CHAPTER- II

REVIEW OF LITERATURE

2. Review of literature

2.1 Interaction of *Leishmania* parasites with macrophages:

Macrophages are the major components of innate immune system described by Elia Metchnikoff and it provides the first line of defense to protect the body from invading pathogens. The main function of macrophage is to engulf the pathogens such as, protozoan parasites and microbes that enter into the system by the process known as phagocytosis. In response of phagocytosis of pathogens and these immune cells activates various cytokines, chemokines, and growth factors for clearance of pathogens [1]. Macrophages are phagocytic cells responsible for the management of pathogens, and in case of adaptive immunity these cells are key regulators of recognition, processing, and antigen presentations to T cells [2]. Macrophages play a crucial role in the internalization and infection of *L. donovani* parasites. Infection process by *L. donovani* promastigotes into the host macrophage cells can be summarized into the following steps, (i) Phagocytosis of infective promastigotes by the interaction of the cell surface molecules of parasite and macrophage, (ii) Transformation promastigotes into amastigotes is mediated by the parasite virulence factors by downregulating host macrophage immune activation avoiding phagosomal maturation, (iii) Intracellular survival and replication of the amastigotes by altering macrophage activation status from Th1 to Th2 phenotype and impairment of defensive expression of pro-inflammatory cytokines and associated molecular signalling pathways, and (iv) Lastly, the infected macrophages, containing amastigotes, can serve as a reservoir for the parasite, allowing it to incorporate into the other tissues and organs through the bloodstream and lymphatic system contributes to the spread of the infection to other parts of the body. The destiny of the parasites inside infected macrophage is determined by the balance between the host and parasite factors that regulate the activation/deactivation of macrophages. *Leishmania* and macrophage interaction has been associated to a variety of parasite and macrophage surface molecules. The binding and attachment of promastigotes to macrophage surfaces involves the complement receptors (CR)1, CR3 (Mac-1), fibronectin receptor, and mannose-fucose receptor (MR) [3]. Macrophage polarization between M1 and M2 phenotpe

critically controls the fate of infection and disease progression by *Leishmania* promastigotes [4,5]. Lipophosphoglycan (LPG) is the primary determinant of promastigote uptake and phagocytosis into the host macrophages through competent membrane-associated receptors, present in macrophage cells which are independent of macrophage inflammatory status. The priming effect of LPS and IL-4 on parasite clearance or parsite survival through type 1 interferons is reported previously [6]. Interaction of parasite and host macrophage cell surface molecules-mediated altered immune activation facilitates the downregulation of pro-inflammatory Th1 cytokines TNF-α, IL-1β, and IFN-γ which promotes parasite burden and survival in the host macrophages, and on the other hand, parasites promotes exaggerated induction of T_{reg} cytokines IL-10 and TGFβ further results into arginase-1 upregulation and parasite survival [7].

