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Review Article

Leishmania Interferes with Host Cell Signaling to Devise a Survival Strategy

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The protozoan parasite *Leishmania spp.* exists as extracellular promastigotes in its vector whereas it resides and replicates as amastigotes within the macrophages of its mammalian host. As a survival strategy, *Leishmania* modulates macrophage functions directly or indirectly. The direct interference includes prevention of oxidative burst and the effector functions that lead to its elimination. The indirect effects include the antigen presentation and modulation of T cell functions in such a way that the effector T cells help the parasite survive by macrophage deactivation. Most of these direct and indirect effects are regulated by host cell receptor signaling that occurs through cycles of phosphorylation and dephosphorylation in cascades of kinases and phosphatases. This review highlights how *Leishmania* selectively manipulates the different signaling pathways to ensure its survival.

1. Introduction

Leishmaniasis, caused by the protozoan parasite of the genus *Leishmania*, is an infection that occurs primarily in the tropical and subtropical regions of the world. *Leishmania* is a dimorphic protozoan parasite that resides as an extracellular flagellate-promastigotes—in its sand fly vector and as an intracellular aflagellate-amastigotes—in macrophages of its mammalian host [1]. Leishmaniasis is characterized by the parasite-induced immunosuppression executed not only by active subversion but also by immune deviation such that the resulting immune responses suppress the antileishmanial immune response further. Because macrophages are not only the host cells for the parasite but also sentinels of the immune system, these cells are targeted by the parasite for immune modulation to ensure their survival. The parasite interferes with the signaling system of the cell such that the effector functions triggered by various cell surface receptors are either actively suppressed or are altered to result in the immune responses that promote parasite survival. A variety of mechanisms potentially contributing to mononuclear phagocyte deactivation during intracellular infection have been identified [2]. Of considerable interest is the evidence that intracellular pathogens are able to impair cell signaling pathways required for host cell activation that may eventuate in their elimination [2, 3]. Cell signaling is regulated by two principal classes of enzymes, protein kinases and phosphoprotein phosphatases [4, 5] (Figure 1).

As the signals are transduced in cascades of kinases and phosphatases through cycles of phosphorylation and dephosphorylation, the parasitic interference often targets these signaling intermediates [6]. Herein, we will analyze the alterations in the signaling of some receptors in *Leishmania*-infected macrophages and will associate those alterations with the altered responsiveness of the macrophages to the ligands of those receptors.

2. *Leishmania* Modulates the Receptor Responsiveness in Macrophages

2.1. Regulation of CD40 Responsiveness and Mitogen Activated Protein Kinase Family. The interaction between CD40, a costimulatory molecule expressed on macrophages, B cells, and dendritic cells [7], and its ligand CD40 ligand (CD154) on T cells [8] results in Th subset skewing to Th1 type. Consistent with the proposition that Th1 cells are responsible for protection against *Leishmania major* infection, the CD40-deficient

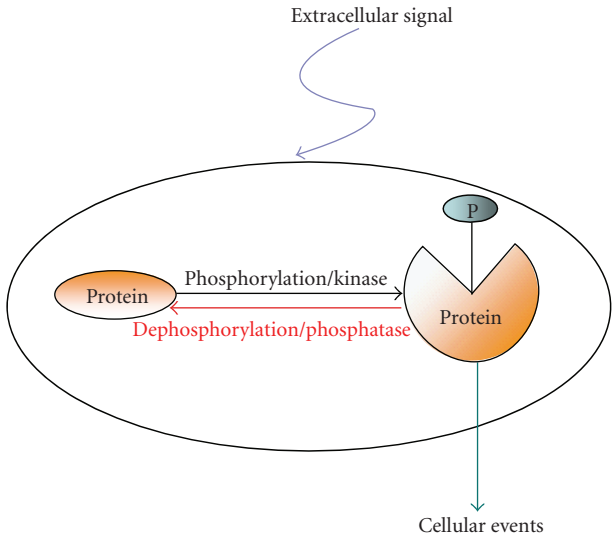

Figure 1: General principles of signal regulation by kinases and Phosphatases: the phosphorylation and dephosphorylation of the protein, the former being catalyzed by a kinase, and the latter by a phosphatase. Phosphorylation usually causes a conformational change in the protein.

mice fail to develop a Th1 response and are susceptible to *Leishmania* infection [9]. The susceptibility to *Leishmania* infection can be prevented by IL-12 administration in these mice suggesting that CD40–CD154 interaction is required for the production of IL-12, which polarizes the Th cells to Th1 type [9–11]. Thus, the host-protective function of CD40 was attributed to setting a Th1 bias [9, 10, 12]. Beside their role in Th1 immune response, CD40-CD40L interactions were also shown to stimulate macrophages to produce a number of cytokines and inflammatory mediators including nitric oxide (NO), which plays a key role in parasite killing [13]. As CD40-L binds to CD40, it triggers the signal through several signaling intermediates [14] to result in mitogen-activated protein kinase (MAPK) phosphorylation [15, 16]. The MAP kinases play an important role as signal kinases and their activity is elicited upon phosphorylation of threonine and tyrosine residues in a Thr-X-Tyr motif in their regulatory domain and thereby controls the activation status of transcription factors [17]. There are three major groups of MAP kinases in mammalian cells—the extracellular signal-regulated protein kinases (ERK) [18], the p38MAP kinases [19], and the c-Jun NH2-terminal kinases (JNK) [20]. MAPKs phosphorylate selected intracellular proteins, including transcription factors, which subsequently regulate gene expression by transcriptional and posttranscriptional mechanisms [21]. Each of these kinases is regulated by other upstream kinases [22]. These three families of MAPKs form three parallel signaling cascades activated by distinct or sometimes overlapping sets of stimuli. Activated by mitogens and growth factors, the ERKs mediate signals promoting cell proliferation, differentiation, and survival. JNK and p38 MAPKs are predominantly activated not only by stress such as osmotic changes and heat shock but also by inflammatory cytokines TNF-α and IL-1β and bacterial lipopolysaccharide (LPS) [23–25].

