Critical Review

Recent Advances in Understanding *Leishmania donovani* Infection: The Importance of Diverse Host Regulatory Pathways

Amrita Saha
Moumita Basu
Anindita Ukil *

Department of Biochemistry, University of Calcutta, Kolkata, West Bengal, India

Abstract

Invasion of host cell by pathogens induce various intracellular signalling pathways. The host cell through the initiation of these signalling circuits desperately wants to get rid of the pathogen, whereas the pathogen tries to subvert these defence strategies to create an environment for their successful survival. *Leishmania spp*. is not an exception. *Leishmania* have to evolve a range of strategic mechanisms to neutralize macrophage defensive arsenals which enable the parasite to replicate within the phagolysosome of infected host. Understanding these signalling mechanisms in detail will not only improve our basic knowledge of host-pathogen interaction but will also help us to develop effective drug targets not only against leishmaniasis but also for many other macrophage associated diseases. © 2018 IUBMB Life, 70(7):593–601, 2018

Keywords: Leishmania*; macrophage; cytokine; negative regulators; apoptosis; reactive oxygen species; NAD(P)H oxidase; inflammasome; curdlan; 18β-glycyrrhetinic acid*

IINTRODUCTION

Visceral leishmaniasis (VL), a fatal disease, is caused by the intracellular protozoan parasite *Leishmania donovani*. This intelligent parasite invades and survives within the host macrophages, the "phagocytic cells" that are recruited to destroy the invading pathogens. To survive and replicate within the

Abbreviations: *BAK, Bcl-2 homologous antagonist/killer; Bcl-2, B-cell lymphoma 2; cIAP1/2, cellular inhibitor of apoptosis 1/2; COX, cyclooxygenase; CREB, cAMP (cyclic adenosine monophosphate)-response element binding protein; DAMP, danger-associated molecular patterns; DC, dendritic cells; EP, E-prostanoid; EPAC, exchange protein activated by cAMP; FOXO, forkhead box protein O1; GRA, 18β-glycyrrhetinic acid; GSK-3, glycogen synthase kinase 3; HO-1, heme oxygenase-1; IRAK1/4, interleukin receptor-associated kinase 1/4; IRAK-M, interleukin-1 receptor-associated kinase M; JAK/STAT, janus kinase/signal transducer and activator of transcription; K63, Lys 63; LPG, lipophosphoglycan; LPS, lipopolyssacharide; MAPK, mitogen activated protein kinases; MCL-1, myeloid cell leukemia-1; MYA, million years ago; MyD88, myeloid differentiation primary response 88; NAD(P)H oxidase, nicotinamide adenine dinucleotide phosphate oxidase; NF-κB, nuclear factor-κB; NK, natural killer; NLRP3, NLR(NOD(nucleotide oligomerization domain)-like receptors) family pyrin domain containing 3; NO, nitric oxide; PAMPs, pathogen-associated molecular patterns; PG, prostaglandins; PI3K, phosphatidylinositol-3-kinases; PKA, protein kinase A; PRR, pattern recognition receptor; PTP, protein-tyrosine phosphatases; ROS, reactive oxygen species; SHP, small heterodimer partner; SOCS-1, suppressor of cytokine signalling-1; TAB1, TAK1-binding protein 1; TAK1, TGFβ (transforming growth factor-β)–associated kinase 1; TIR, toll/interleukin-1 receptor; TLR, toll-like receptor; TRAF6, TNF receptor-associated factor 6; TRIF, TIR domain-containing adaptor protein inducing interferon-β; Ubc13, ubiquitin-conjugating enzyme 13; UCP2, uncoupling protein-2; VL, Visceral Leishmaniasis*

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**Address correspondence to: Dr. Anindita Ukil, Department of Biochemistry, University of Calcutta, 35 Ballygunge Circular Road, Kolkata 700 019, West Bengal, India. Tel: 91-33-2461-5455. Fax: 91-33-2461-4849.*
E-mail:u.anindita@gmail.com
Amrita Saha is currently at Infectious diseases and Immunology Division, CSIR-Indian Institute of Chemical Biology, Kolkata, West Bengal, India

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hostile environment of the macrophages, the parasite initially has to overcome the innate immune response of the host involving toll-like receptor (TLR)-mediated signalling and oxidative burst. Impairment of host protective cytokine and chemokine responses happens to be their next target, and at the same time, it has to safeguard its own niche by preventing host cell apoptosis, an ultimate strategy used by the host to prevent pathogen propagation (1). Therefore, the fate of infection depends on the battle between the host's ability to activate immune defensive machineries and the parasite's ability to evade or suppress these host-mounted leishmanicidal responses. The parasite achieves these targets by distortion of multiple host beneficiary signalling pathways in its favour, thereby creating a parasite fostering immunosuppressive environment. In this review, we tried to elucidate, the intricate host cell signalling pathways that are manipulated by the parasite for the establishment of a successful infection. Finally, we tried to shed some light on the immunomodulators which by virtue of their immune activation potential can be efficacious in the treatment of this deadly disease.

