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Chapter

The Tale of Mastering Macrophage Environment through the Control of Inflammasome-Mediated Macrophage Activation and cAMP Homeostasis by the Protozoan Parasite *Leishmania*

Anindita Bhattacharjee, Arunima Biswas and Pijush K. Das

Abstract

Leishmania, being an intelligent protozoan parasite, modulates the defensive arsenals of the host to create a favorable niche for their survival. When the intracellular parasite is encountered by the host, multimeric complexes of inflammasomes get assembled and activated, thereby leading to genesis of inflammatory response. In order to subvert host defensive strategies, *Leishmania* utilizes their cyclic adenosine monophosphate (cAMP) and cAMP-induced response to neutralize macrophage oxidative damage. In this chapter, we summarize our current understanding of the mechanisms of inflammasome activation in macrophages and cAMP homeostasis of the parasite, leading to parasite viability within the macrophages and establishment of infection. Furthermore, we took into account, recent progresses in translating these research areas into therapeutic strategies, aimed at combating macrophage associated diseases.

Keywords: inflammasome, NLRP3, cAMP homeostasis, phagosome, *Leishmania*, macrophage

1. Introduction

Leishmaniases involve a broad spectrum of neglected tropical diseases that are caused by kinetoplastid parasites belonging to the genus *Leishmania*, that is transmitted by female sandflies belonging to the genus *Phlebotomus* [1]. From the clinical manifestations and symptoms occurring in hosts harboring various species of *Leishmania,* three major forms of *Leishmania* can be delineated: visceral, cutaneous and mucocutaneous leishmaniasis. Visceral leishmaniasis is the chronic and often fatal, if left untreated, form of the disease primarily caused by *Leishmania donovani*

and *Leishmania infantum,* infecting visceral organs including bone marrow, liver and spleen. Cutaneous leishmaniasis, generally caused by *Leishmania major*, produces self-healing ulcerative lesions on the skin. Another variant of cutaneous leishmaniasis is mucocutaneous leishmaniasis that is characterized by damaged oro-naso-pharyngeal tissues. The infective stage, i.e., the metacyclic promastigotes, are found to be embedded in a proteophosphoglycan-rich gel derived from the parasite itself in the anterior mid-gut of the sandfly, which plays important role in parasite regurgitation during blood meal feeding of the sandfly [1]. The moment the parasites enter the host system, they are rapidly phagocytosed by the macrophages where they differentiate into aflagellar, nonmotile amastigotes that multiply within the acidic phagolysosomal compartment of the macrophages [2]. In order to survive within the unfriendly environment of the macrophage, *Leishmania* evolved several approaches to counter the microbicidal power of the macrophage and to mount an effective immune response against the parasite. After the primary encounter of the parasites with neutrophils during early stages of host infection, the monocytes and macrophages in the blood stream participate in the act of engulfing *Leishmania* parasites by phagocytosis. Monocytes that express CD11-c on their surface have recently been identified as the major cellular micro-environment for *Leishmania* to proliferate and also acts as a reservoir of *Leishmania*, providing ample number of parasites required for infecting neighboring cells [2]. On the other hand, recruitment and activation of the inflammatory monocytes protect the host system by limiting proliferation of the parasites by reactive oxygen species (ROS) and inducible nitric oxide synthase (iNOS) generation. However, tissue macrophages become the primary host cells responsible for parasite elimination after infection has been established in the tissues. The macrophages are activated by two components of the immune system like interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNF- α), that synergistically induce iNOS expression, the enzyme responsible for production of free radical NO which actively kills *Leishmania* parasites through oxidative stress induction. Apart from the induction of oxidative stress by the macrophage, monocyte-derived macrophages were found to be responsible for activation of inflammasomes and secretion of cytokines like Interleukin-1 β (IL-1β) upon parasitic infection [3]. Inflammasomes are cytosolic complexes that gather upon sensing microbial molecules or cellular stress, leading to cytokine processing and an inflammatory programmed cell death called pyroptosis, activated by caspase-1. Different species of *Leishmania*, when subjected to analysis against inflammasome activation, were found to be capable of inducing the macrophages to release IL-β by activation of NLRP3 (Nod-like receptor), CASP1 (Caspase-1) and ASC (Apoptosis-associated speck-like protein containing CARD) [4]. IL-1β production by activated NLRP3 inflammasome induces specific signaling pathways triggering NO synthesis leading to parasite killing and disease resistance. In a recent study, it is documented that *Leishmania* lipophosphoglycan (LPG) activates Caspase-11 which in turn activates the non-canonical NLRP3 inflammasomes. Strikingly, the amastigote form of the parasite causes lesser activation of inflammasomes owing to the fact that they display fewer LPG on their surface as compared to the promastigote forms. Moreover, the amastigotes destruct the NLRP3 inflammasome activation pathway in the macrophages by targeting histone H3 [5]. Therefore, it can be considered as an excellent strategy adapted by the parasites to combat the hostility offered by the macrophages against them.

Another major modulator of cytological events in *Leishmania* and other related kinetoplastids is cAMP (cyclic adenosine monophosphate), which is one of the primary factors responsible for parasite transformation and parasite survival. cAMP

plays a major role in differentiation-induced cell cycle arrest in G_0/G_1 phase in both *Leishmania* and *Trypanosoma* [6]. It has significant role in modulating promastigote proliferation, flagellar length and motility and regulation of mitochondrial membrane potential [7]. Moreover, the oxidative burst that the parasites encounter as the first line of defense in mammalian macrophage is associated with elevation of cAMP in the parasite triggering cell cycle arrest and stage differentiation [8]. Thus, it is quite evident that there is a delicate interconnection between the immune response offered by the macrophage upon *Leishmania* infection and the cAMP homeostasis inside the parasite that prevents their killing withing the host. This chapter will discuss the host–parasite interaction, inflammasome activation by the infected macrophages and cAMP homeostasis within the parasite leading to parasite survival.

2. Epidemiology of leishmaniasis

Visceral leishmaniasis, also known as kala-azar, is a deadly disease and in over 95% of instances it is found to be direful when left untreated. Approximately 50,000–90,000 new incidents of visceral leishmaniasis seem to occur globally each year in countries like Bangladesh, Brazil, China, Ethiopia, India, Kenya, Nepal, Somalia, South Sudan and Sudan. Cutaneous and mucocutaneous leishmaniasis occur in South American countries, Mediterranean river basin, the Central Asia and Middle East. Over 90% of mucocutaneous leishmaniasis cases occur in Bolivia, Brazil, Ethiopia and Peru [9]. The diseases collectively affect more than 1 million people every year, visceral leishmaniasis being the main reason for the death of a vast population of about 20,000–40,000 people annually [10]. Apart from humans, the primary host of the diseases, dogs, rodents, other mammals and few reptilian species act as the reservoir hosts of leishmaniases [11].