Several studies show that MAPKs are actively repressed and cannot be activated when *Leishmania*-infected macrophages are stimulated with a variety of agonists. Inhibition of MAPK phosphorylation resulted in less expression of IL-12 and iNOS2 (inducible nitric oxide synthetase type 2), the enzyme that catalyzes the production of NO [26, 27] which has been shown to play crucial role in the development of immunity to *Leishmania* [28]. In naive macrophages, *Leishmania donovani* promastigotes failed to activate the phosphorylation of p38 MAPK, ERK1/2, and JNK, as well as the degradation of IκB-α [29] affecting the activation of proinflammatory cytokines. The parasite surface molecule LPG has been implicated in the inactivation of MAPKs, since phagocytosis of LPG-deficient *L. donovani* promastigotes caused MAPK activation, without the requirement for subsequent macrophage stimulation [29].

One of the studies showed that ERK and p38 MAPKs play differential roles in the regulation of LPS-stimulated inducible NO synthase and IL-12 gene expression [30]. LPS stimulated ERK, JNK, and p38 MAP kinases in J774 macrophages but with different activation kinetics. It was also demonstrated that p38 plays an essential role in the induction of inducible NO synthase, and ERK MAP kinases play only a minor role in promoting NO generation by using inhibitors selective for ERK (PD98059) and p38 (SB203580). It was also demonstrated that synthetic *Leishmania* lipophosphoglycans act by stimulating ERK MAP kinase to inhibit macrophage IL-12 production thus promoting parasite survival and thus underlining the physiological relevance of these regulatory signals [30].

In addition, the CD40-induced p38MAPK phosphorylation, iNOS2 expression, and antileishmanial function were impaired in *Leishmania*-infected macrophages but were restored by anisomycin, a p38MAPK activator, suggesting a crucial role of p38MAPK in CD40 signaling. Anisomycin's effects were reversed by SB203580, a p38MAPK-specific inhibitor, emphasizing the role of p38MAPK in CD40-induced iNOS2-dependent leishmanicidal function. Thus anisomycin's ability to restore CD40 signaling and eliminate amastigotes not only highlighted the susceptibility of amastigotes to killing after p38MAPK activation but also suggested a potential use of anisomycin as an antileishmanial drug [31].

While interference with CD40-induced p38MAPK is consistent with the general suppressive scheme of parasitism, the observation does not explain the CD40-induced IL-10 production from macrophages [32] and increased IL-10 production from *Leishmania*-infected macrophages [33]. Since IL-10 is a suppressive cytokine, these observations support the proparasitic role of IL-10 but contradict our results. This is because *Leishmania* interference with the CD40 signaling through MAPK, if it were associated with IL-10 production as well, would inhibit IL-10 production and clearly, that was not the case. So, it is possible that there are other signaling pathways or MAPKs carrying the CD40 signal and associate with IL-10 production. Indeed, it was observed

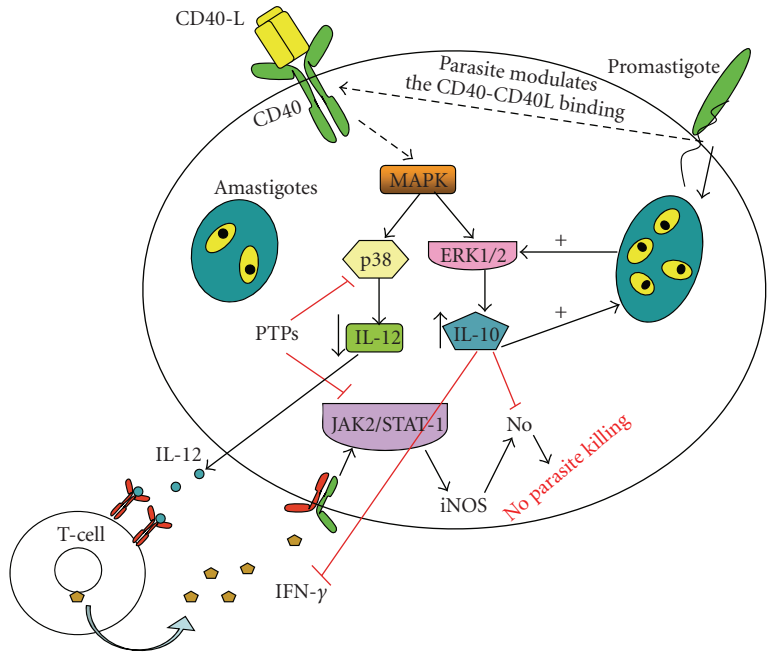

Figure 2: Modulation of CD40 responsiveness in *Leishmania*-infected macrophages: crosslinking of anti-CD40 antibody activates p38 MAPK-induced leishmanicidal function via iNOS2 induction. *Leishmania* infection downregulates CD40-induced p38 MAPK phosphorylation and uses the capability of this receptor to signal along an ERK1/2-dependent pathway to produce the proparasitic Th2 cytokine IL-10 from macrophages. iNOS2: inducible nitric oxide synthase 2; NO: nitric oxide; PTP: Protein tyrosine phosphatases.