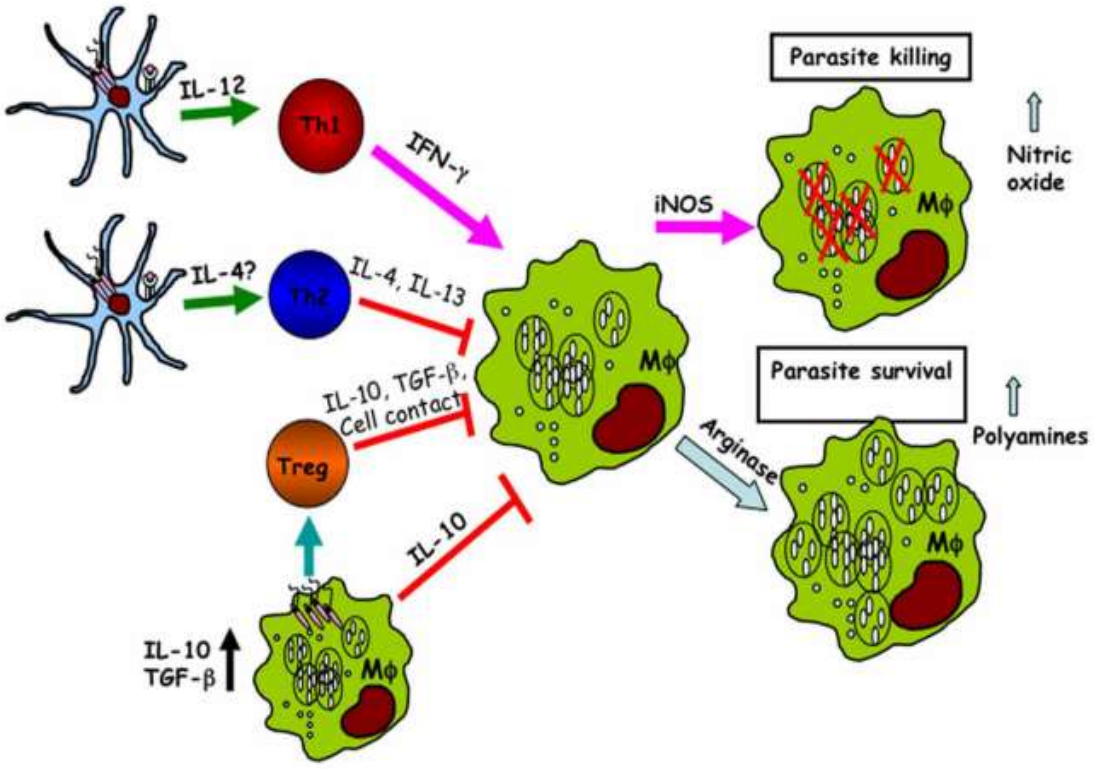

Fig 2.1: Macrophage regulates the infection *Leishmania* outcome. Source: This image was adopted from Liu, D., et al. The early interaction of *Leishmania* with macrophages and dendritic cells and their influence on the host immune response. *Frontiers in cellular and infection microbiology*, 2, p.83, 2012.

2.2 Role of Th1 and Th2 cytokines in leishmaniasis:

The balance between Th1 and Th2 responses determines how *Leishmania* infection will turn out. A dominant Th1 response, which aids in regulating parasite replication, is typically linked to disease resistance. A strong Th2 response, on the other hand, impairs the host's capacity to effectively manage the infection and is linked to infection progression [4,5]. Pro-inflammatory cytokines such as IFN-γ, IL-2, IL-12, and TNF-α plays protective role in parasitic infection, whereas, IL-10, IL-6, IL-17, and TGF-β are reported for their role in parasite survival and disease progression [8].

Th1 subpopulation of lymphocyte activation of M1 macrophages is primarily categorized based on the cytokine production, particularly IFN-γ and TNF-α. These two major cytokines are essential for the removal of intracellular parasites and the death of parasites in macrophage cells as a protective and inflammatory response to oxidative stress. In contrast, the activation of Th2 lymphocytes results in the production of two important cytokine modulators, IL-4 and IL-13, which induces the M2 profile, which is determined by the synthesis of polyamines through the activation of arginase and the production of urea and L-ornithine. This M2 profile favors intra-macrophagic parasite survival and proliferation, which results in the systematic progression of disease [9-11]. The immune response to visceral leishmaniasis (VL), with the parasites *Leishmania donovani* or *Leishmania infantum*, is significantly influenced by interleukin-4 (IL-4). IL-4 has a variety of roles in the setting of VL and can influence how the infection develops in both positive and negative ways. Immune response that is Th2-dominated predominates in the beginning phase of infection in VL. Moreover, IL-4 can decrease the Th1 response together with other Th2 cytokines including IL-10 and IL-13. This suppression of the Th1 immune response might make it more difficult for macrophages to activate and eliminate intracellular parasites [12]. According to earlier research, IL-4 pre-incubation causes macrophage phenotype to change from effective phagocytosis to impaired phagocytic activity in *N. meningitidis*. This is mediated by downregulating Akt phosphorylation and altering the MAPK signaling pathway. Treatment with IL-4 increased absorption and led to intracellular parasite death in *Trypanosoma cruzi*. Research has already demonstrated that in *L.*

major-infected BALB/c mice, early IL-4 neutralization causes a full elimination of IL-4-producing CD4+ T cells and a reduction in IFN-producing CD8+ T cells. Together, these T cell-mediated responses result in the elimination of the *L. major* parasite and the development of immunopathology [13-15]. The Toll-like receptor 4 (TLR4) on immune cells is strongly activated by the gram-negative bacteria's lipopolysaccharide (LPS). Through the NF-κB transcription cascade, TLR4 activation causes the synthesis of pro-inflammatory mediators such as TNF-α and IL-1β, but *Leishmania* LPG mediates the downregulation of these pro-inflammatory cytokines for their survival inside macrophage cells [16]. Immune tolerance and the reduction of excessive inflammation are two functions of regulatory T cells (Tregs). Treg cytokines, such as TGF-β and IL-10, help to suppress the pro-inflammatory Th1 response, which enables the parasite to elude immune deactivation.