3. Parasite infection and activation of parasite-dependent host biology modulation

3.1 Initial events of parasite infection and macrophage activation

The disease manifestation of VL strictly relies on host immunocompetency ranging from asymptomatic forms to severe disease conditions, which if left untreated, can be fatal. Clinical conditions such as HIV infection or immunosuppression due to any drug treatment leading to immunodepression in host weaken the efficiency of the host immune system to deal with the infection and permit recurrence of the disease. The contrast in host–parasite interactions between cutaneous and visceral leishmaniasis is quite noteworthy, suggesting the role of infecting species of *Leishmania* in diversification of complexity of immune responses to the pathogen [12]. Resistance and susceptibility of the host to the infection is associated with various genetic factors impacting both severity of the disease and its diagnosis in case of visceral leishmaniasis. One of the most crucial factors that determine the clinical outcome of the disease is host immune response upon parasite entry. One of the components of innate immunity, the complement system, is activated immediately after penetration of the dermis by the promastigotes, resulting in effective clearance of about 90% of all injected parasites within a moment or two [13, 14]. Cells of the innate immune system including dendritic cells, NK (natural killer) cells, T cells and the cytokines they release,

altogether participate to mount immune response against *Leishmania*. IL-12 released by the dendritic cells activates NK cells that produce IFN-γ that enhance killing mechanisms in macrophages, the main target of *Leishmania* [15]. Apart from interleukins and interferons, tumor necrosis factors (TNFs) play a pivotal role in both parasite clearance and tissue damage in liver and spleen, respectively. *Leishmania* parasites modify the chemokine profiles of the host–parasite persistence is promoted. IFN-γ release triggered by Th1 response correlates with infection resistance by induction of some leishmanicidal mechanism in the phagocytes. However, Th2 response results in infection susceptibility and disease manifestation due to replication of intracellular parasite as macrophage activation is inhibited [16]. Thus, symptoms and extent of disease manifestation largely depends on immunocompetency of the host and the fine interplay between the pathogen and the immunological and genetic characteristics of the infected host.

3.1.1 Recognition and uptake

During the parasite's transfer from its vector to the vertebrate host, neutrophils and macrophages are quickly recruited to the sand fly bite site [17]. The parasites secret proteophosphoglycans inside sandfly midgut which acts as a potent stimulator for recruiting macrophages at the site of infection as found in both *L. mexicana* and *L. infantum* [18, 19]. Parasites initially infect the neutrophils but since neutrophils are unsuitable for parasitic differentiation into the amastigote forms, they start infecting the macrophages, their actual site of transformation [20–22]. As a result, the macrophage is a crucial host cell for parasite infection and persistence. The parasite flagellum initiates the contact between the promastigote and the macrophage. This may induce the parasite to release intrinsic survival factors, hence modulating phagocytic activity of the macrophages [20]. The complexity of *Leishmania*-macrophage interaction largely depends on the type of macrophage receptors involved with parasite recognition. Complement receptor-mediated uptake of the parasite results in parasite survival within the macrophage phagosomes because of inhibition of oxidative burst and inflammation, and lysosomal markers like Cathepsin D and LAMP1 accumulation. On the other hand, increased inflammatory conditions developed by fibronectin receptor-mediated uptake leads to parasite clearance. Other receptors including mannose receptors and FCγ receptors trigger inflammatory responses and NADPH oxidase activation in the phagosomes, respectively [23].

After the event of parasite recognition by macrophage cell surface, promastigotes are internalized by cholesterol-rich caveolae for both *L. chagasi* and *L. donovani* uptake [24–26]. However, lipid microdomains is found to have no effect on amastigote phagocytosis, highlighting the importance of membrane lipid-microdomains in the phagocytosis of *Leishmania* by the mammalian macrophages [24]. It is demonstrated in recent studies that lipid microdomain synthesis is promoted by *Leishmania* promastigotes by conversion of sphingomyelin into ceramide, the main constituent of lipid microdomain, by the activation of host acid sphingomyelinase [27]. *De novo* ceramide production is induced by the parasite at late stage of infection leading to disruption of lipid microdomain and impaired antigen presentation [27]. Moreover, *L. donovani* targets a macrophage transcription factor, SREBP2, which promotes parasite internalization and persistence through regulation of macrophage membrane cholesterol and mitochondrial ROS generation [28]. As a result, precise modulation of lipid microdomains is likely to be one of the important actors in host–parasite interactions in *Leishmania* infection.

3.1.2 Phagosome maturation and parasite differentiation

Following phagocytosis of the promastigotes by the macrophages and their internalization into the phagosome, the parasites fuse with lysosomes and adapt to the hostile environment where they must survive for disease manifestations. Despite the fact that this is one of the most difficult habitats for most infectious pathogens, *Leishmania* is one of the few protozoan parasites that can survive and reproduce in such conditions. Phagosomes that contain *L. donovani* promastigotes showed reduced fusogenicity towards lysosomes and late endosomes [29, 30]. LAMP1, a lysosome marker, was employed to the parasitophorous vacuoles leading to reorientation of the parasites with their cell body facing the macrophage nucleus and the flagellum in the direction of the periphery of the cell. This orientation of the parasites endorsed the parasitophorous vacuole to move outwards causing cell injury, accelerated lysosome docking followed by exocytosis. Some of the parasite containing lysosomes might fuse with the parasitophorous vacuoles promoting transformation of promastigote into amastigote, whereas cell injury might disrupt the integrity of the plasma membrane and the capacity of the host to fight infection [31].

Differentiation from promastigote to amastigote is triggered by an increase in temperature from 22–37°C and a decrease in pH from 7.2 to 5.5 in mammalian phagolysosomes. Furthermore, iron uptake followed by hydrogen peroxide generation has been found to be a significant trigger for parasitic differentiation in *L. amanzonensis* [32, 33]. *Leishmania* iron transporter (LIT1) triggers the conversion of ROS into hydrogen peroxide by the enzyme iron superoxide dismutase; hydrogen peroxide being the major trigger for promastigote-to-amastigote differentiation to occur [34]. From previous studies, it was well documented that there is a close association between this transformation and the elevated levels of cAMP and cAMP-dependent Protein Kinase A (PKA) within the parasite [35]. cAMP plays a very crucial role in the transformation and regulation of the cell cycle of the parasites. cAMP acts as a cyto-protector which increase peroxide neutralizing capacity of the parasite increasing their chance of survival and is also known to trigger G_1 arrest [8]. Several isoforms of leishmanial phosphodiesterases (LdPDEA and LdPDED) also showed roles in controlling the cAMP levels during transformation along with the leishmanial receptor adenylate cyclases (LdracA and LdracB) and pyrophosphatases (V-H$^+$PPase) proving beyond doubt the importance of cAMP signaling cascade in the parasite [35, 36].

3.1.3 Macrophage activation: host: parasite interaction

Macrophages, apart from acting as a phagocytic cell, respond to and regulate different signaling molecules [37]. Circulating monocytes are the precursors of tissue macrophages that secrete various antimicrobial and immunoregulatory molecules capable of inactivating pathogens through ROS and NO generation [38–40]. Monocyte–macrophage lineage show notable plasticity and can modify their physiology according to the environmental stimuli giving rise to diversified cell population with different functions [41, 42]. The state of activation of macrophages can be changed in response to different cytokines, growth factors and microbial molecules. When stimulated by TNF-α or IFN-γ or lipopolysaccharide, macrophages undergo classical activation that is characterized by surface marker CD80 expression [43, 44]. On the other hand, macrophages undergoing alternative activation is induced by IL-4 and IL-13 by the activation of a common receptor, IL-4R [45]. Production of high levels IL-13, CCL14, CCL17, CCL18, CCL22, IL-10, TGF-β, urea, and ornithine which

is an essential substrate for both polyamine and collagen synthesis, are observed in alternatively activated macrophages [44, 46, 47]. One of the mechanisms for the establishment of intra-macrophage parasite infection is the inhibition of host defense mechanism which is achieved by inhibiting inflammatory cytokine secretion and apoptosis. One such target of *L. donovani* promastigotes is AKT signaling and its downstream components, β-catenin and FOXO-1, modulation of which inhibits both cytokine production and apoptosis [48]. Thus, the initial interaction of *Leishmania* with macrophages leads to their activation and polarization that is essential for the survival of the parasite inside the macrophage and establishment of disease manifestation.