that CD40 induced ERK-1/2 activation, inhibition of which resulted in decreased CD40-induced IL-10 production [21]. In *Leishmania*-infected macrophages, CD40-induced ERK-1/2 activation was increased suggesting a reciprocal interaction between the p38MAPK and ERK-1/2 activation [34]. Indeed, inhibition of one MAPK activated the other MAPK. In macrophages, higher strengths of stimulation induced p38MAPK phosphorylation but weaker strengths resulted in ERK-1/2 phosphorylation [34]. During *Leishmania* infection, the level of CD40-induced ERK1/2 phosphorylation and IL-10 production increases, whereas p38MAPK activation and IL-12 production decrease, demonstrating a reciprocal modulation of the CD40 signaling pathway by the parasite [34] (Figure 2). IL-10 produced during the infection inhibits CD40-induced IL-12 production by impairing p38MAPK activation [34]. Neutralization of CD40-induced IL-10 enhances the antileishmanial functions of CD40. Thus, the anti-leishmanial function of CD40 is self-limited by induction of IL-10. The work of Yang et al. also demonstrated a critical role for ERK activation in the induction of IL-10 production by *Leishmania* and showed that parasite immune complexes bind to macrophage FcγR and induce this activation via the macrophage FcγR [35].

Although first observed in *Leishmania* infection in macrophages, the same principle of differential CD40 signaling holds true in dendritic cells and in tumor models [36–38]. In one study it was shown that infection with *L. amazonensis* amastigotes inhibited the ability of DCs to undergo proper maturation in vivo characterized by significantly low CD40 surface expression and significantly decreased IL-12p40 production through activation of the MAP kinase ERK1/2 [39].

While the differential CD40 signaling and its selective manipulation by *Leishmania* solved the apparent paradox of inducing counteractive cytokines by CD40 stimulation, the question remained to be solved is how a single receptor induces reciprocal signaling pathways and counteractive effector functions. We have shown that such differential signaling depends on the composition of the signalosomes assembled on the membrane. When CD40 binds TRAF-2,3,5, it signals primarily through p38MAP kinase whereas binding to TRAF-6 signals primarily through ERK-1/2. We have shown that cholesterol influences the assembly of distinct CD40 signalosomes. Depletion of membrane cholesterol inhibited the assembly of the p38MAP kinase inducing CD40 signalosome but enhances the ERK-1/2 activation [40]. Consistent with these observations, *Leishmania* is found to deplete membrane cholesterol and enhance CD40 binding to TRAF-6 [40]. However, how *Leishmania* interferes with macrophage cholesterol metabolism remains to be elucidated.

It was demonstrated that the *Leishmania* surface molecule, lipophosphoglycan, stimulates the activation of ERKs, JNK, and the p38 MAP kinase simultaneously but with differential kinetics in J774A.1 macrophage cell line. It was shown that both p38 and ERK MAP kinase activation appears to be necessary for AP-1 activation by LPG and it also induced IL-12 production and generation of nitric oxide demonstrating that *L. donovani* LPG activates pro-inflammatory, endotoxin-like response pathway in J774A.1

macrophages [41]. A recent report [42] pointed to the importance of the metalloprotease GP63 in regulating several important signaling proteins, contributing to downstream changes in global protein tyrosine phosphorylation levels as well as a specific effect on p38 MAPK activation. p38 was inactivated upon infection in a GP63-and protein degradation-dependent manner, which likely involves cleavage of the upstream adaptor TAB1 [42].

2.2. TLR Responsiveness. Of the growing number of receptors involved in the recognition of pathogen-associated molecular patterns (PAMPs) [43], TLRs are considered key players of the innate immune response [44, 45]. This family of receptors is comprised of thirteen members that recognize most of the molecular patterns on pathogens. The recognition of the ligands results in the secretion of inflammatory mediators such as TNF-α and IL-12 as well as the induction of iNOS2 expression [45–47], leading to host protection.

Following the recognition of a PAMP, the adaptor myeloid differentiation factor 88 (MyD88) is recruited to the TIR (toll-interleukin 1 receptor) domain of the TLR [48]. Next, IL-1 receptor-associated-kinase-1 (IRAK-1) is recruited to the complex and is phosphorylated by IRAK-4 and by autophosphorylation. IRAK-1 dissociates from MyD88 to interact with TRAF6 and activates various cascades, ultimately leading to the activation of MAP kinase pathways, the translocation of NF-κB to the nucleus as well as the secretion of proinflammatory cytokines [49, 50]. Another pathway, termed "MyD88-independent", is implicated in signaling following engagement of TLR3 and TLR4. This cascade uses TRIF as an adaptor protein and allows the translocation of NF-κB to the nucleus and the activation of MAP kinase pathways with a slower kinetics as well as the activation of IP-10 and IFN-α/β via the activation of IRF3 [51, 52].