In leishmaniasis control IFN-γ plays a crucial role in host macrophage cells. The potential that the parasite may have hampered this route is not completely ruled out by *Leishmania*-induced macrophage malfunction, such as faulty NO and MHC expression in response to IFN-γ. Studies have actually explained this deactivation of macrophages to *Leishmania*-mediated disruption of the JAK2/STAT1 pathway. It has been reported that *L. donovani* infection reduces IFN-γ induced tyrosine phosphorylation and preferentially reduces IFN-γ induced Jak1 and Jak2 activation and phosphorylation of Stat1 in both differentiated U-937 cells and human monocytes [17-19]. It has also been demonstrated that *L. donovani* infection in host macrophages reduces the expression of IFN-γR alpha subunit and induces the transitory expression of cytokine signaling 3 (SOCS3), which has also been demonstrated to negatively affect IFN-γ signaling. Recently, it was shown that *L. donovani* amastigote inhibits IRF-1 expression while having no impact on the levels of STAT1 protein. Subsequent studies shown that the IFN-induced STAT1α connection with the nuclear transport adaptor importin-5 is hampered in macrophages that have been infected with *L. donovani* amastigote and as a result of this reduction of IRF-1 production leads to the poor nuclear translocation of STAT1 [20-22]. In order to aid in its intracellular survival, *L. donovani* infection triggers the endogenous release of IL-10 by selectively impairing PKC-mediated signal transduction [23].

2.3 Involvement of TLR4 receptor in parasite internalization:

Toll like receptors are pattern recognition receptors (PRRs) that specifically targets for the pathogen-associated molecular patterns (PAMPs) and regulates the innate responses to infections. TLRs trigger innate reactions in different ways, causing inflammatory cytokines to be produced by macrophages, various subtypes of dendritic cells (DCs), and type I interferons (IFN) to be produced by inflammatory monocytes, macrophages, and DCs [24]. Infected macrophages also lose their ability to respond to successive challenges with the TLR4 ligand LPS, a trait connected to the phosphoglycans of parasites [25,26]. Cumulative research findings suggested that few *Leishmania*-derived molecules could precisely activates TLR2, TLR4, and TLR9. In macrophages, *L. major* activates the promoter region of IL-1 but not IL-6, IL-8, or IL-10 through a route that is reliant on MyD88 [27]. The formation of the protective IL-12-mediated Th1 response against *L. major* in C57BL6 resistant mice was later found to need MyD88-dependent pathways, since MyD88/mice infected with *L. major* exhibited a nonprotective Th2 response [28]. Infection displayed larger non-healing lesions and low plasma levels of IL-12 which indicates efficient anti-parasite immunity associated with TLR-mediated responses. Despite the absence of ulcerating lesions, it was also discovered that the greater vulnerability of MyD88/ mice to *L. major* infections was associated with increased levels of IL-4 [29]. The fact that SOCS1 directly inhibits TLR4 signaling pathways [30] demonstrates how *L. major* initial activation of TLR2 might eventually result in the suppression of other TLR responses. Studies revealed that TLRs are involved in the recognition of the parasite and their intracellular burden during VL is based on the negative association between the expression of TLR2 and TLR4 and due to IL-12 or IFN-γ expression [31]. Through parasite-dependent contact, *L. donovani* infection of human THP1-derived macrophages reduces TLR2 and TLR4-stimulated IL-12 release and increases IL-10 production. In context, of the significance of the TLR4 receptor in VL, earlier studies demonstrated that TLR4 promotes TGF-β1-regulated expression of SHP-1 and ubiquitin-edited enzyme A20 that serve to increase *L. donovani* infection. As a result of the inhibition of MAPKp38 phosphorylation and activation of ERK1/2 phosphorylation, human THP-1 derived macrophages infected with *L. donovani* promastigotes suppressed TLR2 stimulated IL-12 release with