EVOLUTIONARY HISTORY OF *LEISHMANIA*

Leishmaniasis has a long history, dating to 2,500 B.C., with several primitive descriptions of the disease having been found in ancient writings and recent molecular findings from ancient archaeological material. The first *Leishmania* fossil record was Paleoleishmania proterus, a digenetic *Leishmania* species associated with a blood-filled female of the sandfly *P. burmitis* in Burmese fossil amber (2). Lysenko in 1971 proposed a Palaearctic origin of the genus *Leishmania* (3). Fossil evidence indicates that both phlebotomine sandflies and murid rodents originated in the Palaearctic (4), indicating *Leishmania*, along with its vectors and reservoirs, could have evolved in the Palaearctic during the Cenozoic period and dispersed to the Nearctic during the Oligocene (Eocene), when the Bering land bridge was intact. These species then dispersed into the Neotropics across the Panamanian land bridge during the Pliocene, when the climate was sufficiently warm to permit further dispersal of *Leishmania* (3,5).

GEOGRAPHICAL HISTORY OF *LEISHMANIA*

The disease Leishmaniasis has four main clinical forms according to the location of the parasite in mammalian tissues—visceral, cutaneous, diffuse cutaneous and mucocutaneous Leishmaniasis (6). Geographical history of *Leishmania* species unfolds many interesting facts but these facts are still subjects of controversy. The genus *Leishmania* has presumably evolved before the division of the supercontinent Pangea in the Mesozoic era (252-66 MYA) (7). But, the distinct region of origin of various *Leishmania* species remains as a subject matter of debate. According to the concept of dichotomis of species based on molecular trees, in the old world, *L. donovani* seems to have originated in Eastern Africa, the region said to be of human origin. However, *Leishmania major*, causative agent of cutaneous leishmaniasis could have originated in the Saharan region of North Africa. On considering the genetic polymorphisms and clonality of *Leishmania*, it was hypothesised that the parasite existed in America before the separation of Gondwana (100 MYA). The division of Gondwana further led to the formation of two subgenera *Vianna* (New World) and *Leishmania* (Old World). But again these theories lack any confirmatory data (5).

SURVIVAL STRATEGY OF *LEISHMANIA* INSIDE THE HOST

Being an obligate intracellular pathogen, *Leishmania* has to survive inside the host cell environment. The primary host of *Leishmania* is macrophage, one of the most important cell in mammalian innate immune system. Therefore, to replicate and establish infection inside macrophages, *Leishmania* has to adopt several mechanisms for undermining macrophage defence and make its niche more conducive. The various strategies adopted by this intelligent unicellular parasite have been described below.

Manipulation of Receptors

Whenever a macrophage cell encounters a microbial invader, the first line of defence put forward by the host is different families of pattern recognition receptors (PRRs), which detect distinct conserved motifs on various pathogens, collectively termed as pathogen-associated molecular patterns (PAMPs). These PRR may be either membrane bound receptors or cytosolic. Among the various membrane bound PRRs, TLRs have been studied most extensively.

Toll-Like Receptors. TLRs are hallmarks of cellular receptors that mount up early innate immune response to invading pathogens. TLR recognize highly conserved extracellular motifs such as PAMP and danger-associated molecular patterns (DAMPs). PAMPs are solely expressed by the pathogens, while DAMPs are molecules that are secreted by the dying or necrotic cells. Recognition of PAMP by TLR, serve as the primary line of host defence leading to the clearance of the pathogen. Till date, 13 TLRs have been described detecting distinct motifs of various pathogens. Upon binding to the specific ligand, TLR recruit adaptor proteins such as myeloid differentiation primary response 88 (MyD88) or TIR (Toll/interleukin-1 receptor) domain-containing adaptor protein inducing interferon-β (TRIF) (8). Elimination of the microbial pathogen involves arrays of signalling events leading to leukocyte recruitment, microbicidal activity, generation of type I interferons (interferon-α and interferon-β) and proinflammatory cytokines (tumor necrosis factor [TNF]-α, interleukin [IL]-12 and IL-1β). The role of TLR in leishmaniasis has gradually been deciphered. The first ever studies relating TLR and *Leishmania* was found to be MyD88 dependent for the expression of IL-1α cytokine (9). A large