3.2 Inflammasome activation by *Leishmania*: mastering the macrophage environment exploiting macrophage biology

Upon detection of pathogenic organisms, the cytoplasm of the cells of innate immunity assembles multiprotein complexes called inflammasomes that cause an inflammatory programmed cell death called pyroptosis. The nucleotide-binding domain leucine-rich repeat protein (NLR) family is the widely studied inflammasome that is activated by cell membrane damage-inducing pathogens and molecules (**Figure 1**) [49]. Caspase-1 activation is promoted upon NLRP3 activation and oligomerization which leads to ASC polymerization exposing the CARD domains of the ASC, leading to recruitment of Caspase-1 through CARD/CARD interaction. The NLRP3 inflammasome undergoes both canonical and non-canonical activation. Initially, TLR (toll-like receptors) or TNFRs are stimulated by microbial components or TNF-α leading to numerous inflammatory gene transcription, Nlrp3, Casp11 and Il1b for instance. But inspite of the presence of a first signal, a second signal is required for canonical NLRP3 activation that occurs via pore formation by the microbial toxins, and subsequent rupture of the host cell membrane, resulting in K^+ efflux
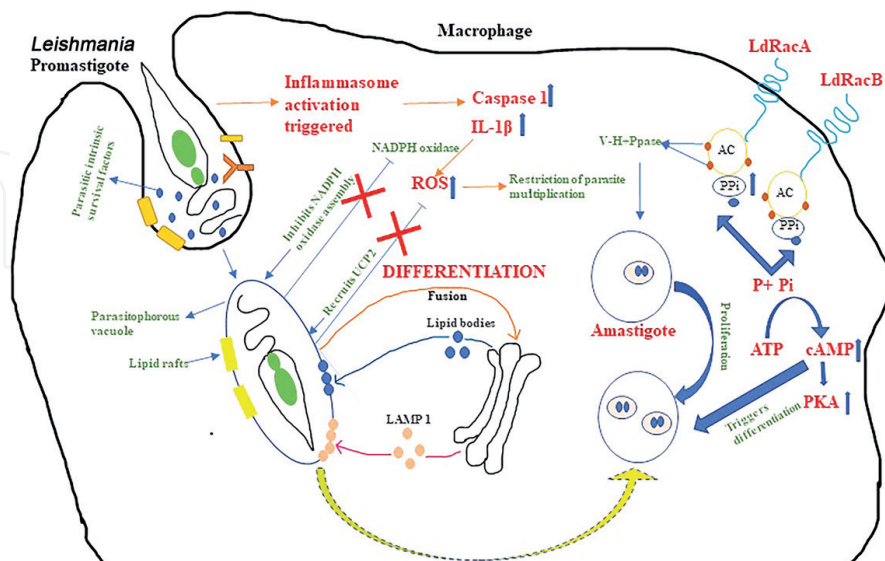

Figure 1.
Interaction of Leishmania *promastigotes with macrophage receptors triggers phagocytosis of the parasite and release of intrinsic survival factors by the pathogen that modify phagosome synthesis and results in inhibition of proinflammatory pathways. The schematic diagram is the summary of the fine interplay between inflammasome-induced and cAMP-mediated differentiation of the parasite through modulation of ROS and activation by Ld receptor adenylate cyclases (LdRacs) by acidocalcisomal pyrophosphatase (V-H$^+$PPase) resulting in upregulation of cAMP, respectively. PPi = inorganic pyrophosphate pool, AC = acidocalcisome.*

and decrease in K⁺ concentration the cytoplasm [50]. Apart from potassium efflux, lysosomal cathepsin and ROS production is also essential for canonical activation of NLRP3. In contrast, non-canonical activation of NLRP3 inflammasomes is promoted by Caspase-11 which is activated by bacterial LPS. Different species of *Leishmania* use different strategies of limiting or inhibiting inflammasome activation in the macrophages. *L. mexicana* and *L. major*, as demonstrated by Shio et al., use GP63, a virulence factor that inhibit IL1b production in THP-1 cells, either by ROS inhibition or by inflammasome components cleavage [51]. Inhibition of NLRP3 inflammasome by *L. donovani* is achieved by A20, a negative regulator of NF-κβ and UCP2, mitochondrial uncoupling protein 2 manipulation by inhibiting ROS generation [52]. Leishmania infection induces the expression of UCP2 leading to downregulation of ROS generation by the macrophage, thus probably averting ROS-mediated inactivation of protein tyrosine phosphatases which in turn suppresses defense mechanism of the infected macrophages [53]. In two recent findings, *L. donovani* and *L. amazonensis* was shown to transcriptionally inhibit the components of inflammasome in infected macrophages [52, 54]. Though inflammation activation might not be fully blocked by the pathogens, but the magnitude or extent of inflammasome activation is surely noticeably limited by *Leishmania* as compared to other infectious pathogens like bacteria or protozoan parasites like *Trypanosoma cruzi* and *Toxoplasma gondii* [4].

3.3 The NLRP3 inflammasome and its activation during *Leishmania* sp. infection

Initial stage of macrophage infection by *Leishmania* triggers inflammasome activation in the infected macrophages prompting Caspase-1 activation and production of IL-1b. The role of NLRP3 inflammasome in Caspase-1 activation was established by the absence of the process in NLRP3 or ASC deficient cells [55]. Macrophage inflammasome activation restricted parasite multiplication by Caspase-1 and IL-1b production and stimulation of ROS generation via p47phox and arachidonic acid-NADPH oxidase signaling pathway in *L. infantum* [56]. In conjunction with these findings, it was revealed that *L. amazonensis* triggered Dectin-1 activation, which resulted in Syk kinase activation and ROS production by NADPH oxidase, a step required for NLRP3 activation in macrophages [57]. Furthermore, inhibiting ROS and NADPH oxidase during the primary infection was sufficient to prevent inflammasome activation, demonstrating that the earliest signals for inflammasome activation occur during parasite phagocytosis [57]. These findings highlight the role of ROS in the activation of NLRP3 during *Leishmania* infection. Lipophosphoglycan (LPG) from several *Leishmania* species has recently been demonstrated to activate caspase-11 in macrophages, suggesting non-canonical NLRP3 inflammasome activation [58]. It was also observed that mitochondrial phosphatase phosphoglycerate mutase family member five, a protein involved in restricting *Leishmania* multiplication in macrophages, is required for the production of IL-1b in response to *Leishmania* infection, implying that this protein is involved in the NLRP3 inflammasome activation [59]. Compounds that activate inflammasomes and promote IL-1b production, such as polyester poly (lactide-co-glycolide acid) nanoparticles infused with an 11 kDa *Leishmania* antigen, the antiprotozoal drug diterpene kaurenoic acid, and the conventional anti-leishmanial drug Amphotericin B, have also been shown to limit parasite replication in macrophages [60, 61]. In a visceral Leishmaniasis model, anti-IL-1b treatment inhibited parasite clearance by Amphotericin B. These investigations show indisputably that the inflammasome is activated as a result of *Leishmania* infection and plays a key role in limiting proliferation of *Leishmania* in macrophages.