increased production of IL-10. This further advanced to the intracellular proliferation and establishment of infection. The macrophage SHP-1 has also been altered by *L. donovani* and *L. major* to reduce the activity of kinases such as IRAKs involved in downstream TLR signaling [33]. In LPS-stimulated macrophages, parasites extended the induction of iNOS or COX-2 expression, increased PGE2 and NO generation, and upregulated arginase-1 expression. TLR4 was also required for the activation of iNOS, COX-2, and arginase-1, providing evidence for the idea that this TLR suppresses inflammation in macrophages during *L. mexicana* infections, eventually inhibiting the synthesis of IL-12 [34].

2.4 Role of parasite lipophosphoglycan (LPG) in leishmaniasis:

Lipophosphoglycan (LPG) is a prominent glycoconjugate present on the surface of promastigote stage of *L. donovani*. The relationship between the host and the parasite in both vertebrate and invertebrate hosts depends on this pleiotropic virulence factors. LPG inhibits the complement system, promotes macrophage opsonization, hinders phagolysosome maturation, and prevents protein kinase C activation in the early stages of infection. LPG is composed of a Gal1,4Man-PO4 repeating polymer that is joined to a lysophosphatidylinositol membrane anchor [35]. Toll-like receptors (TLRs), including TLR2 and TLR4 are effectively activated by the LPGs of several *Leishmania* species. Purified LPG from *L. major* stimulates TLR2 and causes NF-κB to translocate to the nuclear envelope through MyD88. Purified LPG from *L. infantum* and *L. braziliensis* has antagonistic effects on New World species of *Leishmania*. Pro-inflammatory LPG from *L. braziliensis* and *L. mexicana* activates the ERK-1/2, JNK, and p38MAPK through TLR2. The capacity of *L. infantum* LPG to progressively induce JNK and p38 following MAPK activation is an intriguing aspect of the pathway activation profile, as opposed to the relatively transitory profile associated with *L. braziliensis* LPG. It has been found that NO, TNF-α, and IL-6 to be induced by *L. amazonensis* LPGs through TLR4 and but were not capable of NF-κβ translocation [36-44]. LPG is transferred from the parasite to the host macrophage membrane during phagocytosis, where it accumulates in the peri-phagosomal region and hinders the union of the phagosome and lysosome. According to reports, LPG slow down phagosomal maturation by

integrating into the lipid rafts of the host macrophage cell membrane [45]. Reports displayed that CRP receptor mediated *L. donovani* promastigote phagocytosis facilitates defective activation of macrophages, resulting into parasite survival and replication by modulation of host defensive attributes [46]. The Rho-family GTPase Cdc42's aberrant retention at the phagosome is the cause of the peri-phagosomal buildup of F-actin mediated by LPG. Research studies unveiled that by overexpressing the dominant-negative Cdc42N17 mutant in RAW 64.7 macrophages further prevented LPG-mediated peri-phagosomal F-actin accumulation. LPG also prevents Protein Kinase C (PKC)-α from adhering to the phagosome membrane. PKC-α was demonstrated to play a key role in peri-phagosomal F-actin breakdown. It has yet to be proven if PKC-α exclusion by LPG from phagosomes harboring *L. donovani* promastigotes is responsible for the peri-phagosomal buildup of F-actin [47,48].

Reports highlighted that LPG is a essential for changing host immune activation in *L. donovani* by preventing phagosome-lysosome maturation, preventing NADPH oxidase assembly, and upregulating IL-10 and TGF-β, which in turn causes arginase-1 induction and persistent parasite survival without negatively affecting the host microenvironment. Because lpgl-deficient mutant *Leishmania* was unable to infect mice and establish the infection in macrophages, it has been suggested that the LPG molecule in promastigote plays a crucial role in the formation of macrophage infection [49]. LPG-TLR interactions increase ERK phosphorylation with decrease in p38MAPK phosphorylation that modifies the production of reactive oxygen species and nitric oxide and reduces the release of pro-inflammatory cytokines [50-55].