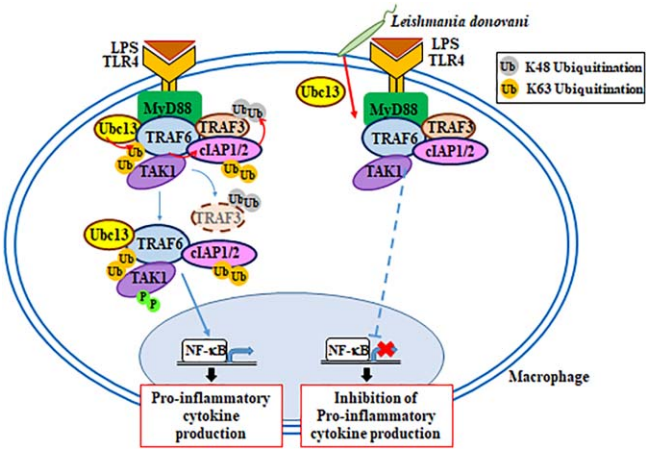

FIG 1 *Modulation of TLR4 signalling pathway by Leishmania donovani upon infection. LPS upon binding to its cognate receptor TLR4 triggers the recruitment of MyD88, which is followed by formation of a signalosome complex consisting of TRAF6, cIAP1/2, Ubc13 and TRAF3. Ubiquitination at K63 of TRAF6 by Ubc13 enables it to ubiquitinate cIAP1/2, which in turn ubiquitinates TRAF3 at K48, leading to its proteasomal degradation. This leads to subsequent release and translocation of the entire complex to the cytosol, resulting in TAK1-dependent activation of NF-κB. Leishmania donovani infection hampers the association of Ubc13 to the complex, causing defective K63-linked ubiquitination of TRAF6. This leads to impairment of TRAF3 ubiquitination and subsequent reduction in TRAF3 degradation. As a result, TRAF3, along with the signalosome complex, persists in the membrane, resulting in inappropriate TAK1 activation, which ultimately dampens the proinflammatory responses of macrophage cell.*

body of evidence indicates significant role of TLR4 in abrogation of *Leishmania* survival in both the phases of immune response: innate and adaptive (9,10). *Leishmania*, the intracellular parasite, has the capacity to dampen host generated TLR-mediated pro-inflammatory response by inhibiting the functioning of the signalling molecules present in the cascade. Binding of lipopolyssaccharide (LPS) to TLR4 results in the formation of a signalosome complex comprising of MyD88, interleukin receptor-associated kinase 1/4 (IRAK1/4), TNF receptor-associated factor 6 (TRAF6), ubiquitin-conjugating enzyme 13 (Ubc13) and cellular inhibitor of apoptosis 1/2 (cIAP1/2) (11). Ubiquitination at Lys 63 (K63) position of TRAF6 triggers ubiquitination of cIAP1/2, followed by TRAF3 ubiquitination at K48, leading to the proteasomal degradation of TRAF3 (11). These signalling events sets free the remaining complex, which then translocates to the cytosol and through downstream kinase activation ultimately results in the nuclear transport of nuclear factor-κB (NF-κB) and transcription of target genes. *Leishmania* infection interferes with the signalosome complex assembly by leading to dissociation of Ubc 13 from the complex (12). Absence of Ubc 13 results in faulty ubiquitination and inactivation of TRAF6 and cIAP1/2, thereby preventing TRAF3 degradation (12) (Fig. 1). As a result, the complex remains bound to membrane and cannot activate downstream signalling required for generation of proinflammatory responses (Fig. 1). Lipophosphoglycan (LPG), isolated from *L. major,* was found to be a TLR2 ligand activating TLR signalling pathways on human natural killer (NK) cells leading to the enhancement of TNF-α and interferon (IFN)-γ and ultimately resulting in clearance of the parasite (13). Despite carrying glycosylphosphotidylinositol-anchored glycophospholipid molecule LPG on their surface, *Leishmania* evades TLR2-mediated cytokine response thereby escaping host defence strategies. The study by Srivastav et al. revealed that *Leishmania,* despite possessing LPG, exploits host deubiquitinating enzyme A20, which hampers ubiquitination of TRAF6 (14). Deubiquitinated TRAF6 cannot form TRAF6–TAK1 assembly complex and cannot activate TAK1-mediated NF-κB activation (14). This was in line with another study reporting A20 deficient macrophages conferring elevated pro-inflammatory response upon stimulation (15). Intriguingly, some studies have also reported that *Leishmania* LPG inhibits fusion of phagosomes and lysosomes (16,17). LPG was also found to dampen TLR9 and TLR2 signalling, the latter by upregulating suppressor of cytokine signalling-1 (SOCS-1) and SOCS-3 (18,19).

