Role of exogenous chemokines as immunotherapeutic tool against visceral leishmaniasis.

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Visceral leishmaniasis (VL), caused by the protozoan parasite, *Leishmania donovani*, is associated with immunological dysfunctions. Recent observations in humans and mice suggest a rapid change in chemokine expression, pointing towards their possible involvement in the disease process. Chemokines form an integral part of the host defense against pathogens and the release of chemokines is regarded as a crucial step in cellular recruitment thereby facilitating the initiation and maintenance of inflammatory responses that is required for counteracting the microbial invaders. Here in this review, we have highlighted the importance of the exogenous CC and CXC chemokines as potential immunotherapeutic tools to counteract *Leishmania* induced pathogenesis.

Key words: Leishmania; chemokines; macrophages; immune response.

1. Visceral leishmaniasis (VL):

1.1 Overview:

Leishmaniasis, a tropical disease, is one of the major health problems facing mankind which occurs in the skin as localized cutaneous (CL) and mucocutaneous (MCL) leishmaniasis to diffuse cutaneous leishmaniasis (DCL), whereas in the viscera they range from subclinical to potentially fatal disease [1]. World Health Organization estimates an incidence of 12 million cases among 350 million at risk and an annual incidence of 1.5 million to 2 million new cases of CL and 500,000 new cases of VL [2]. Leishmaniasis is caused by protozoan parasites belonging to the genus *Leishmania*. These parasites are transmitted by the bite of a Phlebotomine sandfly [3]. VL, the most severe form of leishmaniasis, is caused by *Leishmania donovani* and *Leishmania chagasi* in the Old and New worlds, respectively [4, 5] and is associated with high parasite numbers and the absence of an effective T helper cell type 1 (Th1) immune response [1].

2. Intracellular survival of *Leishmania* parasites:

*Leishmania* has the ability to survive in the macrophageal lysosomal compartment, an intracellular site normally inhospitable to invading microorganisms [4]. Intracellular parasitism of macrophages by *Leishmania* culminates in all the clinical and pathological symptoms associated with leishmaniasis and is favored by several parasites derived factors [6-8]. The inflammatory reactions initiated by the host macrophage during the entry of *Leishmania* occur in two stages. Firstly, during the initial uptake and phagocytosis of promastigotes, the macrophage produces toxic free radicals, including superoxide anions (O$_2^-$) [9, 10]. Exposure to O$_2^-$ has been reported to be fatal to the *Leishmania* promastigotes [9-11] but its generation is inhibited by *L. donovani* infection [12, 13] which appears to be dependent upon cell surface molecules, lipophosphoglycan (LPG) and glycoprotein (GP) 63 [14, 15]. Secondly, even after infection is established, the quiescent macrophage can be activated to kill intracellular amastigote form of *L. donovani*. This second anti-leishmanial event occurs via the NO generation after activation of macrophages by CC chemokines, interferon (IFN)-γ or tumor necrosis factor (TNF)-α along with lipopolysaccharide (LPS) [16-19]. Infected macrophages incubated with purified *Leishmania* surface molecules, LPG or glycosylphosphoinositol (GIPL) lose their ability to activate inducible nitric oxide synthase (iNOS) [20, 21].

2.1 Cytokine and chemokine mediated regulation of disease:

During *Leishmania* infection, cytokines regulate the host response. The important inflammatory cytokines released by *Leishmania* infected macrophages include interleukin (IL)-1β, TNF-α and IL-12. Additionally, Th1 cells produce IFN-γ whereas; Th2 cells produce IL-4. Dendritic cell derived IL-12 by and natural killer (NK) cell derived IFN-γ are also critical in determining the outcome of the disease [22, 23]. Cytokines have a direct involvement in chemokine production and can also precede the expression of some chemokines, which in turn, induces the production of additional...
inflammatory mediators. Cytokines exert a secondary effect on leukocyte recruitment by inducing the expression of several chemokine genes [24]. In leishmaniasis, cytokines seem to synergize with leishmanial elements to regulate chemokine production. TNF-α and IL-1β, together with macrophage inflammatory protein (MIP)-1α, regulate Langerhans cell-mediated transport of Leishmania from the infected skin to regional lymph nodes (LN) in murine CL [25]. IL-12 is required for the induction of Th1-related chemokines such as lymphotactin, IFN-γ-inducible protein 10 (CXCL10 or IP-10) and monocyte chemotactic protein 1 (MCP-1) in LN of resistant L. major-infected mice [26]. Interestingly, Th1- and Th2-derived cytokines can have antagonistic effects on chemokines. Monokine induced by IFN-γ (MIG) and CXCL10 are more selectively induced by IFN-γ [27]. The Th2-related cytokines IL-4 and IL-13 induce macrophage-derived chemokine (MDC) and CCL6 production in macrophages, and this production is inhibited by IFN-γ [28, 29].