Substantial studies demonstrated that different receptors mediate the uptake and phagocytosis of *Leishmania spp.* by macrophages, although the initial signaling events are unknown [53]. As LPG of *Leishmania* promastigotes interacts with NK cell-expressed TLR2 [54], it is possible that the leishmanial LPG may interact with the macrophage expressed TLR2 and modulates cellular functions to ensure its survival within the host cell. For example, *L. major*-induced IL-1α expression was substantially decreased in MyD88-deficient mice [55]. Similarly, the genetically resistant C57BL/6 mice became susceptible to *Leishmania* parasite in absence of MyD88 due to increased level of IL-4 and decreased level of IFN-γ and IL-12p40 [56]. Furthermore, silencing of TLR2, TLR3, IRAK-1, and MyD88 expression by RNA interference also revealed the involvement of both TLR2 and TLR3 in the production of NO and TNF-α by macrophages in response to *L. donovani* promastigotes [57]. TLR2-mediated responses are dependent on Galβ1, 4Manα-PO_4 containing phosphoglycans, whereas TLR3-mediated responses are independent of these glycoconjugates. TLR3 also plays a role in the leishmanicidal activity of the IFN-γ-primed macrophages [57]. It is quite possible that *Leishmania* may modulate MyD88 expression and recruitment to TLRs resulting in altered TLR responsiveness of the infected macrophages.

An impaired resistance to *L. major* was also reported in TLR4-deficient mice. Compared to wild type controls, the growth of parasites in the cutaneous lesions was drastically increased in mice from a resistant background carrying a homozygous mutation of the tlr 4 gene (TLR4 e/e) as early as one day after inoculation of *L. major*. Later in the infection, an enhanced arginase activity leads to the production of compounds essential for parasite proliferation in macrophages and its increase in mutant mice indicating that TLR4 signaling could enhance the microbicidal activity of macrophages harboring parasites [58]. Results from studies comparing TLR4 deficient mice with TLR4 and IL-12β2 double deficient mice suggested an IL-12 independent role of TLR4 in anti-*Leishmania* immunity [59]. The IL-12 dependent NK cell IFN-γ response was severely compromised in TLR9-deficient mice as well. In studies with *L. infantum* infection, in mature dendritic cells- (mDCs-) depleted mice, the IFN-γ response was abolished due to low IL-12 production that could be rescued by CpG and IL-12 [60]. *L. major* is also shown to modulate TLR9 signaling for activating NK cells [61]. Likewise, *L. donovani* infection caused suppression of TLR2- and TLR4-stimulated IL-12p40, with an increase in IL-10 production in cells of monocyte/macrophage lineage by suppressing p38MAPK phosphorylation and activating ERK-1/2 phosphorylation through a contact-dependent mechanism [62]. These studies imply how *Leishmania* modulates the TLR responsiveness that might help their survival in macrophages.

2.3. IFN-γ Receptor Responsiveness. It is widely accepted that IFN-γ plays a critical role in controlling *Leishmania* infection by inducing macrophage leishmanicidal activity as well as by favoring Th1 development [63, 64]. The biological functions of IFN-γ are mediated via IFN-γR-(IFN-γ receptor-) mediated pathway involving receptor-associated kinases JAK1/JAK2 and STAT-1 [65, 66]. IFN-γ binding to the receptor activates JAK1/JAK2 kinases and phosphorylates STAT-1, which translocates to the nucleus and enhances transcription of IFN-γ-induced genes to increase macrophage microbicidal activity (Figure 3). IFN-γR is comprised of IFN-γRα and IFN-γRβ chains. While IFN-γRα chain plays a critical role in ligand binding, IFN-γRβ is required for IFN-γ signal transduction [67]. The critical role of IFN-γR in development of IFN-γ-mediated host immunity is evident in studies showing that IFN-$\gamma R^{-/-}$ mice are highly susceptible to pathogens such as *Mycobacterium avium* [68], *Listeria monocytogenes* [69], *Candida albicans* [70], and *Plasmodium berghei* [71]. It has been shown that IFN-$\gamma R^{-/-}$ mice are highly susceptible to high as well as low dose *L. major* infection indicating that IFN-γR is essential for control of cutaneous leishmaniasis [72]. *L. donovani* has also been shown to attenuate IFN-γR expression in human monocytes [73]. Some of the important macrophage functions suppressed by *Leishmania* which are IFN-γ inducible are NO production, MHC class II expression. One of the major results of *Leishmania* infection

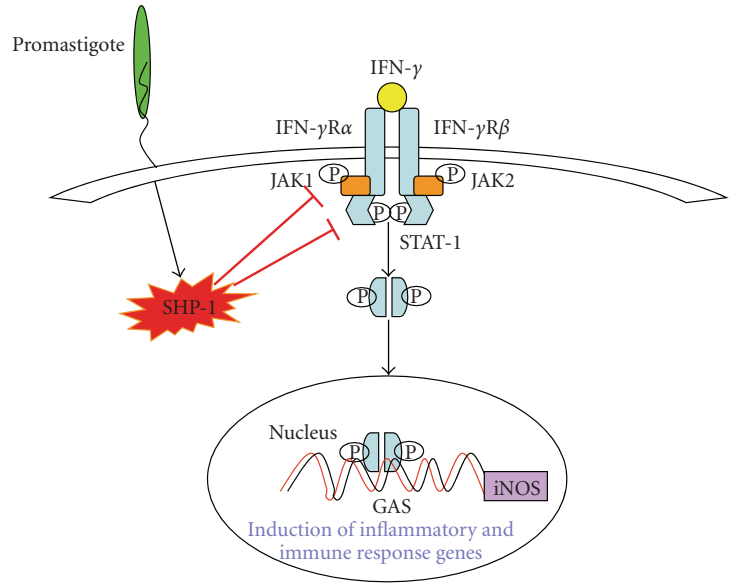

Figure 3: STAT1 phosphorylation regulation through IFNγ receptor: infected macrophages display reduced levels of total and phosphorylated JAK1 and JAK2 and attenuate IFN-γ induced STAT-1 phosphorylation in macrophages, aiding the parasites in escaping host immunity. GAS: Interferon gamma-activated site.