Inflammasomes. Activation of the cytosolic multiprotein inflammasome complex is another genre of innate immune response against invading pathogens. Several families of PRR that recognize intracellular PAMP are important components in the inflammasome complex, comprising of nucleotide-binding domain, leucine-rich repeat containing proteins (also known as NOD-like receptors, NLR) and an adaptor protein apoptosis-associated speck-like protein containing a C-terminal caspase recruitment domain (20). Upon sensing stimuli, NLR oligomerize to constitute caspase-1–activating scaffold. Activated caspase-1 (previously known as interleukin-1β converting enzyme) subsequently cleave the pro-forms of the pro-inflammatory IL-1 family of cytokines into their bioactive or matured forms IL-1β and IL-18 leading to the production of adhesion molecules and chemokines, which in turn trigger immune and inflammatory responses. Among the caspase-1 activating inflammasomes, the most well-characterized is pyrin domain containing NLR (NOD (nucleotide oligomerization domain)-like receptors) family pyrin domain containing 3 (NLRP3) of NLR family, which gets activated in response to a wide range of stimuli, including bacterial and viral pathogens as well as endogenous danger signals (21). The activation of the canonical NLRP3 inflammasome comprises of two signals. The first or priming signal induces the increased expression of NLR protein and transcription of pro-IL-1β via NF-κB, as low levels of these proteins fail to trigger inflammasome activation. The second activation signal is derived from generation of reactive oxygen species (ROS), ion or membrane perturbations, or extracellular ATP which induces the oligomerization and formation of an inflammasome complex leading to the production of matured IL-1β (22). A study reported that the NLRP3 inflammasome provides protection in *L. amazonensis* infected C57BL/6 mice (23). On the contrary, *L. major* and *L. mexicana* significantly inhibit NLRP3 inflammasome activation and IL-1β production (24). Our laboratory has shown

that *L. donovani* inhibits IL-1β production in macrophages (25), whereas inhibition of NLRP3 inflammasome activation, leading to impairment of IL-1β synthesis has been documented in increasing resistance to *L. major* infection in BALB/c mice (26). These contradictory results may be due to use of different *Leishmania* species and versatile genetic background of the host. Recently, a study on *L. amazonensis* demonstrated that ROS induced via NAD(P)H oxidase in macrophages during the early stages of *L. amazonensis* infection is critical for NLRP3 inflammasome activation (27). However, our work has documented that upon *L. donovani* infection, transcription of NLRP3 gets inhibited due to the upregulation of NF-κB inhibitor A20 (25). Moreover, *L. donovani* simultaneously upregulates mitochondrial uncoupling protein-2 (UCP2) (28), thereby inhibiting ROS production and ROS-mediated pro-IL-1β processing (25). Similarly, infection by *L. donovani* results in shutting down the production of IL-1β at the transcription and translational level (25). Furthermore, small hairpin RNA-mediated knockdown of either A20 or UCP2 in infected mice induced inflammasome-mediated IL-1β production and significantly decreased liver and spleen parasite burden, creating a host favourable anti-leishmanial milieu (25). Consistent with this observation, the study by Shio et al. (24) also reported that expression of the metalloprotease GP63 is indispensable for *Leishmania*-mediated inhibition of NLRP3 inflammasome activation and dampening of IL-1β secretion.

Neutralization of Antimicrobial Molecules

Oxidative Burst. Immune cells like neutrophils and macrophages, whenever come across a foreign particle, rapidly release ROS (superoxide radical $O_2^{\cdot -}$ and hydrogen peroxide H_2O_2). NAD(P)H oxidase is the main ROS producing enzyme of the macrophages. Upon stimulation, this multi-subunit enzyme complex NAD(P)H oxidase gets assembled and activated on the membrane leading to generation of ROS. Neutralization of this ROS production is important for intracellular survival of pathogens (29). Regarding *Leishmania* infection, few reports have suggested that infection with amastigote form of *Leishmania* resulted in limited superoxide production (30,31). Another study has shown that infection with live *L. donovani* led to rapid downregulation of the ROS level in infected macrophages indicating active suppression of ROS by the parasite (32). These observations indicated a possible role of macrophage antioxidant enzymes during *Leishmania* infection. Super oxide dismutase has been found to promote *Leishmania* survival within host macrophage (33). The role of another antioxidant enzyme heme oxygenase-1 (HO-1) has been found to be responsible for successful survival of various intracellular pathogens like *Mycobacterium abscessus* and *Salmonella typhimurium* (34,35). The enzyme has also been found to be associated with infection by various *Leishmania spp*. (36,37). It was found that in macrophages infected with *L. pifanoi* amastigotes, there was a significant increase in HO-1 level as early as 1 h post-infection and the level sustained for several hours (37). Induction of HO-1 level led to increased parasite loads in mouse and human macrophages (36). Apart from host antioxidant enzymes, the membrane LPG of *L. donovani* promastigotes has been found to be responsible for decrease in superoxide radical production by inhibiting the assembly of functional phagosomal NADPH oxidase complex excluding the cytosolic components $p47^{phox}$ and $p67^{phox}$ of NADPH oxidase (38).