4. *Leishmania* evades host defense

4.1 Curbing inflammation

To counter host defenses, *Leishmania* exploits a multitude of intervention mechanisms by targeting various signaling pathways in macrophages. Even before the parasites are inoculated by the sand fly vector, the proteophosphoglycan rich secretory gel produced by the parasites promotes *L. mexicana* viability by increasing alternative activation and arginase activity in the macrophages [18]. Interestingly, *Leishmania* amplified the production of the proinflammatory cytokines IL-1, TNF, MIP-1, and MCP-1 as well as the anti-inflammatory cytokine IL-10 induced by leishmanial LPS [62]. Murine peritoneal macrophages infected with *L. donovani* promastigotes has recently been observed to stimulate expression of PPARγ of the host, which is believed to suppress inflammation and guard the host from irreparable damage. The parasite was easier to remove when PPAR was inhibited [63]. *Leishmania* also activated host PTPs (protein tyrosine phosphatases) such as PTP1B, TC-PTP, PTP-PEST, and SHP-1. PTP activation causes a wide range of beneficial events for the parasite, including a decrease in proinflammatory processes, a decrease in IL-12, NO, TNF, phagolysosomal maturation, and antigen presentation by class II MHC molecules [64, 65]. TRAF3 is another newly discovered target of *L. donovani* promastigotes. In RAW 264.7 cells and bone marrow-derived macrophages, the parasite blocked TRAF3 (TNF receptor-associated factor) degradation to impede TLR4-mediated inflammatory response in the host. TLR4 activation requires TRAF3 degradative ubiquitination. TRAF3 knockdown by shRNA reduced parasite load [66]. The preceding investigations demonstrate the wide range of host targets that *Leishmania* uses to avoid macrophage activation and the consequent proinflammatory response. As *Leishmania* develops infection and multiplies within the macrophage, the macrophage may ultimately undergo apoptosis. The parasite postpones apoptosis of macrophage but eventually uses the apoptotic cell to propagate to nearby non - infected macrophages with limited access to extracellular immune recognition systems. Hence, when it is at its most susceptible phases, *Leishmania* skillfully manipulates its host to escape immune detection and associated inflammation.

4.2 Interfering with host cell signaling

Leishmania-infected macrophages lack events associated with activation and are insensitive to IFN-γ [51]. It has also been found that IFN-γ receptors are downregulated by *L. donovani* promastigotes and promote the expression of the cytokine signaling suppressor SOCS3 [67]. As a result, *L. donovani* promastigotes may effectively turn off the main signaling cascade of one of the most essential macrophage activators. *L. donovani* amastigotes, like promastigotes, suppressed IFN-induced MHC class II and iNOS expression. Infection with *L. donovani* amastigotes, on the other hand, reduced IFN-induced gene expression without changing STAT1 activation. Rather, amastigotes decreased IFN-induced nuclear translocation of STAT1 through interfering with STAT1's interaction with karyopherin importin-α5 [68]. The fundamental mechanics are yet unknown.

4.3 Avoiding oxidative damage

The reactions of *Leishmania* to oxidative stress is different for different *Leishmania* species and host cell they infect [69]. For instance, peritoneal macrophages infected with *L. major*, when elicited by thioglycolate, triggered ROS generation by stationary

phase promastigotes whereas the same was inhibited in macrophages infected with *L. amazonensis* [70]. To combat oxidative stress, various Leishmania species employ a variety of strategies. *L. donovani* axenic amastigotes, for example, were able to reduce both intracellular and mitochondrial ROS by inducing mitochondrial uncoupling protein 2 (UCP2) upregulation, hence ROS inhibition [53]. *Leishmania* can also escape oxidative damage by inhibiting the assembly of phagolysosomal membrane NADPH oxidase and the formation of ROS within the parasitophorous vacuoles [71]. The parasite's insertion of the surface glycolipid lipophosphoglycan (LPG) in the phagosomal membrane may further hinder recruitment of the NADPH oxidase components to the parasitophorous vacuoles [30]. In addition to directly damaging the parasite's oxidative damage caused by ROS promotes macrophage apoptosis, destroying the parasite's replicative niche.

4.4 Countering antigen presentation

Antigen cross-presentation is a significant aspect in pathogen immunity. It entails presenting phagocytosed cargo-derived foreign proteins on class I MHC for cytotoxic CD8+ T cells recognition and a systemic immune response coordination. The macrophage, as a specialized antigen presenting cell (APC), contributes in the cross-presentation of proteins generated from *Leishmania*. In macrophages infected with *L. donovani* or *L. major*, the parasite strategically escapes immune responses offered by the host by inducing SNARE VAMPS cleavage, thus preventing antigen cross-presentation [34]. No such inhibition is observed in *L. donovani* amastigote infected macrophages. VAMP8 disruption inhibited assembly of NADPH oxidase, resulting in more effective phagosomal acidification and proteolysis, and thereby reducing presentation by MJHC class I and activation of T cells [71–73]. The parasite also inhibited antigen cross-presentation by disrupting membrane lipid microdomains [74]. Indeed, infected cells had lower membrane cholesterol levels, and the defect in antigen presentation could be repaired with liposomal administration of exogenous cholesterol. Liposomal cholesterol was also found to be enhancing ROS and RNI degeneration, as well as expression of proinflammatory cytokine and intracellular parasite death, and was linked to cellular stress and ROS-induced apoptosis in peritoneal cells infected with *L. donovani* promastigotes [75].

4.5 Inducing autophagy

L. amazonensis amastigotes and stationary phase promastigotes increased intracellular parasite survival by autophagy induction in peritoneal exudate cells or macrophages derived from bone marrow. Autophagy inhibitors like 3-methyladenine (3MA) or wortmannin lowered parasite burden, but autophagy inducers like rapamycin or starvation had little effect or increased parasite load [76, 77]. Autophagy induction was related with a decrease in NO, emphasizing the importance of this mechanism in infection establishment.