is the inhibition of the JAK2/STAT1 signaling cascade. Infected macrophages show defective phosphorylation of JAK1, JAK2, and STAT1 on IFN-γ stimulation [73, 74]. This inactivation depends on the activation of phosphotyrosine phosphatases (PTPs), in particular the PTP, SHP-1. One study has shown that inactivation of JAK2/STAT1 is caused by the negative regulation of the IFN-γ receptor in infected cells [75]. However, this was not observed following *L. amazonensis* amastigote infection, where IFN-γ-dependent regulation of MHC class I was not affected by infection, indicating that the primary signaling lesion lays downstream of the IFN-γ receptor [76]. Certain bacterial and viral pathogens have been shown to evade host immunity by downregulating IFN-γRα expression on effector cells [77–79]. Similarly, both *L. major* and *L. mexicana* suppressed IFN-γRα and IFN-γRβ expression, reduced levels of total Jak1 and Jak2, and downregulated IFN-γ-induced Jak1, Jak2, and STAT1 activation, with the effects more profound with *L. mexicana* infection than *L. major*. In addition *L. mexicana* preferentially enhanced tyrosine phosphorylation of dominant negative STAT1β, which may be one of the several survival mechanisms used by this parasite to evade the host defense mechanisms [80]. Recently it was shown that infection of DCs with *L. amazonensis* parasites resulted in multiple alterations in innate signaling pathways, including a protease- and proteosome-dependent decreased phosphorylation of STAT1, 2, 3 and ERK1/2, and markedly reduced expression of interferon regulatory factor-1 (IRF-1) and IRF-8. Furthermore, it was shown that alterations in intracellular signaling and suppression of IL-12 production were caused by direct effects of amastigotes rather than by the induction of endogenous IL-10 [81].

2.4. IL-10 Receptor Responsiveness. IL-10 is a homodimer with 160aa and belongs to class II α-helical cytokine. IL-10 is produced by many cell types including T cells, monocytes, and macrophages. IL-10 interacts with its tetrameric receptor complex consisting of two IL-10R1 and two IL-10R2 polypeptide chains [82]. IL-10 is a potent immunosuppressant of macrophage functions, suppresses the production of proinflammatory cytokines by activated monocytes/macrophages, and enhances B lymphocyte proliferation and antibody secretion. IL-10 decreases expression of MHC classes I and II affecting antigen presentation [83] and reduces the transcription and translation of proinflammatory cytokines TNFα, IL-12, and IL-18 from macrophages [84]. IL-10 also suppresses the induction of iNOS2 that catalyzes the production of NO, the leishmanicidal free radical [85–87]. IL-10 affects T cells mostly in an indirect manner, by its effects on antigen presenting cells [88].

IL-10 is a critical cytokine in determining the host susceptibility to *Leishmania* infection. In murine models of cutaneous [89] and visceral [90] leishmaniasis, IL-10 contributes to disease progression. IL-10-deficient or anti-IL-10 receptor antibody-treated mice are relatively resistant to *Leishmania* infection [91], while the administration of exogenous IL-10 [92] or the induction of endogenous IL-10 exacerbates the disease [93]. It has been reported that the susceptibility of BALB/c mice to *L. major* infection is dependent on IL-10 as IL-4R alpha$^{-/-}$ BALB/c mice, despite the absence of IL-4/IL-13 signaling remains highly susceptible to *L. major* infection [94]. Similarly, the IL-10 levels in patients with *L. donovani* infection directly correlate with the disease severity [95].

It was demonstrated that macrophage IL-10 is turned-on by *Leishmania* amastigotes itself and the virulence factor responsible for this induction was found to be host IgG [33]. The host IgG present on the surface of *Leishmania* amastigotes interacts with FcγR on the surface of macrophage to trigger signaling events that leads to the induction of IL-10; however, unopsonised amastigotes fail to do so. IL-10 produced by infected macrophages prevents macrophage activation and thus inhibits production of proinflammatory cytokine IL-12 and TNFα. FcγR KO (mice lacking all FcγRs) mice similar to IL-10 KO mice are resistant to *L. mexicana* infection and also control parasites as a result of strong IFNγ response [96]. Ligation of FcγR on macrophages with IgG on the parasite surface induces IL-10, which in turn suppresses IFNγ response and NO production in *L. mexicana* infection. Further it was also shown that FcγR III is crucial for macrophage to produce IL-10 and disease progression in *L. mexicana* infection [97].