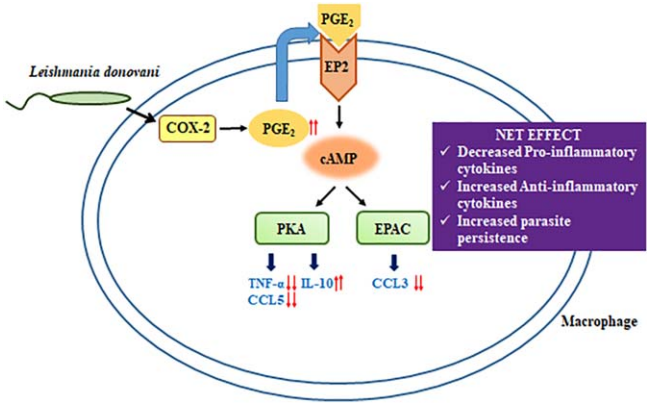

FIG 2 *Role of PGE_2 in the modulation of cytokines and chemokines in Leishmania infected macrophages. L. donovani upon infection causes COX-2-dependent upregulation of PGE_2 production. PGE_2 causes increase in cellular cAMP level through the activation of EP2 receptor. Increased cAMP causes activation of its downstream kinases, PKA and EPAC. PKA leads to decrease in TNF-α and CCL5 levels, whereas EPAC downregulates the chemokine CCL3, thereby maintaining a parasite conducive environment.*

Induction of Immunosuppressive Host Molecules

To inhibit macrophage defence mechanisms, the parasite exploits various immunosuppressive molecules of the host. These molecules play an important role in negatively modulating macrophages defensive arsenals and favour parasite survival. These molecules could be a lipid or a protein or a membrane receptor, and all of them able to deactivate/modulate macrophage activation signalling.

Prostaglandins E_2. Prostaglandins are lipid mediators that play central roles in multitude of infections (39,40). Amongst the various prostaglandins (e.g., prostaglandin D_2 (PGD_2), prostaglandin E_2 [PGE_2] and PGF_2), prostaglandin F_2 (PGE_2) is most well-studied and characterised. PGE_2 exerts its effect by binding to one of its cognate receptors, namely, E-prostanoid (EP)1–4 (41). Different species of *Leishmania* have been reported to activate PGE_2 production (42,43). The upregulation of PGE_2 production in case of *L. donovani* infection has been reported to be the result of action in a concerted manner by TLR2, phosphatidylinositol-3-kinases (PI3K), phospholipase C, extracellular signal-regulated kinase and the calcium-calcineurin-nuclear factor of activated T-cells 2 pathway (42). COX-2 is the rate limiting enzyme for PGE_2 biosynthesis. Both the mRNA and protein level of COX-2 were found to be upregulated upon *L. donovani* infection but in case of *L. major* infection although the mRNA level of COX-2 was significantly