4.6 Exploiting macrophage environment to activate its antioxidant defense mechanism through cyclic nucleotide signaling pathway

Following phagocytosis by macrophages in the early stages of infection, the parasites are subjected to severe oxidative stress as a result of a respiratory burst offered by the macrophages releasing ROS and RNS [78, 79]. Superoxide dismutase, peroxidoxin, and trypanothione reductase are three *Leishmania* components involved in antioxidant defense against ROS and RNIs. The parasites are rendered

susceptible to death in the ROS producing macrophages upon the disruption of the above-mentioned genes [80–83]. Furthermore, resistance against oxidative damage is induced when the organism is pre-exposed to environmental stress [78, 84]. In many unicellular eukaryotes, the cAMP response has been linked as one of the key environmental sensing machineries related with environmental stress response. Despite the fact that *Leishmania* parasites are subjected to extreme stress, which is a trigger for differentiation, and the relevance of cAMP in parasite differentiation has been documented, knowledge about the function of cAMP in *Leishmania* pathogenicity and resistance against oxidative stress is still obscure. A high temperature of 37°C, a low pH of 5.5 and nutritional starvation, all these collectively trigger cell cycle arrest in promastigote resulting in differentiation into aflagellar amastigote forms inside the macrophage. Occurrence of the major antioxidant gene upregulation was confirmed by analyzing the mRNA expression of these genes under stressed condition and maximum elevation of Ldpxn1 level was found indicating the activity of antioxidant genes in *Leishmania* during stress [8]. Not only that, antioxidant gene protein upregulation was also observed in *Leishmania* treated with cAMP analogues, suggesting a crucial role of cAMP in promoting antioxidant defense in *Leishmania*. Chemical or genetic inhibition of intracellular cAMP resulted in a decrease in the mRNA and protein levels of these anti-oxidant genes. The role of cAMP in parasite infectivity was reinforced by the discovery that promastigotes with a steadily overexpressed pde gene had a lower ability to infect IFN-γ activated macrophages than normal cells. The cAMP response is most likely one of several environmental sensing tools connected with *Leishmania* differentiation, and many other biochemical reactions may collaborate with cAMP in the process of differentiation resulting in parasite transformation.

5. Role of cAMP in survival and infectivity of the parasites

In eukaryotes, cAMP, a second messenger which is formed from ATP by the membrane- bound enzyme, receptor adenylate cyclases (RAC), is a key component that controls a wide range of cellular activities such as cytoskeletal modeling, cell proliferation, virulence, cellular differentiation, and death [85]. There have been reports of various isoforms of both membrane-bound receptor adenylate cyclases and soluble adenylate cyclases in *Leishmania* [86]. cAMP has been found to be involved in signal transduction processes that occur during transformations not only in *Leishmania*, but also in other similar kinetoplastid protozoa. In *Trypanosoma brucei* [87] and *Trypanosoma cruzi* [88], various life cycle phases have variable intracellular cAMP concentrations. Owing to the importance of cAMP and the role it plays in the transformation of kinetoplastid parasites, receptor adenylate cyclases in *L. donovani* have further been investigated. In *L. donovani*, the occurrence of receptor adenylate cyclase (RAC) has also been revealed, and a membrane associated RAC-A has been established to be functional when exposed to phagolysosome conditions (PC) actively catalyzing cAMP production [89]. In addition to the direct action of LdRAC-A in stress-induced intra-cellular cAMP generation, intracellular PPi and pyrophosphatases also perform a significant role in modulating cAMP concentration in the cell. A higher PPi concentration might be modulating adenylate cyclase reactions and thus, inhibits cAMP generation in the parasites (**Figure 1**) [90]. Aside from pyrophosphatases, which control intracellular cAMP synthesis by receptor adenylate cyclases, other aspects of cAMP regulation must be investigated. Intramacrophage cAMP concentration is increased upon infection-induced Prostaglandin E2 production in

the *Leishmania*-infected macrophage [91]. Intracellular cAMP level is maintained by phosphodiesterases (PDEs) which are universal enzymes that hydrolyze cAMP to 5′-AMP or cGMP to 5′-GMP to terminate the cyclic nucleotide signaling pathway, are the only mechanism for the cell to get rid of the cAMP generated for managing various cellular activities [92]. Cytosolic PDE activities decrease throughout stage differentiation, although the activity of membrane bound PDEs remains constant. This data suggests that PDEs have a crucial function as a regulating factor during stage differentiation of *Leishmania* [93]. Not only membrane-bound phosphodiesterases, but cytosolic phosphodiesterase, PDED, is also important in the regulation of cAMP homeostasis by enhanced hydrolytic property through PKA-mediated activation of PDED [94]. PDED plays a role in the regulation of PKA activity and in turn maintains cAMP homeostasis in the parasite when initially exposed to stress conditions.

Despite the fact that cAMP-dependent protein kinase (PKA) exists and functions in eukaryotes, the role of PKA in cAMP signaling in this specific parasite is still ambiguous. PKA, being the first downstream effector of cAMP in the RAC pathway, facilitate γ-P transfer to particular ser/thr residue from ATP [95]. Upon exposure of *Leishmania* to environmental stress, a significant upregulation of PKA activity, concomitantly with cAMP level increase, is observed, suggesting a connection between cAMP and PKA during parasite differentiation. PKA activity of five distinct *Leishmania* species was shown to be relatively high in logarithmic, as well as stationary phase promastigotes, with *L. amazonensis* being the most active and *L. donovani* being the least active [96]. A regulatory subunit of cAMP-dependent PKA, LdPKAR1, have been identified in *L. donovani* which plays significant role in metacyclogenesis, intra-macrophage survival and enhanced infectivity of the parasite [97]. Thus, cAMP is emerging as a key regulator of cytological processes in kinetoplastida parasites [98]. Despite the discovery of multiple cyclic nucleotide binding effector molecules, there is still lack of complete understanding on cAMP signaling from receptor to effector.

6. Conclusion

Being an intracellular parasite, *Leishmania* has adopted various ways to enter the host cells efficiently and then subsequently try to neutralize the hostile environment of macrophages. Although various strategies have been adopted by this parasite to establish successful infection, we are mainly concerned about stressing the role of inflammasome activation in host cells and cAMP homeostasis of the parasite in this chapter. To summarize, it can be stated that recent advances in research have increased our understanding of the role of cAMP signaling in kinetoplastid parasites such as *Leishmania* and its relationship to parasite infectivity. These findings shed light on the activity of many enzymes involved in cAMP metabolism. It can be suggested that modulating cAMP levels in the parasite might be one of the methods for controlling leishmaniasis, and that the molecules involved could be explored as powerful therapeutic targets against the leishmaniases.

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Conflict of interest

The authors declare no conflict of interest.

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References

[1] Rogers ME. The role of Leishmania Proteophosphoglycans in sand Fly transmission and infection of the mammalian host. Frontiers in Microbiology. 2012;**3**:223

[2] Heyde S, Philipsen L, Formaglio P, Fu Y, Baars I, Höbbel G, et al. CD11c-expressing Ly6C+CCR2+ monocytes constitute a reservoir for efficient Leishmania proliferation and cell-to-cell transmission. PLoS Pathogens. 2018;**14**(10):e1007374

[3] Kelley N, Jeltema D, Duan Y, He Y. The NLRP3 Inflammasome: An overview of mechanisms of activation and regulation. International Journal of Molecular Sciences. 2019;**20**(13):3328

[4] de Carvalho RVH, Zamboni DS. Inflammasome activation in response to intracellular protozoan parasites. Trends in Parasitology. 2020;**36**(5): 459-472

[5] Lecoeur H, Prina E, Rosazza T, Kokou K, N'Diaye P, Aulner N, et al. Targeting macrophage histone H3 modification as a Leishmania strategy to dampen the NF-κB/NLRP3-mediated inflammatory response. Cell Reports. 2020;**30**(6):1870-1882.e4

[6] Biswas A, Bhattacharya A, Das PK. Role of cAMP Signaling in the survival and infectivity of the protozoan parasite, Leishmania donovani. Molecular Biology International. 2011;**2011**:e782971

[7] Saha A, Bhattacharjee A, Vij A, Das PK, Bhattacharya A, Biswas A. Evaluation of modulators of cAMP-response in terms of their impact on cell cycle and mitochondrial activity of Leishmania donovani. Frontiers in Pharmacology. 2020;**11**:782

