effect in NLRP3 inflammasome. While there is no confirmed direct interaction between GPI anchors and NLRP3, a study indicated that GPIs can activate TLRs to produce proinflammatory cytokines such as IL-1β (59). Moreover, activation of NLRP3 *Plasmodium* infections can worsen CM progression because of its suppressant action on IL-33 production. In addition, activation of NLRP3 can benefit the parasite by reducing both T cell activation and the humoral response, resulting in worsening infection and poorer prognoses. In contrast, lower NLRP3 activation increases the production of IFN-I cytokines and reduces disease symptoms. These results imply that the inhibition of inflammasome activation could be a valuable target in the development of effective malaria treatments.

1.2.2 *Leishmania*

Leishmania is an intracellular parasite that can cause leishmaniasis, a tropical and subtropical infectious disease. *Leishmania* is transmitted to humans by the bite of sandflies such as *Phlebotomus* and *Lutzomyia* (60). Leishmaniasis has been characterized by WHO as one of the seven most important tropical diseases and is prevalent in North East Africa, Southern Europe, the Middle East, South eastern Mexico, and Central and South America. It is a complex disease, with significant clinical

and epidemiological diversity and gives rise to a broad spectrum of symptoms and in some cases can lead to death (61). Worldwide, 1.5 to 2 million new cases occur each year, resulting in 70,000 deaths.

Leishmania progresses through two main developmental stages each with its own morphology: promastigotes and amastigotes. Promastigotes are able to move within the gut of the sand fly, while amastigotes live intracellularly in mammalian cells such as macrophages. More than 20 different *Leishmania* species are known to cause disease in humans like *L. major, L. mexicana, L. amazonensis, and L. brasilliensis* are the cause of cutaneous infections of the skin. However, the most severe and sometimes fatal disease is caused by *L. donovani and L. infantum.* These species infect the host systematically, resulting in visceral leishmaniasis which accounts for a total of 70,000 deaths (62). Clinical manifestations are influenced by the species of *Leishmania* and the immune response of the host, and range from localized cutaneous infections to the potentially lethal visceral form (63).

After transmission of Leishmania parasites by sandflies, clinical manifestation of the infection requires mechanisms that allow the parasites to proliferate in the mammalian host and attack, resulting in initiating the innate and adaptive antileishmanial defence*. Leishmania* parasites' ability to challenge the host's immune response and eventually establish a chronic infection, makes the disease extremely difficult to treat. The rapid clearing of pathogens, and further shaping of the adaptive immune response, is vital for controlling infection and improving disease outcomes (64). Both innate and adaptive immunity are therefore essential for the host's defence against *Leishmania*. The innate response is initiated by a complex interaction between parasitic molecules, such as lipophosphoglycan (LPG), glycoprotein-63 (GP63), and glycosylphosphatidylinositol (GPI), and the receptors of the antigen-presenting cells (APCs) (65). This interaction represents a type I immune response, and involves the production of IL-12 followed by IFN-γ-secreting. This leads to the initiation of the macrophages' microbicidal mechanisms (66). Adaptive immunity is essential for improving disease outcomes and to fully eliminate the infection and create long-lasting response memories against re-infection by *Leishmania* (37). Several proinflammatory cytokines are secreted during the adaptive phase, such as TNF-α, IFN-γ, IL-1β, IL-12, and IL-18 which together form an inflammatory response regulating parasite growth and infection outcome (67).

Recent advances in research have indicated crucial role for NLRP3 inflammasomes during Leishmaniasis.NLRP3 inflammasomes exert strong control over IL-1β and IL-18 production, and these cytokines are considered key mediators during *Leishmania* infections both *in vitro* and *in vivo* (68, 69). One study infected mouse macrophage with different *Leishmania* species, such as *L. amazonensis, L. braziliensis,* and *L. Mexicana and* found induction of caspase-1 activation and IL-1β production was dependent on the NLRP3 inflammasome. Furthermore, NLRP3 knockout mice were found to be extremely susceptible to *L. amazonensis* infection in comparison to WT control mice, indicating the protective role of NLRP3 inflammasome activation. This protective role involves IL-1β production, and therefore NO secretion, which contributes to the *Leishmania* killing mechanism (70). In contrast, infection of C57BL/6 mice with the *L. major Seidman strain* (*LmSd*) (isolated from a patient with chronic lesions), results in unhealed lesions, uncontrolled parasite growth and full destruction of the ear

dermis. This is accompanied by IL-1β production within dermal cells and remarkable neutrophil recruitment to the infected skin. Similarly, the severity of lesions in tegumentary leishmaniasis (TL) patients has been associated with increased expression of AIM2, which is part of the NLRP3 inflammasome (71, 72). However, mice deficient in NLRP3, ASC, and caspase-1/11, or lacking IL-1β or IL-1 receptors, have better lesions repair and parasitic elimination, due to the absence of IL-1β - which affects neutrophils' local enrolment and therefore suppresses inflammation (72). The production of IL-1β dependent on NLRP3 inflammasome activation may be limits neutrophil recruitment, and causes non-healing forms of cutaneous leishmaniasis in commonly resistant mice. In contrast, a study found that infecting susceptible BALB/c mice with *L. major* induced severe footpad swelling and parasite burden, whereas NLRP3−/− BALB/c mice showed considerably reduced footpad swelling and the parasite burden. This suggests NLRP3 activation has a negative impact on BALB/c mice during infections with *L. major.* The authors propose that IL-18 might promote *L. major* survival by suppressing Th1 cell responses (73).

In another study aiming to understand the role of the NLRP3 inflammasome in Th1/Th2 responses during leishmaniasis, knockout BALB/c mice for NLRP3, ASC, or caspase-1, displayed deficient IL-1β and IL-18 production and were resistant to cutaneous *L. major* infection. This study also indictes that the production of IL-18 enhances disease susceptibility in BALB/c mice by stimulating anti-inflammatory cytokine production. Neutralization of IL-18 in these animals lowered the *L. major* burden and reduced footpad swelling (74). These studies all suggest that IL-18 neutralization could be a potential pharmacological approach in the treatment of leishmaniasis patients.

Several studies have been conducted to increase our understanding of the underlying mechanisms of NLRP3 activation during leishmaniasis. For instant, Inflammasome activation during the onset stages of *L. amazonensis* infection in macrophages, seems to require ROS production through the NADPH oxidase mechanism, and the engagement of Dectin-1 and a C-type lectin receptor via spleen tyrosine kinase (Syk) signals. Therefore, inflammasome activation in response to *L. amazonensis* is decreased by the deficiency of NADPH oxidase, Syk, focal adhesion kinase, and proline-rich tyrosine kinase 2, as well as by the absence of Dectin-1. Further experiments confirmed this using Dectin-1 knockout mice, where Dectin-1 inflammasome activation was found to be important in controlling the parasite burden in macrophages, and improving resistance to *L. amazonensis* infection *in vivo* (75). An alternative pathway has been suggested to participate in the NLRP3 inflammasome activation that helps control *L. amazonensis* infection. This pathway is facilitated by the P2X7 receptor and LTB4 and depends on the production of IL-1β via non-canonical NLRP3 inflammasome activation (76). This is supported by the finding that inflammasome genes like IL-1β, NLRP3, and P2RX7, are upregulated in localized cutaneous leishmaniasis (LCL) patients (77). Furthermore, Carvalho et al. found that the parasite membrane glycoconjugate lipophosphoglycan (LPG) triggers the NLRP3 inflammasome pathway via caspase-11 activation in macrophages and *in vivo* (78). These studies propose possible pathways for the activation of the NLRP3 inflammasome during *Leishmania* infection and improve our understanding of the immunological role of NLRP3 activation in the host's immune response.

Understanding these mechanisms is important for the development of new therapeutic strategies to limit leishmaniasis progression **(Figure2)**.

As discussed, different species of *Leishmania* can suppress the production of IL-1β both *in vitro* and *in vivo.* In this context, Shio et al., found that *L. mexicana* reduces IL-1β macrophage production through its virulence factor GP63 (the metalloprotease expressed by all *Leishmania* species). Also, the reduction of IL-1β production has been associated with the inhibition of reactive oxygen species (ROS) secretion, which has been linked to NLRP3 inflammasome activation. This ROS suppression is thought to result from damaged PKC-mediated protein phosphorylation. This finding indicates that the *Leishmania* surface GP63 molecule can significantly suppress NLRP3 inflammasome activation, resulting in a reduction of IL-1β production (79). *Leishmania*, therefore, employs a unique protective mechanism to manipulate the host's immune response. A subsequent study found that BALB/c mice infected with *L. donovani* produced IL-1β when given the antileishmanial drug Amp B. In contrast, administering the anti-IL-1β antibody to infected Amp B-treated mice increased the parasitic burden. This suggests that *Leishmania* is able to inhibit NLRP3 inflammasome activities, which in turn suppresses caspase-1 activation, and therefore IL-1β maturation, which is accompanying with reduction in NF-κB activity (80). This study also used gene silencing of A20 (a negative regulator of NF-κB signaling) or UCP2 (mitochondrial uncoupling protein 2) in macrophages infected with *Leishmania* and concluded that *Leishmania* utilizes A20 and UCP2 to prevent inflammasome activation, resulting in their multiplication (80). Furthermore, the *Leishmania* RNA virus (LRV) is a key virulence factor related to the progression of mucocutaneous leishmaniasis, a severe form of the disease (47). A study that combined data from humans and animals revealed that LRV stimulates TLR3 and TRIF to trigger type I IFN production, resulting in autophagy. This leads to Autophagy related 5 (ATG5) expressions which mediated the breakdown of NLRP3 and ASC, thus reducing NLRP3 inflammasome activation in macrophages (48). Also, it is suggested that LRV inhibits caspase-11 activation and IL-1β production dependent on both TLR3 and ATG5. Therefore, this signalling pathway utilized by LRV is in the parasite's favour by increasing its survival and pathogenicity (81). It is clear that *Leishmania* develops several mechanisms to escape the host's immune response by targeting NLRP3 inflammasome activation resulting in suppression of inflammatory response **(Figure3)**.

In summary, while the knockout mice studies indicate that NLRP3 activation during leishmaniasis is important for infection control, several studies have shown that the lack of NLRP3 also leads to a reduction in infection severity and mortality. It seems, therefore, that multiple factors, such as the parasite species and susceptibility of the host to infection, influence NLRP3 activation and lead to its dual action during *Leishmania* infection. Antileishmanial therapeutics will need greater research into the molecular pathophysiology of NLRP3 inflammasome activation in response to viral leishmaniasis.

1.2.3 *Toxoplasma gondii*

Toxoplasma gondii (*T. gondii*) is an intracellular parasitic organism able to infect all warm-blooded animals, including humans (where it infects about one-third of the global population) (82). Most

immune-competent individuals infected with *T. gondii* are asymptomatic or experience only mild and self-limiting illness (83). However, extremely virulent strains of *T. gondii* can result in ocular disease in immune-competent adults (82). Immunocompromised individuals may develop severe complications associated with *T. gondii* infection (83). Infection during pregnancy with *T. gondii* is particularly serious as congenital toxoplasmosis may develop, resulting in abortion or neonatal mortality (84).

The immune response to *T. gondii* infection is complex due to the high level of heterogeneity in the genetic backgrounds of hosts, and the diverse virulence of parasite strains (85). Immune responses during infection involve early production of pro-inflammatory cytokines, such as IL-12, to induce the production of IFN-γ by natural killer (NK) cells, CD4+ T cells, as well as CD8+ T cells [4]. IL-12 and IFN-γ are crucial in facilitating parasite death and controlling its growth (86).The adaptive immunity against *T. gondii* infection involves maintaining a balance between the cell-mediated and humoral immune response actions of Th1 and Th2 cells. The Th1 response provides a strong protective role, characterised by activation of dendritic cells (DC) to produce IL-12 (87). Th1 cells also produce IFN-γ as well as TNF-α cytokines, which stimulate the macrophages' killing mechanisms against intraocular parasites (88).

Initiating the innate immune response is essential for controlling *T. gondii* infection. Limiting parasite proliferation appears to involve a defensive inflammasome-mediated response (89). *In vitro*, infecting murine bone marrow-derived macrophages with *T. gondii* activates the NLRP3 inflammasome, causing an increase in IL-1β production. Furthermore, infecting knockout mice for NLRP3, caspase-1/11, IL-1R or the adaptor protein ASC, causes a reduction in IL-18 secretion and an increase in the parasitic burden that eventually leads to host death (61). The activation of NLRP3 in a human fetal small intestinal epithelial infected with *T. gondii*, was mediated by P2X7R and resulted in IL-1 β production, and therefore inhibited *T. gondii* proliferation (90). Infecting macrophages with *T. gondii* have been found to activate P2X7R and limit parasite proliferation. This P2X7R activation pathway involves the initiation of NADPH-oxidase-dependent ROS production, and activating an inflammasome, resulting in increased IL-1β secretion and ROS generation (91). Furthermore, a study showed that NLRP3 was an inflammasome sensor activated during *T. gondii* infection in primary human peripheral blood cells, and its activation is mediated by the release of intracellular potassium (92). It is suggested that *T. gondii* activates the NLRP3 inflammasome in primary human peripheral blood monocytes via the Syk-CARD9/MALT-1-NF-κB signalling pathway, resulting in IL-1β production (93).

Several studies explore the potential parasitic components that can impact NLRP3 activation during *T. gondii* infection. For instance, the soluble total Ag (STAg) derived from *T. gondii* strain RH, has been shown to stimulate NLRP3 activation and thereby increase IL-1β secretion *in vitro* (94). A recent study revealed that the *T. gondii* secretory protein, rhoptry protein 7 (ROP7), can bond with the NACHT domain of NLRP3 in differentiated THP-1 cells, causing significant up-regulation in NF-κB expression and therefore inflammasome hyper activation via the IL-1β/NF-κB/NLRP3 pathway (64). More recent study on THP-1 cell line treated with Profilin from *T. gondii* (TgP) reported that an increase in NLRP3

expression resulting in IL-1β production (95). In contrast, Kim et al., found that Dense granule proteins 9 (GRA9), a secretory protein produced by *T. gondii,* is involved in disrupting the formation of the NLRP3 inflammasome. The protein blocks the binding of apoptotic speck-containing (ASC)-NLRP3, and suppresses the effect of NLRP3 (96). In contrast, different *T. gondii* effector proteins, like GRA15, promote the NLRP3 inflammasome activation, resulting in IL-1β and IFN-γ production in THP-1 cells. This induces iNOS expression and NO secretion, causing the inhibition of IDO1 expression and therefore increased *T. gondii* growth in hepatocytes (97). Additional effector proteins, such as GRA35, GRA42, and GRA43, also have a key role in *T. gondii* infection through pyroptosis stimulation and IL-1β production in Lewis's rat BMDMs. However, whether such effector proteins have direct interactions with NLRP3 has not been proven (98) **(Figure 3)**.

Regarding the involvement of inflammasome during the chronic stage of toxoplasmosis. Studies on the immune response to *T. gondii* at the chronic infection stage have found a vacuolar antigen of the parasite present in the host's macrophages. This suggests that proliferation of the parasite is controlled through a unique pathway involving NLRP3 induction of CD8 T cell IFN-γ responses [123].

The research discussed here indicates that *T. gondii* products are able to activate the NLRP3 inflammasome, which then produces IL-1β to control the infection. In contrast, the absence of it or any of its components, results in increased parasitic growth and mortality, implying that NLRP3 activation during toxoplasmosis serves a protective function. This review also enhances our understanding of the NLRP3 activation mechanism during *T. gondii* infection, data of value for the development of drugs to improve infection outcomes. However, further studies are required to understand the role of other parasitic products in the NLRP inflammasome's activation.

1.2.4 Entamoeba histolytic

Amoebiasis is a parasitic disease that infects the large intestine of humans caused by an extracellular parasitic protozoan, *Entamoeba histolytica (E. histolytica)* (99). According to the WHO, 500 million people worldwide are infected with Entamoeba; only 10% of these individuals are infected with *E. histolytica,* while the remaining are infected with non-pathogenic species like *Entamoeba dispar* and *Entamoeba coli*. Annually, amoebiasis can result in 40,000–100,000 deaths, which makes it the fourth protozoan infection causing death (100). In general, the transmission route of *E. histolytica* to a host is by ingesting contaminated water or food due to faecal excretion of cysts or person-to-person contact (101). *E. histolytica* is a virulent pathogen that is able to secrete molecules to break down and kill the host tissues and cells, in addition to engulfing red blood cells (99). It infects the intestinal tract of humans, causing amoebiasis, which is clinically asymptomatic; however, an invasive host's intestinal may result in the disease manifesting including abdominal pain, watery or bloody diarrhoea and weight loss (102). In some cases, amoebas can breach the mucosal barrier of the intestine and travel to other organs, like the liver, lung, and, in some cases the brain, resulting in amoebic abscesses (103). *E. histolytica* is predominantly found in the large intestine without initiating symptoms; however, in

unknown conditions, the amoebae attack the mucosa and epithelium, causing intestinal amoebiasis, causing tissue lesions that progress to abscesses and a host acute inflammatory response (104).

Establishing an amoebic infection includes a critical balance between the parasite pathogenicity and immune response. Amoebas live in the outer mucus layer of the intestinal tract, where they can feed on gut bacteria. However, the reasons by which amoebas attack the host tissues are not completely known. After amoebas invade the tissues, the immune system triggers a response against the parasite (105). Nevertheless, the key immune mechanisms against amoebas are still poorly understood (106). Several studies with *E. histolytica* showed that trophozoites bind to TLR-2 and TLR-4 in human colonic cells through the carbohydrate recognition domain of the Galactose/ N-acetylgalactosamine (Gal/GalNac) lectin and the lipopeptidophophoglycan (LPPG) located in the parasite surface. By acting as pathogen-associated molecular patterns (PAMPs), these amebic molecules trigger the classical TLR signalling pathway, prompting NFkB activation and increased expression of TLRs followed by inflammatory cytokines production (107). That includes IL1β, IL-6, IL-8, IL-12, IFN-γ, and TNF-α, which further regulate the functions of the host immune response (108). Furthermore, the secretion of *E. histolytica* macrophage migration inhibitory factor (MIF) (EhMIF) is vital for initiating the intestinal inflammation during amoebic invasion (109). Macrophages are as well play a vital role in defence against amoebiasis via their production of a variety of inflammatory cytokines, such as IL-1β, IL-6, and IL-12 as well as NO, resulting in *E. histolytica* prolifration reduction (110-112). As part of the innate response during amoebic infection Prostaglandin E2 (PGE2) of *E. histolytica*, induces the secretion of IL-8, a potent neutrophil chemoattractant (113). An additional pro-inflammatory cytokine produced during amoebic infections is TNF-α, and its production is associated with *E. histolytica*-induced diarrhoea in children as well as tissue damage in the amoebic liver abscess in mouse models (114, 115). Therefore, it can be suggested that amoeba-induced inflammatory response results in tissue injury that can favour amoeba invasion.

Adaptive immunity also plays a significant role in the host defence against *E. histolytica.* A study in C3H mice infected with *E. histolytica* found that the diminution of CD4+ cells significantly reduced both parasite growth and inflammation, which also correlated with a decline in IL-4 and IL-13 production (116). Thus, this study indicates that the importance of CD4+ T cells in mediating inflammation also contributes to the disease progress. Moreover, the type of cytokines produced from T cells might impact the disease outcome; for example, IFN-γ as a pro-inflammatory has a protection role during amebiasis via initiating the killing mechanisms of neutrophils and macrophages to control amoebicidal activity (46, 74, 89, 173). In contrast, IL-4 is an anti-inflammatory involved in the acute phase and during amoeba's invasion (115, 117, 118). IL-10 is an additional cytokine with a central protective role during intestinal amoebiasis by triggering resistance to intestinal amoebiasis in B6 mice (119). Furthermore, CD8+ cytotoxic T cells can cause death to amoebas either directly or through the production of IL-17 (120). However, Treg cells have been identified in a model of amoeba infection, and their role is characterized by participating in the control and resolution of the inflammatory response to *E. histolytica* infection (169). Together, these studies suggest that cell-mediated immune responses have a significant contribution against *E. histolytica* infections. Therefore, the immune

system-activated inflammation seems a double-edged sword: it can defend the host from *E. histolytica* invasive infection or stimulate severe tissue damage, facilitating *E. histolytica* distribution.

It was found that the NLRP3 inflammasome activation played a significant role during *E. histolytica* infection leading to IL-1β/IL-18 production and parasitic clearance from tissue (121). Noticeable, NLRP3 inflammasome activation by *E. histolytica* does not trigger pyroptosis, which is a normal strategy of the host to remove intracellular parasites, as an alternative inflammasome can facilitate cell death leading to delay in the suppression of parasitic invasive, which can be unfavourable to innate host defences (122, 123). Following *E. histolytica* invasion into the lamina propria, macrophages immigrate to the site of infection and orchestrate robust inflammatory responses. This response includes priming and activating the NLRP3 inflammasome component genes, leading to the production of IL-1β/IL-18. It was found that Gal/GalNAc lectin can activate NF-κB and MAP kinase-signaling pathways in macrophages, resulting in upregulation of the transcription of proinflammatory cytokines in addition to NLRP3 inflammasome components and pro-IL-1β (124). A study in macrophages found that Gal/GalNAc is equivalent to LPS in upregulating the pro-IL-1β and NLRP3 expression and achieves the priming requirements for NLRP3 activation, which all need NF-κB activation. Thus, this study suggested that Gal/GalNAc as soluble ligands may trigger TLRs in macrophages (122) **(Figure 2)**.

Exceptionally, *E. histolytica*-prompted NLRP3 inflammasome activation includes direct interaction of intact live *E. histolytica* by Gal/GalNAc lectin-mediated binding (122). As a result of NLRP3 activation, the recruitment and activation of caspase-1 will occur, causing the cleaving of the precursor IL-1β/IL-18 into their bioactive form. Several studies also found that the release and processing of IL-1β in response to *E. histolytica* is caspase-1 dependent, since inhibition of caspase-1 using specific inhibitors reduce the release of these pro-inflammatory cytokines (125). Furthermore, the stimulating molecular mechanism of inflammasome and caspase-1 activation involves the formation of an intracellular bridge between cysteine proteinases containing an arginine-glycine-aspartate (RGD) binding with macrophages $\alpha 5\beta 1$ integrin (121). In supporting this, a study showed that when the Gal/GalNAc lectin interactions with macrophages, both $\alpha 5\beta 1$ integrin and NLRP3 are enrolled into an intracellular junction, enabling EhCP-A5 RGD domain to directly cooperate with $\alpha 5\beta 1$ integrin. Subsequently, this activation will trigger Src family kinase phosphorylation and opening of pannexin-1 (Panx1) channel to facilitate the rapid extracellular release of ATP. The free ATPs then return to macrophages via P2X7 receptors to initiate the second signal for NLRP3 inflammasome complex formation (126). Therefore, *E. histolytica* is also able to induce NLRP3 activation in a two-signal event; the first signal involves the direct *E. histolytica* interaction that is facilitated by the Gal/GalNAc lectin forms an immune cell synapse to induce EhCP-A5 RGD linking with $\alpha 5\beta 1$ integrin. The second signal occurs due to the extracellular ATP release acting in an autocrine manner via host P2X7 receptors to stimulate downstream signal transduction events. A study proposed that an EhCP-A5 RGD, expressed on the trophozoite surface and as secreted molecules, is essential for contact-dependent inflammasome activation (121). Notably, *E. histolytica* can induce inflammasome activation via the

efflux of potassium since the blocking of K+ channel activity causes IL-1β inhibition (127). Also, Nlrp3−/− and Asc−/− mice showed reduced colonic production of IL-1β in response to live *E. histolytica* (121). Li et al., also found that trophozoites abundantly secrete peroxiredoxins (Prx) during host cell invasion, and Prx C-terminal is considered as the main functional domain that can trigger NLRP3 in macrophage, and its activation pathway involves the binding of Prx with either TLR4 or P2X7 receptors (128). These findings suggest that *E. histolytica* can trigger the activation of NLPP3 inflammasomes in a priming and activating fashion, resulting in an inflammatory response **(Figure 2)**.

However, a study suggested that the lipid mediator prostaglandin E2 (PGE2) could modulate inflammatory response by inhibiting transcription of different pro-inflammatory genes such as IL-1β, TNF-α and IL-8, therefore suppressing NLRP3 inflammasome (121). Mechanically, following PGE2 signal transduction coupling with the EP4 receptor, adenylyl cyclase will be activated, resulting in up-regulated intracellular concentration of cyclic adenylyl monophosphate (cAMP) (129). Then, the Protein Kinase A (PKA), the key mediator of cAMP signalling kinase, directly phosphorylates the Ser295 position of NLRP3 and turns off its ATPase activities (121). Therefore, the self-oligomerization of NLRP3 and the inflammasome complex assembly are inhibited (130) **(Figure 3)**. Thus, the Production of PGE2 plays a crucial role in disease pathogenesis and immune evasion during *E. histolytica* via inhibition of inflammasome activation. However, the mechanism of how this inhibition occurs and whether this is beneficial for *E. histolytica* are not yet clear.

Collectively, it has become well understood that as an extracellular parasite, *E. histolytica* can activate the NLRP3 inflammasome complex via direct interaction with the live parasite and macrophages. However, intercellular interaction also can include the activation of NLRP3 via Gal/GalNAc mediated adherence with macrophages, facilitating an intercellular bridge between EhCP-A5 and $\alpha5\beta1$ integrin, resulting in the rapid extracellular release of ATP that will eventually activate the NLRP3 inflammasome. Although PGE2 suppresses the activation of NLPR3, the actual immunological impact of this inhibition on either the host or parasite is still vague. It is clear that NLRP3 activation is one of the vital innate events during *E. histolytica,* resulting in an inflammatory response that will control the infection's progression. However, compared to another parasitic infection discussed here, it is noticeable that most studies regarding the NLRP3 inflammasome-*E.histolytica* interaction were *in vitro* studies. Thus, supplementary *in vivo* studies are required to evaluate the interaction between the parasite and the host's immune response within the complex network of biological influence and cross-regulatory pathways during amoebiasis. Like the influence of *E. histolytica* bioenvironmental, in which colonic cells are typically exposed to pathogenic and commensal organisms within the colon. Also, it is crucial to understand the overall impact of inflammasome activation or inhibition on either the host response or infection progression. Therefore, it can be proposed that the NLRP3 inflammasome action needs further investigation by applying additional *in vivo* experiments, including NLRP3 knockout animal models. Knowing the immunological functions of NLRP3 during amoebiasis will benefit in preventing the disease pathogenesis of *E. histolytica* infection.

1.2.5 *Trypanosome cruzi*

Trypanosome cruzi is the parasite that causes Chagas disease, a potentially fatal infection that can affect the heart and gastrointestinal tract (131). The WHO estimates that Chagas disease is the most important parasitic disease in the Americas, accounting for five times as many infections as malaria. In 2015, WHO estimated that 7 million people were infected, the majority living in Latin America, with 25 million at high risk of contracting the chronic form of the disease (132). The spread of Chagas disease beyond the geographical areas it was once confined to, has transformed it into a global healthcare issue (133). *T. cruzi* is normally found in the guts of hematophagous triatomine bugs, and transmission occurs when infected bug faeces contaminate the bite site or mucous membranes of the host. *T. cruzi* can also be transmitted by transfusion, tissue transplants, and congenitally (134, 135). *T. cruzi* strains are classified into seven different type units (DTUs), TcI toTcVI and TcBat, whose virulence, and pathogenicity in the vertebrate host, differ greatly (136). Most patients are asymptomatic or have mild or nonspecific symptoms such as fever (135). However, 1% of patients develop severe acute disease (Chagas disease), with potentially fatal symptoms that include acute myocarditis, pericardial effusion, and meningoencephalitis (134, 137).

To establish chronic infection, *T. cruzi* triggers a complex response in the host immune system (138). Experimental models have shown that *T. cruzi* surface glycoproteins (mucins) and/or glycophospholipids (GIPLs), can activate the innate immune cells to produce IFN-γ, TNF-α, IL-1β, and IL-6. These cytokines stimulate the production of NO and superoxide by macrophages which cause parasite death [135]. When mice are infected with *T. cruzi*, IFN-γ and IL-12 trigger protective adaptive immunity, including the parasite-specific Th1 response (139). Generally, it is found that the resistance against acute experimental *T. cruzi* infection involves the activation of several innate immune receptors, such as Toll-like and Nod-like receptors, and the NLRP3 inflammasome (140).

As previously established, NLRP3 is an essential immunological component during *T. cruzi* infection. A study in mice evaluated the influence of *T. cruzi* virulence (low, medium, high) on the expression of several innate immune mediators, including NLRP3, and concluded that highly virulent *T. cruzi* strains upregulate the expression of NLRP3, caspase-1, IL-1β and iNOS mRNA in heart muscle more than strains with low or medium virulence. These effects may be responsible for the myocarditis and increased mortality associated with some *T. cruzi* infections (141). A study by Goncalves et al., demonstrated that *T. cruzi* infection triggers IL-1β production in an NLRP3- and caspase-1-dependent manner in peritoneal macrophages (PMs), and that cathepsin-B was required for the activation of NLRP3. Importantly, NLRP3-/- and caspase1-/- mice were found to host more *T. cruzi* parasites than MyD88-/-and iNOS-/-mice (which are susceptible models for *T. cruzi* infection), showing that the NLRP3 inflammasome contributes to acute infection control. In addition, when these NLRP3 and caspase-1 knockout mice were infected with *T. cruzi*, they decreased NO production and limited macrophage-mediated parasite killing (142). These data demonstrate how the activation of NLRP3, and subsequent NO production, functions as a unique effector-killing mechanism to control *T. cruzi* infections. As described earlier, in 1% of patients *T. cruzi* infection develops into Chagas disease,

which may result in life-threatening meningoencephalitis. Of particular relevance to this aspect of *T. cruzi* infection, one study found that NLRP3 is activated within the microglia. This activation results in IL-1β and NO secretion which contributes to the pathogenesis of *T. cruzi* infection within the CNS (143).

It appears that NLRP3 has a critical function in regulating infection, hence various research has been undertaken to fully understand its activity. One mechanism proposed for the activation of the ASC/NLRP3 pathway by *T. cruzi*, includes K^+ efflux, lysosomal acidification, ROS production and lysosomal impairment. One study also observed that ASC and caspase-1 knockout mice infected with *T. cruzi* had higher mortality and heart inflammation, suggesting that inflammasomes play a critical role in host resistance to the parasite (144). Another study utilised wild-type (WT), ASC -/-, and NLRP3 -/- macrophages, as well as human macrophages, and suggested that *T. cruzi* infection provokes delayed activation of inflammatory cytokine gene expression and IL-1β production in NLRP3 -/- macrophages. However, these macrophages showed significant reductions in intracellular parasite proliferation compared to WT controls. This study also found that caspase-1/ASC inflammasomes play a key role in the activation of IL-1β /ROS and NF-kB signalling of cytokine gene expression in human and mouse macrophages, which contributes to the control of *T. cruzi* infection (145). Research in human THP monocyte-derived macrophages found *T. cruzi* to strongly suppress TXNIP expression, an anti-oxidant inhibitor that facilitates caspase-1 activation upon recruitment to NLRP3 inflammasome (146). Furthermore, rapamycin-pretreated macrophages infected with *T. cruzi* have been found to show much greater NLRP3 and mitochondrial ROS (mtROS) expression compared to control cells. However, when mtROS production was inhibited in rapamycin-pretreated infected macrophages from NLRP3 KO mice, the parasitic replication significantly increased. Suggesting that mTOR suppression during *T. cruzi* infection triggers NLRP3 activation and mtROS production, causing macrophage inflammatory response that regulates *T. cruzi* proliferation (147). These data suggest that NLRP3 inflammasome activation can be induced by *T. cruzi* resulting in inhibition of mTOR production and consequent limiting of parasite replication. The activation of the inflammasome is a specific strategy that necessarily influences inflammatory outcomes.

It is clear that macrophages are one of the major cell types mediating the recognition and modulation of immune responses during *T. cruzi* infection. For example, *T. cruzi* can facilitate the macrophage galactose-C type lectin (MGL 1) receptor to initiate the innate immune response. Stimulating MGL1 knockout macrophages *in vitro* with *T. cruzi* antigen (TcAg) has been shown to reduce procaspase-1, caspase-1, and NLRP3 inflammasome expression. (148) **(Figure 3)**. This finding reveals a possible mechanism for the NLRP3 activation pathway in macrophages during the immune response to *T. cruzi.* IL-1β production by macrophages is crucial for T cell activation during *T. cruzi* infection. Paroli et al., investigated the role of NLRP3 and caspase-1/11 in the differentiation and activation of T cells during acute infection with a *T. cruzi-Tulahuen* strain (149). They found that during infection, NLRP3−/− and C57BL/6 WT mice showed similar parasitemia and survival rates, although the parasite burden was greater in the livers of NLRP3−/− mice than WT mice. Suggesting that NLRP3 is not

needed for regulating parasitemia, but is still crucial for improved parasite clearance from the liver. Importantly, they found that the differentiation of T helper and cytotoxic T lymphocyte phenotypes depended on whether the mice were deficient in NLRP3 or caspase-1/11. Notably, caspase-1/11−/− mice showed a significant decrease in the number of IFN-γ- and IL-17-producing CD4+ and CD8+ T cells, which are linked to higher parasite loads and lower survival (149). These results imply that NLRP3 pathway activation is vital for assembling an appropriate T cell response during *T. cruzi* infection.This finding reveals a possible mechanism for the NLRP3 activation pathway in macrophages during the immune response to *T. cruzi.* Autophagy is one of the effector mechanisms that limit *T. cruzi* infection. For example, a study showed that NLRP3 is needed to stimulate an autophagic flux during *T. cruzi* infection by mediating the autolysosome formation in peritoneal macrophages (PMs) from C57BL/6 WT mice, thereby limiting *T. cruzi* replication (148).

It is obvious that NLRP3 inflammasome activation is influenced by the strain of *T. cruzi* during the infection course. In addition, the NLRP3 knockout mice studies show that lacking this inflammasome significantly affects the macrophage-mediated parasite-killing mechanism, resulting in heart inflammation and higher mortality. NLRP3 deficiency also has an impact on the development of T cell responses during *T. cruzi* infection by reducing CD4+ and CD8+ T cell numbers, leading to higher parasite loads and lower survival. Inflammasome activation may contribute to inflammatory responses during *T. cruzi* infection, through its inhibitory effect on mTOR production, which reduces parasite growth. However, no studies have examined the potential role of parasitic surface proteins, such as Mucin and Trans-Sialidase, in either NLRP3 inflammasome activation or inhibition. NLRP3 activation may therefore have other functions during *T. cruzi* infection which remain to be discovered. Understanding these key immunological cellular pathways will help to develop drugs for controlling *T. cruzi* infection and limiting the immunopathology of Chagas disease.

1.2.6 *Helminths*

Helminths are complex, multicellular, parasitic worms occupying a wide range of geographical, ecological, and anatomical niches, and with highly complex life cycles. Helminths are categorised into three classes: nematodes (roundworms) , platyhelminths (flatworms, including trematodes and cestodes), and annelids (segmented worms, including leeches) (150). It is estimated that approximately 2 billion individuals are infected with the parasite, making it the most common human infection in developing countries (151). Moreover, helminths have several invasion routes, including the skin (schistosomes and hookworms) and mosquito bite (filarial worms) , but the most common is via the gastrointestinal tract (152). The disease in humans is normally caused by adult worms, egg deposition in tissues, or migration of larvae or microfilariae. Helminth infections are normally asymptomatic or mild, but immunologically naïve and immunosuppressed individuals can experience severe clinical outcomes (150).

Helminths can form long-term chronic infections during which the host immune response is severely suppressed (153). The remarkable distribution of helminth infections arises from their ability to

manipulate the host immune system by controlling its susceptibility, resistance, and pathogenesis (154). Although protective immunity to helminths in humans is not well understood, animal models of infection have indicated that human immunity is mediated by the Th2 response (154, 155). The latter seems to be targeted by the helminth immunoregulation mechanism as a means to establish a successful opportunistic parasite-host relation (152). Asymptomatic infection shows increased production of anti-inflammatory cytokines such as IL-10 and high levels of circulating T cells expressing the inhibitory marker CTLA-4 (cytotoxic T lymphocyte antigen 4) (156, 157). There is also inhibited production of Th1 inflammatory cytokines, such as IFN-γ (158). However, in severe and deteriorating cases, lymphatic pathology develops with fewer regulatory T cells and increased Th1 and Th17 effector responses - which might explain the severe lymphatic inflammation outcome (159). The relationship between these parasites and the host immune response is highly complex, and a full analysis is beyond the scope of this review.

Activation of the NLRP3 inflammasome plays a key role in helminth infections by provoking Th2 and Th17/inflammatory responses (160, 161). Potential stimuli for NLRP3 activation during infections are helminth products that are either soluble or exosomal, and endogenous signals from inflammation and injured tissue (161). One study found that soluble schistosomal egg antigens (SEA) can activate the NLRP3 inflammasome, resulting in IL-1β production in dendritic cells. SEA protein appears to function as a second signal for inducing proteolytic pro-IL-1β cleavage **(Figure 2)**. Moreover when mice deficient in the central inflammasome adapter ASC, but had NLRP3 molecules infected with *Schistosoma mansoni*, they showed a reduction in IL-1β expression and liver immunopathology (162). In contrast, infecting WT mice with *Schistosoma japonicum* (*S. japonicum*) resulted in high expression of IL-1β, and NLRP3 activation. In examining this activation mechanism, Meng et al. observed that hepatic mouse stellate cells (HSCs) cultured with soluble egg antigen, induced NLRP3 inflammasome formation, which was linked to both redox regulation and lysosomal dysfunction (163). This suggests that NLRP3 inflammasome activation plays a role in initiating the inflammatory action that leads to liver fibrosis associated with *S. japonicum* infection. Several earlier studies have shown that the inflammatory action of NLRP3 inflammasomes during schistosomiasis in the liver could be limited by taurine (a sulfur-containing β-amino acid) (164). In mice infected with *S. japonicum*, taurine was found to suppress activation of the hepatic thioredoxin-interacting protein (TXNIP)/NLRP3 inflammasome, thereby preventing IL-1β production and pyroptosis. The study also found that NLRP3-deficient mice infected with *S. japonicum*, developed hepatosplenomegaly, liver dysfunction, hepatic granulomas, and fibrosis, and showed reduced NLRP3-dependent liver pyroptosis. The authors suggest that taurine's ability to control the activation of the TXNIP/NLRP3 inflammasome pathway might make it an effective preventative of liver pathology during *S. japonicum* infection (165).

Many studies examine the immunological action of NLRP3 during trematode infection. It is found that the FhCL3 helminth-derived molecules of *Fasciola hepatica* can induce non-canonical inflammasome activation in dendritic cells (DCs), resulting in IL-1β and IL-18 production, and this has been associated with the cysteine protease activity of FhCL3 - an independent caspase pathway. The activation of the NLRP3 inflammasome by FhCL3, prompts the adaptive immune response and is characterized by the

secretion of IFN-γ and IL-13 (166). These data indicate that the helminth-derived molecule FhCL3, can activate the NLRP3 inflammasome in a caspase-independent manner. However, Alvarado et al. demonstrated that NLRP3 inflammasome activation can be inhibited by Helminth defence molecule-1 of *F. hepatica* (FhHDM-1) (a cathelicidin-like peptide), resulting in a reduction in IL-1β secretion by macrophages **(Figure 3)**. The inhibitory outcome was associated with lysosomal cathepsin B protease causing IL-1β production and effective Th1 response suppression, eventually parasite survival (167). Moreover, infected NLRP3−/− mice with *Trichinella spiralis*, have been shown to host more larvae than WT mice. In supporting the finding, administration of WT mice with MLES (muscle larvae excretory-secretory products) showed higher levels of IL-4, IL-10, TGF-β, and Tregs population, than NLRP3−/− mice receiving the same treatment. This was carried out *in vitro* by treating WT-DCs with MLES, and resulted in upregulation of CD40 expression and increased production of IL-4, IL-10, TGF-β, and Tregs populations. Conversely, treating NLRP3 knockout cells with MLES, caused downregulation of CD40 expression with increased production of IL-1β, IL-18, IL-10, and TGF-β, but not IL-12p70 (168). This study explained the vital role NLRP3 plays in developing the Th2 and Treg responses of the host defence against *Trichinella spiralis*.

Although NLRP3 inflammasome activation seems to be important to host defences against helminth infections by regulating Th2 and Th17 responses, it can also cause uncontrolled inflammatory action that leads to liver immunopathology. This is confirmed by the NLRP3 knockout studies, where mice lacking the NLRP3 molecule had better disease outcomes. For instance, NLRP3-deficient mice infected with *Schistosoma japonicum* had reduced NLRP3-dependent liver pyroptosis. The absence of NLRP3 could also be favourable to parasite growth, as was indicated in the *Trichinella spiralis* infection studies. That the NLRP3 inflammasome appears to play a dual role in host defences against helminth infection might be due to the complexity of the parasite's life cycle. Currently, there are too few studies to fully determine the role of NLRP3 or its activation mechanism, in the host response to helminth infections. Exploring these areas would therefore be important for a fuller understanding of this inflammasome's contribution to anti-parasitic immune responses.

1.3 Conclusion and Future Perspective

The NLRP3 inflammasome has many effects on the host response during parasitic infection. In some cases, it successfully fulfils its immunological role and protects the host. In others, however, its immunological response may be counterproductive, damaging the host or advantaging the parasite's growth. Since NLRP3 inflammasome activation was found to exert significant control over *Leishmania, T.gondii* and *T. cruzi* infections. In addition, *E. histolytica* as extracellular can stimulate the NLRP3 inflammasome activation via outside-in signalling independent of pyroptosis leading to an inflammatory response against the parasite. Conversely, NLRP3 deficiency is also beneficial to the host, as it limits the infection severity in malaria and leishmaniasis, while its absence affects the *T. cruzi*-killing mechanism of macrophages and the differentiation of T cell responses, resulting in greater parasite burdens. It is demonstrated that NLRP3 activation during helminth infection helps to control

the parasite by triggering Th2 and Th17 responses. However, for some types of helminth species, a lack of NLRP3 can also reduce the parasite burden carried by the host.

This review suggests that different parasitic products might have different effects on the NLRP3 activation, and in some cases these effects could conflict, thereby accounting for the inflammasome's contrary influences. It has been found that plasmodium products like Hz and DNA are capable of stimulating NLRP3 activation. Yet, further studies are needed to determine the potential role of other plasmodium molecules like GPIs and immunostimulatory DNA, in the NLRP3 function. Since the ability of GPIs to activate NF-kB signaling through TLRs resulting in the production of pro-inflammatory cytokines particularly IL-1β, proposing that GPIs might possibly interact with NLPR3 inflammasomes (162). However, further investigation is needed to prove this point. Several Leishmania molecules and their mechanical actions have also been reported in this review, including LPG, which can stimulate NLRP3 activation, whereas GP63 and LRV both suppress it (36, 52, 66). In addition, *T.gondii* products are found to be involved in the activation of NLRP3, such as STAg and ROP7 (51, 163). Conversely, GRA9 proteins show an anti-inflammatory response by suppressing NLRP3 formation (83). In the case of *E. histolytica* the adherence molecules like Gal/GalNAc and EhCP-A5 RGD together are able to mediate NLRP3 inflammasome activation, while the production of PGE2 by the parasite indirectly inhibits it (107, 121). As earlier remarked, compared to other parasitic infections, very few studies have been carried out on the interaction of NLRP3 inflammasomes with *T. cruzi* and helminths molecules **(Table1).**

The evidence here suggests that the NLRP3 inflammasome's interaction with parasites and their molecules *in vivo* remains only preliminary and requires further confirmation. It has been proposed that whether the NLRP3 inflammasome is activated or inhibited during infection depends on the parasite and the host's genetic background. The host immune response, and the parasites' regulation of that response, are vital areas that must be studied to attain the knowledge necessary to develop effective vaccines and treatment approaches to control these infectious diseases. In addition, in this increasingly advanced field, this review may have further new ideas about parasitic molecules' influence on inflammasome actions that provide clear clinical opportunities to develop new therapeutic interventions to treat these diseases.

Conflicts of Interest:

The author declares no conflict of interest.

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Figure 1: Mechanisms of activation of NLRP3 Inflammasomes. The priming signal (Signal 1) is the first phase in inducing the transcriptional upregulation of NLRP3, pro-interleukin β (IL-1β) and pro-IL-18. It begins when Pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), such as LPS, necrosis factor (TNF) and IL-1β bind to Toll-like receptors (TLRs), tumour necrosis factor receptor (TNFRs), Nucleotide Binding Oligomerization Domain Containing 2 (NOD2) and Interleukin-1 receptor (IL-1R) respectively. This results in the transcriptional upregulation of NLRP3, IL1 β, and IL18 via activation of myeloid differentiation primary response protein (MyD88) proteins and transcription factors nuclear factor kappa-light-chain-enhancer (NF-κB). **The activation signal (Signal 2)** is the second signal that triggered by PAMPs or DAMPs, such as adenosine triphosphate (ATP) and crystals which stimulate diverse signaling events including ROS, lysosomal damage and K+ efflux, resulting in oligomerization, and activation of NLRP3 inflammasome complex. The activation of NLRP3 inflammasome leads to two events: (i) When the adaptor protein ASC and inactive pro-caspase-1 couple together, afterwards cleaving pro-caspase-1 into active caspase-1, which sequentially cleaves the pro-IL-1β and pro-IL-18 into their bioactive forms preceding their release. (ii) active caspase-1 also cleaves Gasdermin D into N- GSDMD, therefore pyroptosis induction and IL-1β and IL-18 production. **Non-canonical NLRP3 inflammasome activation** is prompted by the cytosolic LPS detecting by human caspase 4/5 or mouse caspase 11, followed by cleaving and formation of GSDMD membrane pores, leading to potassium efflux, which eventually triggers NLRP3 inflammasomes activation. The activated NLRP3 cleaves the GSDMD to form additional membrane pores and induce the active form of caspase-1, pro-IL-1β and pro-IL-18, resulting in pyroptotic cell death.

Figure 2: Schematic representation of the mechanisms NLRP3 activation by parasitic molecules. *Plasmodium* hemozoin (Hz) is able to stimulate NLRP3 activation via the Src kinase Lyn and the tyrosine kinase Syk. Also, Hz-NLRP3 activation pathway be boosted by the uric acid produced during malaria infection. Hz coated with plasmodial genomic DNA (gDNA), or CpG-oligonucleotides initiate TLR9 translocation leading to NLRP3 activation. While Leishmania membrane glycoconjugate lipophosphoglycan (LPG) initiates the NLRP3 activation through caspase-11 pathway. *T. gondii* induces NLRP3 activation by the soluble total Ag (STAg) rhoptry protein 7 (ROP7), Profilin from *T. gondii* (TgP) and effector proteins GRA15 causing significant up-regulation in NF-κB expression and consequently inflammasome activation via the IL-1β/NF-κB/NLRP3 pathway. Galactose/ N-acetylgalactosamine (Gal/GalNac) lectin of *E. histolytica* promotes NF-κB and MAP kinase-signaling pathways resulting in NLRP3 inflammasome components and pro-IL-1β transcription. *E. histolytica* Peroxiredoxins (Prx) also functions as a key domain that causes NLRP3 activation pathway via the interaction with TLR4 receptor and P2X7 receptor. Also, Gal/GalNAc lectin supports the formation of the intracellular junction between EhCP-A5 RGD domain and $\alpha 5\beta 1$ integrin resulting in activation of Src family kinase phosphorylation and pannexin-1 (Panx1) channel to enable ATP release. This free ATP then signals back via P2X7 receptors for promoting the second signal for NLRP3 inflammasome formation. In addition, Soluble schistosomal egg antigens (SEA) as a second signal can induce the activation of NLRP3 inflammasome and IL-1β. The FhCL3 helminth-derived molecules of Fasciola hepatica also can promote NLRP3 activation and IL-1β and IL-18 production in non-canonical inflammasome dependent manner.

Figure 3: Schematic representation of the mechanisms of NLRP3 inhibition by parasitic molecules. The virulence factor GP63, expressed by all Leishmania species inhibits reactive oxygen species (ROS), resulting in NLRP3 inflammasome suppression. While, Leishmania RNA virus (LRV) activates TLR3 and TRIF to produce type I IFN resulting in autophagy which induce the expression of Autophagy related 5 (ATG5), that able to block NLRP3 and apoptotic speck-containing (ASC) formation. Also, identically, the Dense granule protein 9 (GRA9) of *T. gondii* suppresses the NLRP3 inflammasome activation by blocking the binding of ASC-NLRP3 and causing disruption of the NLRP3 inflammasome formation. The *E. histolytica* lipid mediator prostaglandin E2 (PGE2) inhibits NLRP3 inflammasome via the PGE2 receptor; as a result of PGE2 signal transduction bonding with the EP4 receptor, adenylyl cyclase activated, subsequently increased intracellular level of cyclic adenylyl monophosphate (cAMP). The Protein Kinase A (PKA) mediates cAMP signalling to directly phosphorylate the Ser295 position of NLRP3 and prevent its ATPase function, resulting in NLRP3 oligomerization inhibition. *T. cruzi* antigen (TcAg) decreases NLRP3 inflammasome expression. Helminth defence molecule-1 of *F. hepatica* (FhHDM-1) (a cathelicidin-like peptide) suppresses NLRP3 inflammasome activation and reducing IL-1β secretion.

Table 1. Summary of parasitic molecules and their actions to NLRP3

Parasite Name	Parasite molecules	Action on NLRP3	By means	Result	Ref.
Plasmodium	Hemozoin (Hz)	Activation	Src kinase Lyn and the tyrosine kinase Syk	IL-1β production	(37)
			?	Negatively influences conventional CD8a+ type 1 dendritic cell (cDC1) abundance, phagocytosis	(43)
	Uric acid	Enhance	?	IL-1β production	(38)
	Hz coated with plasmodial genomic DNA (gDNA) or CpG oligonucleotides	Activation	TLR9		(44)
Leishmania	Parasite membrane glycoconjugatelipophosphoglycan (LPG)	Activation	CASP11 activation in macrophages and in vivo		(65)
	GP63 factor	Suppression	?	Reduction of IL-1β production	(52)
	RNA virus (LRV) virulence factor		TLR3 and TRIF	Leading to Autophagy related 5 (ATG5) expressions mediating NLRP3 breakdown	(36)
T. gondii	The soluble total Ag (STAg) derived from *T. gondii* strain RH	Activation	?	Increasing IL-1β secretion *in vitro*	(81)
	Dense granule proteins 15 (GRA15)		?	L-1β and IFN-γ production	(84)
	T. gondii secretory protein, rhoptry protein 7 (ROP7)	Hyperactivation	IL-1β/NF-κB/ NLRP3 pathway	Up-regulation NF-κB expression	(51)
	Dense granule proteins 9 (GRA9)	Suppression	?	anti-inflammation response	(83)
E. histolytica	Gal/GalNAc lectin	Activation	Activate NF-κB and MAP kinase-signaling pathways	Pro-IL-1β	(114)
	EhCP-A5 RGD binding with macrophages $\alpha5\beta1$ integrin		Src family kinase phosphorylation and opening of Panx1	Release of ATP	(118)
	Peroxiredoxins (Prx)		Binding with TLR4 receptor and P2X7		(119)
	Prostaglandin E2 PGE2	Suppression	Coupling E-prostanoid 4 (EP4)	Turned off ATPase affecting self-oligomerization of NLRP3	(113)
T. cruzi	*T. cruzi* antigen (TcAg)	Suppression	?		(103)

Schistosomal	Soluble schistosomal egg antigens (SEA)	Activation	SEA protein functions as a second signal	Resulting in IL-1β production in dendritic cells	(117)
Fasciola hepatia	FhCL3 helminth-derived molecules of Fasciola hepatica	Activation	?	Promoting adaptive immune response,	(121)
	Fasciola hepatica products like FhHDM-1 (cathelicidin-like peptide)	Suppression	?	Reduction in IL-1β secretion by macrophages	(122)