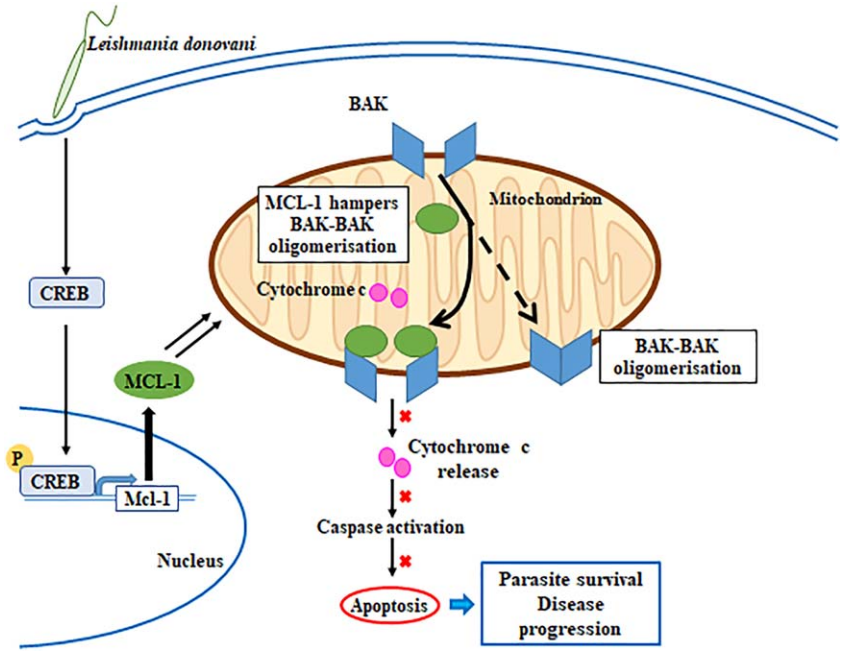

FIG 3 *Upregulation of MCL-1 during L. donovani infection prevents mitochondria-dependent apoptosis. L. donovani upon infection causes CREB-dependent upregulation of macrophage anti-apoptotic protein MCL-1 which gets translocated to the mitochondria. MCL-1 in the mitochondria interacts with pro-apoptotic protein BAK and prevents BAK-BAK homo-oligomer–mediated pore formation in mitochondrial membrane. This prevents cytochrome c release from mitochondria and thereby inhibits mitochondria-dependent apoptosis.*

induced but the protein level remained unaltered (44). Our study revealed that *L. donovani*-activated PGE_2 exerts its downstream function of increasing intracellular cAMP (cyclic adenosine monophosphate) and generating a parasite-favouring environment through activation of its cognate receptor EP2 (32). But, in contrast to this observation, treatment of *L. major*-infected susceptible BALB/c mice with either EP2 or EP4 agonists significantly restricted the parasite load in the draining lymph node (45), suggesting varied receptor activation amongst different species of *Leishmania*. cAMP has long been reported to stimulate anti-inflammatory response. The role of intra-macrophage cAMP in the context of leishmaniasis has been reported in recent years and we have found that cAMP produced via COX-2/PGE_2/EP2 pathway during infection results in inhibition of macrophage microbicidal functions (32) (Fig. 2). This is in accordance with a recent report by Figueiredo et al. (46) that *L. amazonensis* upregulates cAMP of dendritic cells (DCs) via adenosine Adenosine 2B (A2B) receptor. The resultant increased cAMP level suppresses activation of DC and production of both IL-12 and IFN-γ cytokines. The two downstream effector molecules of cAMP are cAMP-dependent protein kinase A (PKA) and exchange protein activated by cAMP (EPAC) (47). Our inhibitor-based study has shown that these two kinases independently modulate cytokine and chemokine activation thereby leading to propagation of infection (32).

Interleukin-1 Receptor-Associated Kinase M. IRAK-M is a negative regulator of TLR-signalling pathway that gets induced upon TLR activation. The expression of IRAK-M is limited to monocytes/macrophages. IRAK-M deficient conditional knockout mice showed decreased *Klebsiella pneumonia* burden (48). Lack of IRAK-M in cells exhibited increased cytokine production upon TLR/IL-1 stimulation and bacterial challenge (49). Moreover, enhanced innate immunity was found in IRAK-M knockout mice as revealed by increased inflammatory responses against bacterial infection. We showed that IRAK-M is exploited by *L. donovani* to exercise a sustained repression on TLR-mediated pro-inflammatory response during established infection (50). In infected macrophages, siRNA mediated silencing of IRAK-M displayed enhanced IRAK1 and IRAK4 phosphorylation, which is mandatory for TLR activation with a concomitant increase in downstream NF-κB activity and reduced parasite burden (50). IRAK-1 inactivation during *Leishmania* infection has been reported in another study where SHP-1 was exploited by *Leishmania* to bind to IRAK-1 and for complete suppression of its intrinsic kinase activity (51).

Inhibition of Apoptosis

Apoptosis is a phenomenon that occurs naturally in multicellular organisms. It serves as the last resort for infected cells to ensure complete elimination of the unwanted guests along with the infected cells. The ability of certain pathogens to prevent host cell apoptosis has emerged as a new theme in the field of pathogenesis. Inhibition of apoptosis of the host cell, that is, pathogen's niche during infection provides the

pathogen with a survival tool to replicate within the physiological address for the establishment of infection.