2.5 Alteration of MAPK signaling pathway by leishmaniasis:

The mitogen-activated protein kinases (MAPKs), a group of serine/threonine-specific protein kinases, regulate the accessory and effector functions by controlling the production of pro-inflammatory cytokines and NO in macrophages [57]. The ubiquitous transcription factors activating protein 1 (AP-1), NF-κB, and IFN regulatory factors (IRFs), among others, are phosphorylated by these kinases once

they are active resulted in response to a variety of signaling cascades [57-59]. It has been shown that *L. donovani* infection in macrophages alters the MAPK pathway, which in turn encourages parasite survival and growth inside the host cell. MAPK mediated impairment of ERK motably attenuates AP-1 and NF-κB activation and the production of NO in infected macrophages [60]. Additionally, these findings are consistent with other studies showing that infection of naive macrophages with promastigotes of *L. donovani* evades activation of MAPKs, impairing the generation of pro-inflammatory cytokines. Moreover, it has also been demonstrated that IFN-γ treatment of macrophages before infection causes the activation of p38 MAPK and ERK1/2 as well as the generation of pro-inflammatory cytokines [61]. Infection with *L. donovani* considerably reduces the expression of TLR2 and TLR4 which stimulates the production of IL-12p40, and boosts the production of IL-10 by inhibiting p38 MAPK phosphorylation and activating ERK1/2 phosphorylation through a contact-dependent mechanism. Furthermore, TLR activation causes the phosphorylation of p38 MAPK and ERK1/2, which produces IL-12 and IL-10, respectively [54, 62, 63].

2.6 Imidazo[1,2-α] pyridine derivatives as potential anti-leishmanial agents:

Currently available first- and second-line of anti-leishmanial medications have serious adverse effects such drug resistance, multi-organ toxicity, and protracted treatment regimens. The heterocycles are a common structural element found in commercially marketed anti-cancer medications [64]. In recent years, heterocyclics, a class of synthetic organic chemicals, have been increasingly popular among researchers as a safer and more effective anti-leishmanial agents. Numerous heterocyclic motifs have been studied in recent years for their potential as strong anti-leishmanial therapeutics [65-70]. The imidazole moiety is a member of the heterocyclic organic molecule with potent anti-microbial, anti-proliferative, anti-tumor, and anti-cancer capabilities, [71,72]. The heterocyclic chemical molecules including triazoles, chalcones, chromone, thiazoles, thiosemicarbazones, indole, and imidazole are gained interest among the researchers for finding effective therapeutic candidates with lesser side effects in managing VL. The hunt for a viable treatment option with fewer side effects for treating VL has sparked interest in

imidazoles. It is widely known that the structural element of imidazopyridine moiety contains a nitrogen heterocycle that is useful in biology. Among the several imidazopyridine derivatives, imidazo[1,2-α] pyridine is particularly notable because it is already well recognized for its anti-proliferative characteristics [73]. Development of imidazo[1,2-α] pyridine derivatives by coupling of imidazo[1,2-α] pyridine with the 2-amino-4H-pyran moiety has already been described and shown to be potent in inducing apoptosis in non-small cell lung cancer [75]. A possible core pharmacophore imidazo[1,2-α] pyridine was reported to reduce the leishmaniasis [74]. In particular, amino pyran, a member of the pyran family of heterocycles, has been studied further for its potential to be both cytotoxic and microbicidal, making it a good candidate for the synthesis of imidazo[1,2-α]pyridine derivatives. The structural element of imidazopyridine moiety contains physiologically active nitrogen heterocycles [73]. One of the several imidazopyridine derivatives is imidazo[1,2-α] pyridine, which is already well-known for its anti-proliferative qualities [74]. In addition, it was found that imidazo[1,2-α] pyridine was employed as a possible central pharmacophore for leishmaniasis mitigation. A good option for the synthesis of imidazo[1,2-] pyridine derivatives is amino pyran, which specifically belongs to the pyran family of heterocycles and has acquired further insight into its cytotoxic and microbicidal potential [76-79]. All of these studies demonstrated the therapeutic value of the heterocyclic molecule imidazole, and its imidazopyridine derivatives may enable us to conduct more effective therapeutic interventions for the management of VL in near future.

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