[8] Bhattacharya A, Biswas A, Das PK. Role of intracellular cAMP in differentiation-coupled induction of resistance against oxidative damage in Leishmania donovani. Free Radical Biology & Medicine. 2008;**44**(5):779-794

[9] Casalle N, de Barros Pinto Grifoni L, Bosco Mendes AC, Delort S, Massucato EMS. Mucocutaneous Leishmaniasis with rare manifestation in the nasal mucosa and cartilage bone septal. Case Reports in Infectious Diseases. 2020;**2020**:8876020

[10] Alvar J, Vélez ID, Bern C, Herrero M, Desjeux P, Cano J, et al. Leishmaniasis worldwide and global estimates of its incidence. PLoS One. 2012;**7**(5):e35671

[11] Ready PD. Epidemiology of visceral leishmaniasis. Clinical Epidemiology. 2014;**6**:147-154

[12] McMahon-Pratt D, Alexander J. Does the Leishmania major paradigm of pathogenesis and protection hold for New World cutaneous leishmaniases or the visceral disease? Immunological Reviews. 2004;**201**:206-224

[13] Domínguez M, Moreno I, Aizpurua C, Toraño A. Early mechanisms of Leishmania infection in human blood. Microbes and Infection. 2003;**5**(6):507-513

[14] Maurer M, Dondji B, von Stebut E. What determines the success or failure of intracellular cutaneous parasites? Lessons learned from leishmaniasis. Medical Microbiology and Immunology. 2009;**198**(3):137-146

[15] Scharton TM, Scott P. Natural killer cells are a source of interferon gamma that drives differentiation of

CD4+ T cell subsets and induces early resistance to Leishmania major in mice. The Journal of Experimental Medicine. 1993;**178**(2):567-577

[16] Sundar S, Reed SG, Sharma S, Mehrotra A, Murray HW. Circulating T helper 1 (Th1) cell- and Th2 cell-associated cytokines in Indian patients with visceral leishmaniasis. The American Journal of Tropical Medicine and Hygiene. 1997;**56**(5):522-525

[17] Peters NC, Egen JG, Secundino N, Debrabant A, Kimblin N, Kamhawi S, et al. In vivo imaging reveals an essential role for neutrophils in leishmaniasis transmitted by sand flies. Science. 2008;**321**(5891):970-974

[18] Rogers M, Kropf P, Choi B-S, Dillon R, Podinovskaia M, Bates P, et al. Proteophosophoglycans regurgitated by Leishmania-infected sand flies target the L-arginine metabolism of host macrophages to promote parasite survival. PLoS Pathogens. 2009;**5**(8):e1000555

[19] Rogers ME, Corware K, Müller I, Bates PA. Leishmania infantum proteophosphoglycans regurgitated by the bite of its natural sand fly vector, Lutzomyia longipalpis, promote parasite establishment in mouse skin and skin-distant tissues. Microbes and Infection. 2010;**12**(11):875-879

[20] Rotureau B, Morales MA, Bastin P, Späth GF. The flagellum-mitogen-activated protein kinase connection in Trypanosomatids: A key sensory role in parasite signalling and development? Cellular Microbiology. 2009;**11**(5):710-718

[21] Peters NC, Sacks DL. The impact of vector-mediated neutrophil recruitment on cutaneous leishmaniasis. Cellular Microbiology. 2009;**11**(9):1290-1296

[22] Mollinedo F, Janssen H, de la Iglesia-Vicente J, Villa-Pulgarin JA, Calafat J. Selective fusion of azurophilic granules with Leishmania-containing phagosomes in human neutrophils. The Journal of Biological Chemistry. 2010;**285**(45):34528-34536

[23] Ueno N, Wilson ME. Receptor-mediated phagocytosis of Leishmania: Implications for intracellular survival. Trends in Parasitology. 2012;**28**(8):335-344

[24] Rodríguez NE, Gaur Dixit U, Allen L-AH, Wilson ME. Stage-specific pathways of Leishmania infantum chagasi entry and phagosome maturation in macrophages. PLoS One. 2011;**6**(4):e19000

[25] Chattopadhyay A, Jafurulla M. Role of membrane cholesterol in leishmanial infection. Advances in Experimental Medicine and Biology. 2012;**749**:201-213

[26] Roy S, Kumar GA, Jafurulla M, Mandal C, Chattopadhyay A. Integrity of the actin cytoskeleton of host macrophages is essential for Leishmania donovani infection. Biochimica et Biophysica Acta. 2014;**1838**(8):2011-2018

[27] Majumder S, Dey R, Bhattacharjee S, Rub A, Gupta G, Bhattacharyya Majumdar S, et al. Leishmania-induced biphasic ceramide generation in macrophages is crucial for uptake and survival of the parasite. The Journal of Infectious Diseases. 2012;**205**(10):1607-1616

[28] Mukherjee M, Basu Ball W, Das PK. Leishmania donovani activates SREBP2 to modulate macrophage membrane cholesterol and mitochondrial oxidants for establishment of infection. The International Journal of Biochemistry & Cell Biology. 2014;**55**:196-208

[29] Vinet AF, Fukuda M, Turco SJ, Descoteaux A. The Leishmania donovani

lipophosphoglycan excludes the vesicular proton-ATPase from phagosomes by impairing the recruitment of synaptotagmin V. PLoS Pathogens. 2009;**5**(10):e1000628

[30] Moradin N, Descoteaux A. Leishmania promastigotes: Building a safe niche within macrophages. Frontiers in Cellular and Infection Microbiology. 2012;**2**:121

[31] Forestier C-L, Machu C, Loussert C, Pescher P, Späth GF. Imaging host cell-Leishmania interaction dynamics implicates parasite motility, lysosome recruitment, and host cell wounding in the infection process. Cell Host & Microbe. 2011;**9**(4):319-330

[32] Mittra B, Cortez M, Haydock A, Ramasamy G, Myler PJ, Andrews NW. Iron uptake controls the generation of Leishmania infective forms through regulation of ROS levels. The Journal of Experimental Medicine. 2013;**210**(2):401-416

[33] Mittra B, Andrews NW. IRONy OF FATE: Role of iron-mediated ROS in Leishmania differentiation. Trends in Parasitology. 2013;**29**(10):489-496

[34] Podinovskaia M, Descoteaux A. Leishmania and the macrophage: A multifaceted interaction. Future Microbiology. 2015;**10**(1):111-129

[35] Vij A, Biswas A, Bhattacharya A, Das PK. A soluble phosphodiesterase in Leishmania donovani negatively regulates cAMP signaling by inhibiting protein kinase a through a two way process involving catalytic as well as non-catalytic sites. The International Journal of Biochemistry & Cell Biology. 2014;**57**:197-206

[36] Biswas A, Bhattacharya A, Vij A, Das PK. Role of leishmanial

acidocalcisomal pyrophosphatase in the cAMP homeostasis in phagolysosome conditions required for intra-macrophage survival. The International Journal of Biochemistry & Cell Biology. 2017;**86**:1-13

[37] Wynn TA, Chawla A, Pollard JW. Macrophage biology in development, homeostasis and disease. Nature. 2013;**496**(7446):445-455