Myeloid Cell Leukemia-1. Myeloid cell leukemia-1 (MCL-1), a member of BCL-2 family, is an anti-apoptotic protein that has been reported to play significant roles during pathogenic invasion (52–54). Both the RNA and protein levels of MCL-1 were found to be upregulated during infection with the virulent strain of *M. tuberculosis* strain but not with attenuated (53,54). Furthermore, MAPK signalling pathway was found to foster increased MCL-1 expression (55). Unmethylated species-specific CpG motifs in LdDNA was found to significantly delay macrophage PCD by upregulation of the anti apoptotic protein Mcl-1 in TLR9-dependent pathway (56). In our recent study, we demonstrated that MCL-1 was markedly upregulated in *Leishmania* infection (57). Infection-induced MCL-1 was found to be localized in mitochondria and its expression regulated by cAMP-response element binding protein (CREB). MCL-1 interacts with the pro-apoptotic protein Bcl-2 homologous antagonist/killer (BAK), inhibiting BAK-BAK homo-oligomer association and subsequent release of cytochrome c from the mitochondrial membrane (57) (Fig. 3). A study with *S. aureus* infection also showed MCL-1 as one of the major players in subversion of host cell apoptosis, leading to disease progression (52). Recently, it was reported that BCL-2 is significantly induced in peripheral blood of VL patients and inhibition of BCL-2 resulted in increased nitric oxide (NO) response thereby reducing parasitic burden (58).

Suppressor of Cytokine Signalling. SOCS family comprises of eight intracellular proteins. These proteins function in a negative feedback loop to inhibit JAK/STAT (Janus kinase/signal transducer and activator of transcription) pathway-mediated cytokine signalling (59). Various pathogens have been reported to tamper with host immune defence response by activation of SOCS proteins leading to suppression of macrophage's microbicidal strategies and recent studies also implicated the role of these proteins in disease pathogenesis (60). We showed significant upregulation of SOCS 1 and 3 in case of *L. donovani* infection and suppression of oxidative burst-mediated host cell apoptosis (61). Egr1-mediated induction of SOCS proteins in infected cells resulted in activation of thioredoxin, which caused stabilization of protein-tyrosine phosphatases (PTPs). PTP in turn inhibited activation of caspase cascade required for apoptosis of host cells. SOCS knock-down cells displayed reduction in disease progression suggesting thereby that *L. donovani* uses differential induction of host SOCS proteins to subvert macrophage apoptotic machinery triggered by parasite internalization-mediated oxidative burst (61). In line with these observations, the work by Bertholet et al. also reported the induction of SOCS3 mRNA by macrophages infected with either *L. major* or *L. amazonensis*, although the level was lower than macrophages infected with *L. donovani* strains (62).

Multifaceted Regulator

AKT Signalling. Host regulatory serine/threonine kinase, AKT plays important role in cell growth, survival, apoptosis and immune regulation (63) and at times used by pathogenic microbes to ensure persistent survival in host cells (64–66). AKT becomes a profitable target of the pathogens as it can restrict both immune activation and host cell apoptosis (63,65). PI3K are phosphoinositide kinases that interact with membrane receptors to phosphorylate membrane inositol phospholipid substrates and produce the second messengers inositol 3, 4, 5-triphosphates. These second messengers, in turn, recruit and activate AKT (67). AKT pathway has been reported to foster cellular survival, inhibiting host cell apoptosis during infection with *Salmonella*, *Toxoplasma* or *Trypanosoma* (64–66). For establishing infection, the intra-macrophage parasite *L. donovani* needs to inhibit host defence parameters like inflammatory cytokine production and apoptosis. Recently, we demonstrated a mechanistic insight into how by exploiting the single host regulator AKT, *Leishmania* inhibit both these defence parameters by simultaneously exploiting two transcription factors, anti-apoptotic β-catenin and pro-apoptotic Forkhead box protein O1 (FOXO-1) (68). *Leishmania* inhibits GSK-3β thereby activating β-catenin and also inactivates nuclear translocation of FOXO-1, thereby ensuring cell survival and immune deactivation (68).The macrophage surface molecules that interact with *Leishmania* leading to the activation of PI3K/AKT pathway have not yet been identified. It is noteworthy that *L. major* infection did not activate PI3K/AKT pathway in Schwann cells (69), but this may not be surprising as Schwann cells lack the molecules required to trigger PI3K/AKT activation.

Potential Compounds as Anti-Leishmanial Agents

There is an urgent requirement for safe, oral and cost-effective drugs for the treatment of VL. Currently two anti-leishmanial front-line therapies are miltefosine and amphotericin B. Delamanid (OPC-67683), is an approved drug for multi-drug resistant tuberculosis, but recently found to be a potent inhibitor of *L. donovani* both *in vitro* and *in vivo* (70). Delamanid was found to have activity superior to miltefosine, with no mice displaying any overt signs of toxicity and exemplified the therapeutic potential of delamanid (70). However, the exact identification of target and the metabolites it produces are currently under study which hinders our ability to accurately predict the outcome of delamanid treatment in VL patients. In summary, the data available suggest that delamanid has the potential to be repurposed as a VL therapy, but additional VL animal model studies exploring the effect of extended delamanid dosing should be investigated. Close congeners (compounds 9, 12, 14 and 18) of naturally occurring β-nitrostyrenes were also found to have *in vitro* antileishmanial activities against intracellular amastigotes (71). These compounds were found to be nontoxic against mammalian macrophages even at a concentration of 25 μM. From the data, it is clearly understood that the alkyl substitution at β position highly influences the biological activity against *L. donovani* promastigotes and amastigotes. However,

detailed mechanistic studies are still under progress. A number of dihydropyrimidine-based derivatives to make specific interactions in *Pteridine reductase 1* (PTR1) active site of *L. major* are also designed recently to study their efficacy as anti-leishmanial compound. These compounds have been found to have potential to eradicate both visceral and topical leishmaniasis (72). But here also the studies are in the very preliminary stages and detailed study is required.

Immunomodulators

Multidrug resistance has become a major concern in combating infection recently. Therefore, emerging repertoire of immunomodulators of natural, synthetic and recombinant origin is a ray of hope for the treatment of various viral, bacterial, parasite and fungi-associated diseases and can prove as an important constituent of effective antimicrobial therapies. The potential significance of immunomodulators in treatment of experimental leishmaniasis gained momentum with the discovery of imiquimod as anti-leishmanial compound (73,74). Imiquimod is an agonist for TLR7, which is present on macrophages and DCs and promotes the development of Th1 immune response (75). After that, several other synthetic compounds such as S_2 complex (an organic complex of copper chloride, ascorbic acid and nicotinamide) (76), acetyl salicylic acid (77) and immunomodulatory peptide from cystatin (78) have been demonstrated to possess both immune-modulating and antileishmanial activities. Curdlan is a β-(1, 3) glucan, reported to possess immunomodulatory and pharmacological properties (79). Curdlan at the dosage of 10 mg/kg/day, almost completely eliminated liver and spleen parasite burden in experimental BALB/c mouse model of VL, owing to the production of disease-resolving Th1 cytokines and Th17 cytokines IL-17 and IL-23 (80). 18β-Glycyrrhetinic acid (GRA), obtained from the root of the medicinal plant licorice (*Glycyrrhizzaglabra L)* has been reported to induce dominant Th1 immunity against *Candida albicans* surface mannan extract (81). Potent anti-leishmanial activity of GRA was found during *in vivo* administration of GRA in BALB/c mouse model of VL which almost completely eliminated liver and spleen parasite burden (82).This anti-leishmanial effect of GRA was found to be mediated through NO upregulation, proinflammatory cytokine expression and NF-κB activation (82). An adipocyte-derived hormone, leptin, was also found to be capable of regulating the immune response, in *L. donovani*-infected mice. It was observed that recombinant leptin treatment reduced splenic parasite burden by increasing NO and pro-inflammatory cytokine production in the splenocytes, indicating host-protecting Th1 response (83). NO-mediated killing of *Leishmania* parasites by tannins and related compounds has also been demonstrated (84). Different plant secondary metabolites and extracts have been found to induce IL-12 up-regulation, indicating the efficacy of many natural resources as anti-leishmanial drugs. Stimulated macrophages also produced IL-18, which along with IL-12 stimulates IFN-γ production and aids in parasite clearance (85,86). *Pelargonium sidoides* extracts have been found to induce IL-18 mRNA during *L. major* infection (87). Licarin A, treated *L. major*-infected macrophages manifestated a decline in IL-6 as well as IL-10 cytokine levels (88).

CONCLUSIONS

We have tried to elucidate the various signalling cascades of the host that are manipulated by *Leishmania* to promote replication inside the macrophages, which are 'sentinels of the immune system'. Some of the mechanisms used by *Leishmania spp*. may also be true for other intracellular pathogens, which similarly inhibit immune cell function for their survival and disease progression. A comprehensive understanding of the different strategies employed by the parasite to neutralize the huge and robust defensive machineries of the host macrophages for successful survival and replication will not only broaden our understanding of the basic biology and pathophysiology of leishmaniasis but also will help in developing effective therapeutic regimens, which may be applicable not only for leishmaniasis but also for other macrophage-associated diseases. Although many decades ago, macrophages have been identified as the host cells for *Leishmania*, but several questions concerning the strategies used and the molecules secreted by these parasites to establish themselves inside the cells, still remain to be answered. However, this review tried to address some of those questions many remained unanswered providing significant scope of research in this area in future.

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