MAPK ERK1/2 has been shown to be involved in induction of IL-10 from macrophages [35]. IgG opsonised amastigotes interact with FcγR on macrophages to induce ERK1/2 activation. The hyperactivation of ERK1/2 results in histone H3 phosphorylation of IL-10 promoter making the promoter accessible to transcription factor, and the result is secretion of high levels of inhibitory cytokine IL-10. However lesion-derived amastigotes alone are not sufficient to induce IL-10; despite their activity to rapidly activate ERK1/2, some inflammatory signal is required for induction of IL-10. This inflammatory stimulus can be small fragments of hyaluronan called as LMW-HA which is a major component of extracellular matrix. Also leishmanial lesions are generally superinfected with bacteria which can provide inflammatory signal through TLR2 or 4. Role of ERK1/2 MAPK in inducing IL-10 has also been demonstrated in CD40-CD40L interaction; lower dose of anti-CD40 stimulation induces more ERK1/2 MAPK phosphorylation and IL-10 secretion in *L. major* infection while inhibition of ERK1/2 reduces CD40 induced IL-10 secretion and disease progression [34].

The IL-10/IL-10R interaction engages the phosphorylation and activation of receptor-associated janus tyrosine kinases, JAK1 and Tyk2, which in turn phosphorylate transcription factor STAT3. It then homodimerizes and translocates to the nucleus where it binds with high affinity to STAT-binding elements (SBEs) in the promoters of various IL-10-responsive genes [98]. STAT3 plays a dominant mediator of majority of IL-10 functions [99]. Overexpression of dominant negative STAT3 suppresses the IL-10 promoter activity while wild type STAT3 leads to enhancement of this activity [100]. The anti-inflammatory functions of IL-10 are STAT3 dependent as in STAT3 deficient murine macrophages IL-10 is unable to suppress LPS-induced TNF-α and IL-6 production [101, 102]. In human macrophages, IL-10 rapidly induces SOCS3 protein expression and this expression requires STAT3 as STAT3 dominant negative human macrophages failed to induce IL-10-mediated SOCS3 expression [103].

3. Alterations of Host Cell Kinases and Phosphatases by *Leishmania*

3.1. Protein Kinase C. PKC, serine-threonine kinases with several isoforms are involved in a wide variety of immune cell functions and are classified as classical, novel, and atypical PKC depending on their structure and cofactor requirement [104]. A number of studies have implicated PKC in the control of host defense against intracellular infections. Indeed, *Leishmania* infection inhibits PKC activation and subsequent intracellular signaling. PKC-dependent oxidative burst activity and protein phosphorylation were found to be markedly attenuated in *Leishmania*-infected human monocytes [105]. Promastigote LPG has been shown to inhibit PKC activation and PKC-dependent phosphorylation of both the PKC-specific VRKRTRLLR substrate peptide and MARCKS (Myristoylated alanine-rich C kinase substrate) [106]. Another PKC substrate protein, MRP (MARCKS-related protein), levels were also found to be decreased in infection with all species or strains of *Leishmania* parasite, including lipophosphoglycan-deficient *L. major* L119 [107]. LPG-mediated inhibition of PKC activation may be due to the ability of LPG to interfere with binding of regulators, including Ca^{2+} and diacyl glycerol; in addition LPG can also block PKC membrane insertion [108]. LPG also inhibits phagosomal maturation, by inhibiting PKC-α dependent depolymerization of periphagosomal F-actin [109, 110]. Further infection with *L. major* inhibited PKC-dependent c-fos and TNFα gene expression [111]. *L. donovani* infection selectively inhibited Ca^{2+}-dependent PKC activity but Ca^{2+}-independent PKC activity was enhanced. *Leishmania* infection reduced the Ca^{2+}-dependent PKC isoform-PKCβ-expression whereas expression of PKC zeta, a Ca^{2+}-independent PKC isoform, was enhanced [112]. This decrease in Ca^{2+}-dependent PKC activity can be due to IL-10 produced by *L. donovani* infection as pretreatment with anti-IL-10 neutralizing antibody significantly restored Ca^{2+}-dependent PKC activity [113]. Infection of macrophages with *L. donovani* enhanced the level of intracellular ceramide largely due to its de novo synthesis and the enhanced ceramide was found to be responsible for the downregulation of classical PKC activity, upregulation of Ca^{2+}-independent atypical PKC-zeta expression, and activity of calcium independent PKC [114]. Also C-C chemokines particularly macrophage inflammatory protein- (MIP-) 1 alpha and macrophage chemoattractant protein- (MCP-) 1 were found to restrict the parasitic burden via the regulation of impaired PKC signaling and induction of free-radical generation in murine leishmaniasis. These chemokines restored Ca^{2+}-dependent PKC activity and inhibited Ca^{2+}-independent atypical PKC activity in *L. donovani*-infected macrophages under both in vivo and in vitro conditions [115]. de Almeida-Amaral et al. reported the presence of protein kinase C-like (PKC-like) protein in *L. amazonensis* and found that this PKC-like protein is activated by phorbol ester (PMA) and has both calcium dependent and independent PKC-like activity. Further they studied the role of this PKC-like protein in modulation of promastigotes $(Na^{++}\ K^{+})$ATPase activity and found that activation of Ca^{2+}-dependent PKC-like protein