[38] Nathan CF, Hibbs JB. Role of nitric oxide synthesis in macrophage antimicrobial activity. Current Opinion in Immunology. 1991;**3**(1):65-70

[39] Forman HJ, Torres M. Reactive oxygen species and cell signaling: Respiratory burst in macrophage signaling. American Journal of Respiratory and Critical Care Medicine. 2002;**166**(12 Pt 2):S4-S8

[40] Franken L, Schiwon M, Kurts C. Macrophages: Sentinels and regulators of the immune system. Cellular Microbiology. 2016;**18**(4):475-487

[41] Gordon S, Taylor PR. Monocyte and macrophage heterogeneity. Nature Reviews. Immunology. 2005;**5**(12):953-964

[42] Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. Nature Reviews. Immunology. 2008;**8**(12):958-969

[43] Sica A, Mantovani A. Macrophage plasticity and polarization: in vivo veritas. The Journal of Clinical Investigation. 2012;**122**(3):787-795

[44] Jaguin M, Houlbert N, Fardel O, Lecureur V. Polarization profiles of human M-CSF-generated macrophages and comparison of M1-markers in classically activated macrophages from GM-CSF and M-CSF origin. Cellular Immunology. 2013;**281**(1):51-61

[45] Gordon S, Martinez FO. Alternative activation of macrophages: Mechanism and functions. Immunity. 2010;**32**(5):593-604

[46] Mills CD. Macrophage arginine metabolism to ornithine/urea or nitric oxide/citrulline: A life or death issue. Critical Reviews in Immunology. 2001;**21**(5):399-425

[47] Tarique AA, Logan J, Thomas E, Holt PG, Sly PD, Fantino E. Phenotypic, functional, and plasticity features of classical and alternatively activated human macrophages. American Journal of Respiratory Cell and Molecular Biology. 2015;**53**(5):676-688

[48] Gupta P, Srivastav S, Saha S, Das PK, Ukil A. Leishmania donovani inhibits macrophage apoptosis and pro-inflammatory response through AKT-mediated regulation of β-catenin and FOXO-1. Cell Death and Differentiation. 2016;**23**(11):1815-1826

[49] Broz P, Dixit VM. Inflammasomes: Mechanism of assembly, regulation and signalling. Nature Reviews. Immunology. 2016;**16**(7):407-420

[50] Di A, Xiong S, Ye Z, Malireddi RKS, Kometani S, Zhong M, et al. The TWIK2 potassium Efflux Channel in macrophages mediates NLRP3 Inflammasome-induced inflammation. Immunity. 2018;**49**(1):56-65.e4

[51] Shio MT, Christian JG, Jung JY, Chang K-P, Olivier M. PKC/ROS-mediated NLRP3 Inflammasome activation is attenuated by Leishmania zinc-metalloprotease during infection. PLoS Neglected Tropical Diseases. 2015;**9**(6):e0003868

[52] Gupta AK, Ghosh K, Palit S, Barua J, Das PK, Ukil A. Leishmania donovani inhibits inflammasome-dependent macrophage activation by exploiting the negative regulatory proteins A20 and UCP2. The FASEB Journal. 2017;**31**(11):5087-5101

[53] Ball WB, Mukherjee M, Srivastav S, Das PK. Leishmania donovani activates uncoupling protein 2 transcription to suppress mitochondrial oxidative burst through differential modulation of SREBP2, Sp1 and USF1 transcription factors. The International Journal of Biochemistry & Cell Biology. 2014;**48**:66-76

[54] Saha G, Khamar BM, Singh OP, Sundar S, Dubey VK. Leishmania donovani evades caspase 1 dependent host defense mechanism during infection. International Journal of Biological Macromolecules. 2019;**126**:392-401

[55] Lima-Junior DS, Costa DL, Carregaro V, Cunha LD, Silva ALN, Mineo TWP, et al. Inflammasome-derived IL-1β production induces nitric oxide-mediated resistance to Leishmania. Nature Medicine. 2013;**19**(7):909-915

[56] Lefèvre L, Lugo-Villarino G, Meunier E, Valentin A, Olagnier D, Authier H, et al. The C-type lectin receptors dectin-1, MR, and SIGNR3 contribute both positively and negatively to the macrophage response to Leishmania infantum. Immunity. 2013;**38**(5):1038-1049

[57] Lima-Junior DS, Mineo TWP, Calich VLG, Zamboni DS. Dectin-1 activation during Leishmania amazonensis phagocytosis prompts Syk-dependent reactive oxygen species production to trigger Inflammasome assembly and restriction of parasite replication. Journal of Immunology. 2017;**199**(6):2055-2068

[58] de Carvalho RVH, Andrade WA, Lima-Junior DS, Dilucca M, de

Oliveira CV, Wang K, et al. Leishmania Lipophosphoglycan triggers Caspase-11 and the non-canonical activation of the NLRP3 Inflammasome. Cell Reports. 2019;**26**(2):429-437.e5

[59] Farias Luz N, Balaji S, Okuda K, Barreto AS, Bertin J, Gough PJ, et al. RIPK1 and PGAM5 control Leishmania replication through distinct mechanisms. Journal of Immunology. 2016;**196**(12):5056-5063

[60] Santos DM, Carneiro MW, de Moura TR, Soto M, Luz NF, Prates DB, et al. PLGA nanoparticles loaded with KMP-11 stimulate innate immunity and induce the killing of Leishmania. Nanomedicine. 2013;**9**(7):985-995

[61] Miranda MM, Panis C, da Silva SS, Macri JA, Kawakami NY, Hayashida TH, et al. Kaurenoic acid possesses leishmanicidal activity by triggering a NLRP12/IL-1β/cNOS/NO pathway. Mediators of Inflammation. 2015;**2015**:392918

[62] Lapara NJ, Kelly BL. Suppression of LPS-induced inflammatory responses in macrophages infected with Leishmania. Journal of Inflammation. 2010;**7**(1):8

[63] Chan MM, Adapala N, Chen C. Peroxisome proliferator-activated receptor-γ-mediated polarization of macrophages in Leishmania infection. PPAR Research. 2012;**2012**:796235

[64] Gómez MA, Olivier M. Proteases and phosphatases during Leishmania-macrophage interaction: Paving the road for pathogenesis. Virulence. 2010;**1**(4):314-318

[65] Liu D, Uzonna JE. The early interaction of Leishmania with macrophages and dendritic cells and its influence on the host immune response.