2.1.1 Modulation of host cell signaling:
Several signaling molecules have been implicated in the regulation of antiparasitic host immune response, including members of the protein kinase C (PKC) super-family and mitogen activated protein kinase (MAPK) family [30-33]. MAPKs are a group of serine/threonine kinases, the enzymatic activity of which is elicited upon phosphorylation of threonine and tyrosine residues in a Thr-X-Tyr motif in their regulatory domain [34]. It includes extracellular signal-related kinase 1 and 2 (ERK1/2), c-jun NH2-terminal kinase (JNK) and p38MAPK. MAPK phosphorylate selected intracellular proteins, including transcription factors, which subsequently regulate gene expression by transcriptional and post-transcriptional mechanisms [35]. 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 low expression of IL-12 and iNOS2 [36, 37] which has been shown to play crucial role in the development of immunity to Leishmania major [38]. CD40-induced p38MAPK phosphorylation, iNOS2 expression, and antileishmanial function were impaired in Leishmania major-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 [39].

Activation of MAPKs depends on PKC [35], suggesting that Leishmania parasites can impair signaling through these enzymes in infected cells. PKC is a calcium and phospholipid dependent serine/threonine kinase that exists as a family of different isoforms having closely related structure (30, 32, 40). The fourteen known isoforms of PKC family have been subdivided into the classical (cPKC; α, β-I, β-II, γ), novel (nPKC; δ, ε, η, θ, μ) and atypical (aPKC; ζ, τ, λ) groups. Infection with L. donovani or Leishmania derived glycolipid, lipophosphoglycan (LPG) accounted for impaired PKC activity [41, 42]. PKC regulates phagosome maturation, as the iso-enzymes α-and β-PKC are associated with phagosomal membrane [43, 44]. Leishmania and Leishmania derived LPG impairs the PKC dependent signal transduction in macrophages thus helping the parasite to survive in the macrophagal microenvironment [12-14, 31]. Accordingly, BALB/c peritoneal macrophages infected with UR-6, LPG deficient attenuated leishmanial parasite [42, 45], enhanced the PKC-β activity. The decrease in Ca2+-dependent PKC β activity might be due to IL-10 produced by L. donovani infection as pretreatment with anti-IL-10 neutralizing antibody significantly restored Ca2+-dependent PKC β activity [45].

3. Chemokines:

3.1 Classification and structure:
Chemokines are a homologous super family of relatively small proteins ranging from 8 to 17 kDa [46, 47]. The super family of chemokines is subclassified on the basis of the arrangement of cysteine residues located in the N-terminal regions of these molecules. These are designated C, CC, CXC, and CXXC, where C represents the number of N-terminal region cysteine residues and X represents the number of intervening amino acids [47-49]. CXC (α) chemokines have their first two consensus cysteines separated by an amino acid; the CC (β) chemokines have their first two cysteines adjacent to each other. The other two minor subfamilies include the CX3C chemokines and the C chemokines [50]. Chemokines exert their effect through guanosine nucleotide-protein coupled receptors (GPCR) [48]. Like all known GPCR, the chemokine receptors have seven transmembrane hydrophobic domains with three intracellular and three extracellular hydrophilic loops.
### Homologue of human and mouse chemokines