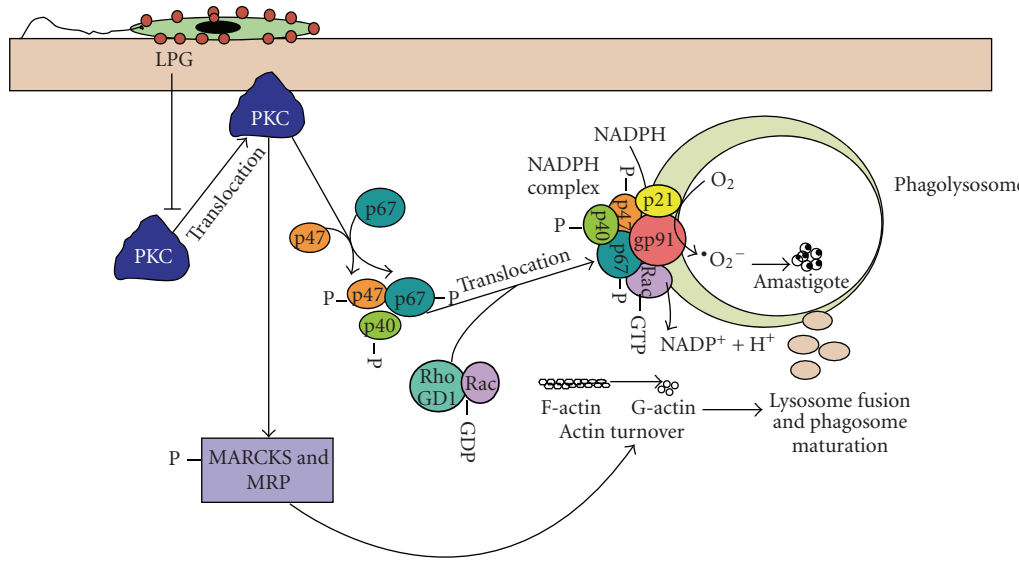

Figure 4: PKC regulation in *Leishmania* infected macrophages: LPG of *Leishmania* inhibits PKC activation and translocation to the membrane. PKC is responsible for phosphorylation of p47phox and p67phox components of NADPH oxidase which are subsequently translocated to phagosomal membrane to form NADPH oxidase complex, which is responsible for superoxide anion generation and hence parasite killing. Further PKC phosphorylates myristoylated alanine-rich C kinase substrate (MARCKS) and MARCKS-related protein (MRP) which are involved in actin turnover and finally in phagosomal maturation and lysosomal fusion resulting in parasite killing. As PKC activation is inhibited by *Leishmania,* this results in subsequent inhibition of all the above mentioned processes thereby favoring parasite survival.

increased $(Na^{++}\ K^{+})$ATPase activity while activation of Ca^{2+}-independent PKC-like protein has inhibitory effect [116]. Recently a study by the same group reported presence of ecto-PKC in different *Leishmania* species. They found higher PKC activity in infective stationary stage of *Leishmania* promastigotes and that this PKC-like plays a critical role in attachment and internalization steps involved in the parasite invasion process [117]. Some of the *Leishmania*-PKC interactions are summarized in Figure 4.

3.2. Other Kinases. PI3K signaling activated by *Leishmania* infection is a negative signaling pathway which helps in progression of disease. It has been shown that PI3K signaling negatively regulates IL-12 production and inhibition of PI3K signaling by specific inhibitor or its downstream kinase Akt reverses the IL-12 blockade in macrophages [118]. $PI3K^{-/-}$ DCs show enhanced IL-12 production and $PI3K^{-/-}$ mice elicit an enhanced Th1 response upon *L. major* infection [119].

4. Modulation of Phosphatases

Leishmania can also activate various molecules that inhibit intracellular signaling cascades (Figure 5) thereby evading host immune machinery to inhibit immune responses.

4.1. SHP-1 Protein Tyrosine Phosphatase. An important negative regulatory molecule of numerous signaling pathways, such as those related to the actions of interferons [120, 121], erythropoietin [122, 123], and many others, is SHP-1 (Src homology 2 domain containing tyrosine phosphatase) which is expressed principally in haematopoietic cells but also in smooth muscle [124] and epithelial cells [125]. Many of the interactions of SHP-1 with its substrates involve the binding of either one or both of its tandem SH2 domains to tyrosine phosphorylated, immunoreceptor tyrosine-based inhibitory motifs (ITIMs). These specialized motifs are known to be present in many signaling molecules [126, 127]. Multiple types of ITIMs exist and display-specific abilities to recruit and activate SH2 containing PTPs.

SHP-1 is responsible for the negative regulation of many signaling pathways in all hematopoietic cell types, by acting in a variety of fashions. For instance, SHP-1 can bind to receptors and dephosphorylate them directly; it can also associate with a receptor and dephosphorylate other members of the receptor binding complex. The PTP can also interact with other cytosolic proteins and tyrosine dephosphorylates them or their associated proteins [126]. Macrophages infected with *Leishmania* in vitro have elevated SHP-1 activity as well as total PTP activity, resulting in widespread dephosphorylation of high-molecular-weight proteins [74]. Furthermore, infection causes colocalization of SHP-1 and JAK2 and prevents tyrosine phosphorylation of JAK2 in response to IFN-γ [74]. Dephosphorylation of JAK1/2, TYK2, and STAT1α, -2, -3, -5 α/β, and -6 has already been documented [121–123, 128, 129]. Forget et al. showed that activation of the host PTP SHP-1 is responsible for the dephosphorylation and inactivation of ERK1/2, as SHP-1-deficient macrophages showed normal JAK2 and ERK1/2 activity following infection with *L. donovani*, and responded to IFNγ by increased NO production [130].

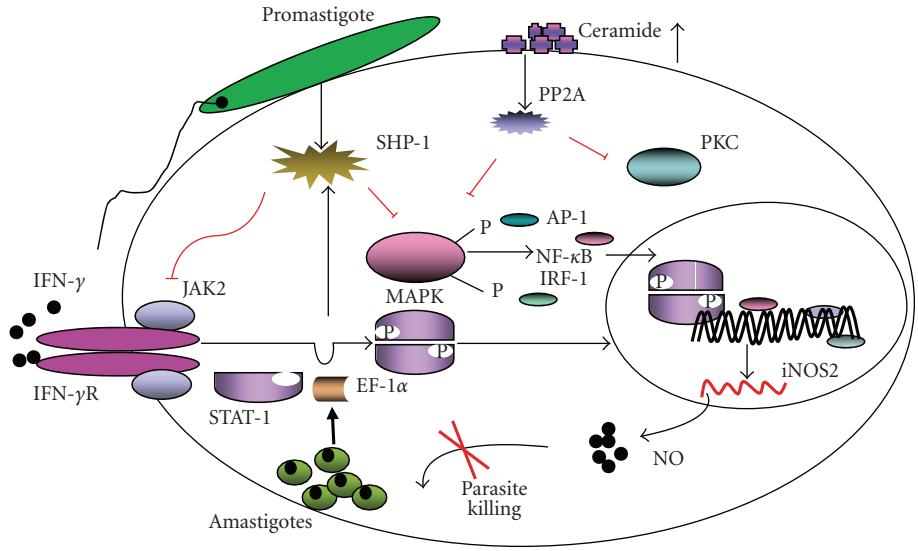

Figure 5: MAPK phosphorylation in *Leishmania*-infected macrophages: *Leishmania* infection of macrophages represses the most important MAPK family members: ERK1/2, p38, and JNK. MAPK inactivation is accompanied by inhibition of transcription factors Elk-1, c-fos, IRF-1, AP-1, and NF-κB and IFN-γ-dependent NO generation.

In viable motheaten mice, whose SHP-1 phosphatase activity is deficient, increased nuclear translocation of the transcription factor NF-κB has been reported [131, 132], which seems to provoke an exacerbated inflammatory response. Macrophages derived from SHP-$1^{-/-}$ mice show elevated iNOS induction and NO generation and are more efficient at killing *Leishmania* [133]. This is reflected in vivo by increased NO generation and reduced parasite load in both SHP-1-deficient mice and mice treated with chemical PTP inhibitors like peroxovanadium [133–135]. Some studies have demonstrated that the inhibition of IFN-γ-dependent phosphorylation cascades following infection is due to activation of host cell tyrosine phosphatases [74, 134]. This was associated with a phenotype of cell deactivation in which MAP kinase signaling, c-FOS, and iNOS expression were each defective. Importantly, inhibition of phosphotyrosine phosphatase activity with sodium orthovanadate before infection prevented development of the deactivated phenotype [136]. Studies aimed at understanding the mechanism responsible for the change in activation state of SHP-1 led to the identification of *Leishmania* EF-1α as a modulator of host SHP-1 and also suggesting it to be a novel virulence factor contributing to macrophage deactivation [137].

Recently one study revealed that upon *Leishmania* infection, SHP-1 is able to rapidly bind to and inactivate a critical kinase (IRAK-1) in TLR signaling pathway. This regulatory binding was shown to be mediated by an evolutionarily conserved motif identified in the kinase. This motif was also present in other kinases involved in Toll signaling and therefore could represent a regulatory mechanism of relevance to many kinases. This work reports a unique mechanism by which *Leishmania* can avoid harmful TLR signaling [138].

4.2. Other Phosphatases. Ceramide is also capable of activating protein phosphatases such as protein phosphatase 1 (PP1) and PP2A [139–141]. It is through these protein phosphatases that ceramide inhibits kinases such as the classical as well as novel PKC isoforms and Akt [138–142]. It was observed that endogenous ceramide generated during leishmanial infection led to the dephosphorylation of protein kinase B (Akt) in infected cells. Ceramide induced the PKCζ-Akt interaction along with the serine/threonine phosphatase PP2A [143].

However a phosphosphotyrosine phosphatase PTEN (phosphatase and tensin homologue deleted on chromosome ten) is reported to play a protective role against *L. major* infection as the mice lacking PTEN are more susceptible to the infection than the WT mice. PTEN deficient macrophages have reduced ability to kill parasites in response to IFN-γ treatment, showing decreased TNF-α production, iNOS expression, and NO secretion but more IL-10 secretion than WT cells. Thus the study shows that phosphatase PTEN is required for efficient clearance of intracellular parasite in macrophages [144].

5. Conclusion

There are multiple ways by which intracellular pathogens like *Leishmania* make use of host cell's machinery in order to survive and replicate. One such mechanism is the distortion of host macrophage's own signaling pathways to selectively repress or enhance the expression of various cytokines and microbicidal molecules and antigen presentation. The interplay between various signaling molecules is complex. As signaling pathways can be pharmacologically manipulated, a better knowledge of their role and the mechanisms whereby

they regulate host immune cell functions and pathogen growth should permit the development of new therapies to control infectious agents.

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