Frontiers in Cellular and Infection Microbiology. 2012;**2**:83

[66] Gupta P, Giri J, Srivastav S, Chande AG, Mukhopadhyaya R, Das PK, et al. Leishmania donovani targets tumor necrosis factor receptor-associated factor (TRAF) 3 for impairing TLR4-mediated host response. The FASEB Journal. 2014;**28**(4):1756-1768

[67] Bhattacharya P, Gupta G, Majumder S, Adhikari A, Banerjee S, Halder K, et al. Arabinosylated lipoarabinomannan skews Th2 phenotype towards Th1 during Leishmania infection by chromatin modification: Involvement of MAPK signaling. PLoS One. 2011;**6**(9):e24141

[68] Matte C, Descoteaux A. Leishmania donovani amastigotes impair gamma interferon-induced STAT1alpha nuclear translocation by blocking the interaction between STAT1alpha and importin-alpha5. Infection and Immunity. 2010;**78**(9):3736-3743

[69] Olivier M, Atayde VD, Isnard A, Hassani K, Shio MT. Leishmania virulence factors: Focus on the metalloprotease GP63. Microbes and Infection. 2012;**14**(15):1377-1389

[70] Almeida TF, Palma LC, Mendez LC, Noronha-Dutra AA, Veras PST. Leishmania amazonensis fails to induce the release of reactive oxygen intermediates by CBA macrophages. Parasite Immunology. 2012;**34**(10):492-498

[71] Matheoud D, Moradin N, Bellemare-Pelletier A, Shio MT, Hong WJ, Olivier M, et al. Leishmania evades host immunity by inhibiting antigen cross-presentation through direct cleavage of the SNARE VAMP8. Cell Host & Microbe. 2013;**14**(1):15-25

[72] Savina A, Jancic C, Hugues S, Guermonprez P, Vargas P, Moura IC,

et al. NOX2 controls phagosomal pH to regulate antigen processing during crosspresentation by dendritic cells. Cell. 2006;**126**(1):205-218

[73] Rybicka JM, Balce DR, Chaudhuri S, Allan ERO, Yates RM. Phagosomal proteolysis in dendritic cells is modulated by NADPH oxidase in a pH-independent manner. The EMBO Journal. 2012;**31**(4):932-944

[74] Chakraborty D, Banerjee S, Sen A, Banerjee KK, Das P, Roy S. Leishmania donovani affects antigen presentation of macrophage by disrupting lipid rafts. Journal of Immunology. 2005;**175**(5):3214-3224

[75] Ghosh J, Guha R, Das S, Roy S. Liposomal cholesterol delivery activates the macrophage innate immune arm to facilitate intracellular Leishmania donovani killing. Infection and Immunity. 2014;**82**(2):607-617

[76] Pinheiro RO, Nunes MP, Pinheiro CS, D'Avila H, Bozza PT, Takiya CM, et al. Induction of autophagy correlates with increased parasite load of Leishmania amazonensis in BALB/c but not C57BL/6 macrophages. Microbes and Infection. 2009;**11**(2):181-190

[77] Cyrino LT, Araújo AP, Joazeiro PP, Vicente CP, Giorgio S. In vivo and in vitro Leishmania amazonensis infection induces autophagy in macrophages. Tissue & Cell. 2012;**44**(6):401-408

[78] Zarley JH, Britigan BE, Wilson ME. Hydrogen peroxide-mediated toxicity for Leishmania donovani chagasi promastigotes. Role of hydroxyl radical and protection by heat shock. The Journal of Clinical Investigation. 1991;**88**(5):1511-1521

[79] Gantt KR, Goldman TL, McCormick ML, Miller MA,

Jeronimo SM, Nascimento ET, et al. Oxidative responses of human and murine macrophages during phagocytosis of Leishmania chagasi. Journal of Immunology. 2001;**167**(2):893-901

[80] Tovar J, Wilkinson S, Mottram JC, Fairlamb AH. Evidence that trypanothione reductase is an essential enzyme in Leishmania by targeted replacement of the tryA gene locus. Molecular Microbiology. 1998;**29**(2):653-660

[81] Barr SD, Gedamu L. Cloning and characterization of three differentially expressed peroxidoxin genes from Leishmania chagasi. Evidence for an enzymatic detoxification of hydroxyl radicals. The Journal of Biological Chemistry. 2001;**276**(36):34279-34287

[82] Ghosh S, Goswami S, Adhya S. Role of superoxide dismutase in survival of Leishmania within the macrophage. The Biochemical Journal. 2003;**369** (Pt 3):447-452

[83] Plewes KA, Barr SD, Gedamu L. Iron superoxide dismutases targeted to the glycosomes of Leishmania chagasi are important for survival. Infection and Immunity. 2003;**71**(10):5910-5920

[84] Miller MA, McGowan SE, Gantt KR, Champion M, Novick SL, Andersen KA, et al. Inducible resistance to oxidant stress in the protozoan Leishmania chagasi. The Journal of Biological Chemistry. 2000;**275**(43):33883-33889

[85] Dremier S, Kopperud R, Doskeland SO, Dumont JE, Maenhaut C. Search for new cyclic AMP-binding proteins. FEBS Letters. 2003;**546**(1):103-107

[86] Hansen BD, Chiang PK, Perez-Arbelo J. Evidence for a membrane

adenosine receptor in Leishmania mexicana mexicana (WR 227). Advances in Experimental Medicine and Biology. 1986;**195**(Pt B):547-551

[87] Mancini PE, Patton CL. Cyclic 3′,5′-adenosine monophosphate levels during the developmental cycle of Trypanosoma brucei brucei in the rat. Molecular and Biochemical Parasitology. 1981;**3**(1):19-31

[88] Rangel-Aldao R, Allende O, Triana F, Piras R, Henriquez D, Piras M. Possible role of cAMP in the differentiation of Trypanosoma cruzi. Molecular and Biochemical Parasitology. 1987;**22**(1):39-43

[89] Sanchez MA, Zeoli D, Klamo EM, Kavanaugh MP, Landfear SM. A family of putative receptor-adenylate cyclases from Leishmania donovani. The Journal of Biological Chemistry. 1995;**270**(29):17551-17558

[90] Seebeck T, Schaub R, Johner A. cAMP signalling in the kinetoplastid protozoa. Current Molecular Medicine. 2004;**4**(6):585-599

[91] Saha A, Biswas A, Srivastav S, Mukherjee M, Das PK, Ukil A. Prostaglandin E2 negatively regulates the production of inflammatory cytokines/chemokines and IL-17 in visceral leishmaniasis. Journal of Immunology. 2014;**193**(5):2330-2339

[92] Conti M, Beavo J. Biochemistry and physiology of cyclic nucleotide phosphodiesterases: Essential components in cyclic nucleotide signaling. Annual Review of Biochemistry. 2007;**76**:481-511

[93] Bhattacharya A, Biswas A, Das PK. Role of a differentially expressed cAMP phosphodiesterase in regulating the induction of resistance against oxidative damage in Leishmania donovani. Free Radical Biology & Medicine. 2009;**47**(10):1494-1506

[94] Gettys TW, Vine AJ, Simonds MF, Corbin JD. Activation of the particulate low km phosphodiesterase of adipocytes by addition of cAMP-dependent protein kinase. The Journal of Biological Chemistry. 1988;**263**(21):10359-10363

[95] Mochly-Rosen D. Localization of protein kinases by anchoring proteins: A theme in signal transduction. Science. 1995;**268**(5208):247-251

[96] Mukhopadhyay NK, Saha AK, Lovelace JK, Da Silva R, Sacks DL, Glew RH. Comparison of the protein kinase and acid phosphatase activities of five species of Leishmania. The Journal of Protozoology. 1988;**35**(4):601-607

[97] Bhattacharya A, Biswas A, Das PK. Identification of a protein kinase a regulatory subunit from Leishmania having importance in metacyclogenesis through induction of autophagy. Molecular Microbiology. 2012;**83**(3):548-564

[98] Tagoe DNA, Kalejaiye TD, de Koning HP. The ever unfolding story of cAMP signaling in trypanosomatids: Vive la difference! Frontiers in Pharmacology. 2015;**6**:185