#### 3.1.1 General functions:

Chemokines regulate distinct signal transduction pathways culminating in a variety of biological functions including integrin activation and cell migration along a chemokine gradient [51]. They provide the directional cues for the movement of leukocytes in development, homeostasis, and inflammation. Leukocytes extravasation from the blood into the tissues is a regulated multistep process involving a series of coordinated interactions between leukocytes and endothelial cells [49, 52]. Extravasated leukocytes follow a gradient of chemokine concentration and come to rest at a site of high concentration [52]. The dramatic increase in the secretion of chemokines during inflammation results in the selective recruitment of leukocytes into inflamed tissue [48, 53]. They play a vital role in the regulation of immunity to various diseases [54-56] where distinct chemokines and chemokine receptor-integrin combinations are associated with particular diseases by controlling the effector cell migration to their respective infected tissue sites [50].
3.1.2 Protective role in different diseases:
Chemokines form an integral part of the host defense against pathogenic invasion. In bacterial and fungal infections, a rapid inflammatory response is required to counteract the microbial invaders. Under such a situation an induction of chemokine has been observed in several animal models as well as clinical studies [57-59]. Chemokines were found to be induced in the lungs of patients with Mycobacterium tuberculosis infection, in which they are secreted by bronchoalveolar macrophage [59]. Recent studies using chemokine have been focused on blockade or use recombinant chemokine treatment for infectious disease. MCP-1, a CC chemokine, restored the impaired free radical generation and proinflammatory cytokines in mycobacterial pathogenesis [60]. MCP-1 also regulates the pulmonary host defense via neutrophil recruitment during E. coli infection [61]. CC chemokines contribute to the formation of mononuclear phagocyte dependent granulomas viz. MIP-1α is found to induce granuloma in mouse infected with Schistosoma mansoni [62]. In MIP-1α knockout mice a pronounced delay in resolution of viral infection was observed, which were associated with substantially reduced recruitment of CD8+ T cells into the infected lung [63]. Studies have suggested a potentially important role of CC chemokines, MIP-1α, MIP-1β, and RANTES have been identified as suppressive factors for HIV-1 by inhibiting the replication of the macrophage tropic HIV strains [64]. Moreover MIP-1α, MIP-1β and RANTES activate human macrophages to kill Trypanosoma cruzi via NO dependent mechanism [65]. Co-injecting CXCL10 with Vaccinia antigens activates the cytotoxic T cells which render protection against subsequent infectious challenge with vaccinia virus [66].

4. Chemokines and Visceral leishmaniasis

4.1 Changes in chemokine expression during VL:
Initial phase of L. donovani infection is marked by an induction of MIP-1α and MCP-1 which attracts the monocytic cells as a source of Th1 mobilizing chemokines such as CXCL10 [67]. This is accompanied by CCR7 downregulation leading to the impairment of DC migration to lymph nodes contributing to disease progression [68]. Moreover in CCR5-/-, MIP-1α/- or CCR2/- knockout mice there was low antigen specific IFN-γ response during early L. donovani infection [69]. During the late phase, L. donovani infected mice undergo a rapid hepatic accumulation of MIP-1α, MCP-1 and CXCL10 [67, 69]. Whereas, VL patients show elevated concentrations of CXCL9 and CXCL10 in their serum during active infection L. donovani which is critical for pathogenesis [70], thereby suggesting differences in chemokine expression in experimental and clinical VL. Leishmania derived LPG alters the migration of monocytes by decreasing the expression of adhesion molecules and inhibiting the induction and release of MCP-1 [71]. The expression of the genes encoding RANTES, MIP-1α, MIP-1β, CXCL10 and MCP-1 is more strongly upregulated in the air pouch lining of viscerotropic L. donovani-infected animals than in that of dermotropic L. major-infected animals [72], suggesting that leukocyte transendothelial migration could be blocked by L. donovani LPG [73].

4.1.1 Exogenous chemokines host protective immune response in L. donovani infected macrophages:
CC chemokine treatment in the form of MIP-1α and MCP-1 conferred significant protection against L. donovani in an in vitro model of VL [74] by inducing pro-inflammatory cytokines, IL-12 and TNF-α, secretion and inhibiting the anti-inflammatory cytokines, IL-10 and TGF-β, in infected macrophages and also restored the impaired antigen presentation capability of infected macrophages [74, 75] which is critical for generating parasite specific T cell responses [76]. CC
exomkines strongly induced iNOS2 culminating in a potent generation of NO in *L. donovani*-infected macrophages and was at par with the concomitant up-regulation of TNF-α release [74]. In addition to NO, MIP-1α and MCP-1 primed parasitized macrophages showed enhanced the chemotactic migration along with significant ROS generation at early time point in *L. donovani* infected macrophages [77] which were similar to the observation made with antileishmanial drug, sodium stibogluconate [78]. These findings point towards a CC chemokine mediated induction of ROS and NO during early and late phase of infection respectively. Silencing of CCR5, a receptor for MIP-1α, leads to low parasite entry in the macrophages along with enhanced production of NO and proinflammatory cytokines in *L. donovani* infected macrophages [79]. Comparative study with CXC chemokines, CXCL10 and CXCL8, showed that CXCL10 but not CXCL8, could restrict the intracellular parasitic growth by restoring the impaired proinflammatory cytokines (IL-12 and TNF-α) and chemokines (MIP-1α and MCP-1) along with the NO release in *L. donovani* infected macrophages [80] which agrees with an earlier finding that *Leishmania* promastigotes inhibits CXCL10 but induces CXCL8 production [81]. But there was an absence of ROS generation in CXC chemokine primed parasitized macrophages [80] which showcase the difference in the functioning between the CC and CXC chemokines.

4.1.1.1 Exogenous chemokines mediated protection against experimental VL:

*In vivo* studies with MIP-1α and MCP-1 showed that CC chemokines treatment led to significant decrease in liver and spleen parasite load in infected BALB/c mice [77]. CC chemokine treatment resulted in reduced IL-10 mRNA expression along with enhanced IL-12p40, IFN-γ, TNF-α and iNOS mRNA expression in both liver mononuclear cells as well as in splenocytes, reflecting a switch of CD4+ T cell differentiation from Th2 to Th1 as evident by the increase in IFN-γ secreting CD4+ T cells [75] as host protection is completely dependent on the activation of CD4+ T cells producing Th1-cytokines [82]. Similar to CC chemokines, CXCL10 treatment could also significantly restrict the parasite growth in liver and spleen of *L. donovani* infected BALB/c mice which were accompanied by a strong induction of Th1 cytokines like IFN-γ and IL-12 along with subsequent abrogation of Th2 cytokines like IL-10 and TGF-β [80, 83]. Additionally, there was a significant increase in IFN-γ secreting CD4+ T cells along with reduction in TGF-β secreting CD4+ CD25+ T regulatory cells in the spleen of CXCL10 treated *L. donovani* infected mice which is critical for the containment of disease progression [83]. *In vivo* CXCL10 administration also activates the Natural killer (NK) cells which contribute to promote the protective immune response against *Leishmania* [84]. The involvement of the CXCR3, receptor for CXCL10, in the disease process was established using CXCR3 (-/-) C57BL/6 mice which show a delayed onset of hepatic inflammation and granuloma formation after *L. donovani* infection. However, they could mount an efficient Th1 response, recruited T cells to the liver, and controlled parasite growth as efficiently as do CXCR3(+/-) C57BL/6 mice [85].

4.1.1.1.1 Exogenous chemokines rescue the parasite impaired signal transduction during VL:

Treatment with exogenous CC chemokines, MIP-1α and MCP-1, restored the impaired PKC activity during the early hours of *L. donovani* infection. These chemokines restored Ca2+-dependent PKC activity and inhibited Ca2+-independent atypical PKC activity in *L. donovani*-infected macrophages under both *in vivo* and *in vitro* conditions [77]. This restoration of classical PKC activity by CC chemokines led to ROS generation via membrane translocation of cytosolic factors p47phox and p67phox [77] which are the essential components required for the assembly of an active NADPH oxidase complex [86]. These changes in classical and atypical PKC isoforms was also observed in splenocytes of CC chemokine treated infected mice [77]. On the other hand, exogenous CXC chemokine, CXCL10 antagonistically regulate the MAPK signaling during the disease process. At an early time point, p38MAPK phosphorylation was much higher in CXCL10–treated *L. donovani* infected macrophages, compared with infected macrophages; in contrast, ERK1/2 phosphorylation in CXCL10–treated *L. donovani* infected macrophages was much lower than that in infected macrophages [80]. This effective regulation of MAPK signaling by CXCL10 was responsible for enhanced iNOS2 induction and NO production [80]. These findings highlight the importance of PKC and MAPK signaling in the antileishmanial functioning of exogenous CC and CXC chemokines.

5. Conclusion:

These studies on leishmaniasis emphasize on the therapeutic role of exogenous CC and CXC chemokines and their receptors which could regulate the host immunity thereby conferring protection. Further research needs to be undertaken to investigate the role of other novel chemokines in restricting *Leishmania* induced pathogenesis either alone or in combination with standard antileishmanial drugs.
Reference:


