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Pro- and Anti-inflammatory Cytokines in Visceral Leishmaniasis

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Abstract

Visceral leishmaniasis is a vector borne disease caused by *Leishmania donovani* complex. Pro- and anti-inflammatory cytokines play a key role in protecting from the pathogenesis of Leishmania infection, and their balance and dynamic changes may control or predict clinical outcome. Pro-inflammatory cytokines are created primarily to amplify inflammatory reactions; triggers the immune response to Leishmania infection. Even though this cytokine is necessary for a protective response, it may also cause excessive inflammation and collateral tissue damage. For this reason anti-inflammatory cytokines counteract the effects of pro-inflammatory cytokines to limit the inflammation present. However, an excessive down regulation of pro-inflammatory cytokines may favor disease progression. During VL/HIV co-infection, there is a decreased production of macrophage activating cytokines such as IFN-γ, IL-12, IL-15 and IL-18 and increased immunosuppressive cytokines like IL-4, IL-10 and TGF-β. Pro-inflammatory cytokine i.e. IL-6 and TNF-α also have been implicated in the pathogenesis of VL/HIV co-infection by inducing HIV replication. In peoples with VL and/or VL/HIV co-infection microbial translocation into the systemic circulation induces an intense pro-inflammatory response, which in turn activates lymphocyte. Thus, continuous and exaggerated activation causes exhaustion of the T-cell compartment and contributing to immunesuppression. Post kala-azar dermal leishmaniasis may also arise as a complication for VL following treatment. Its immunopathogenesis is not well understood, however, IL-10 is widely accepted as an immunosuppressive cytokine involved in the pathogenesis. Recently, IL-17 has contributed significantly to disease pathogenesis by inducing the production of TNF-α and NO.

Keywords: Pro-and anti-inflammatory cytokines; Visceral Leishmaniasis; VL/HIV co-infection; Microbial Translocation; Post Kala-azar Dermal Leishmaniasis

Introduction

Visceral leishmaniasis (VL), also known as 'kala-azar', is a vector borne neglected tropical disease caused by an obligate intra-macrophage protozoan parasite of the *Leishmania donovani* complex [1]. VL ranked as the second in mortality and fourth in morbidity among tropical diseases. It affects up to 0.4 million people and an estimate of 20,000 to 40,000 deaths occur per year. More than 90% of global VL cases occur in six countries: Ethiopia, Sudan, South Sudan, India, Bangladesh, and Brazil [2]. VL is worsened by co-infections with HIV [3]. Ethiopia is a hot spot for VL/ HIV co-infection: around one-third of patients with VL in North-West Ethiopia are HIV positive. Patients with VL and HIV co-infection reveal an increased susceptibility to drug toxicity, increased leishmaniasis relapses and accelerated progression to AIDS [3,4]. There is also a complication of VL after treatment the so called post kala-azar dermal leishmaniasis (PKDL) [5].

Visceral leishmaniasis affects organs rich in lymphocytes such as spleen, liver, bone marrow and lymph nodes [6,7]. The classical clinical manifestation of VL involves prolonged fever, severe weight loss, hepatosplenomegaly, anemia, leucopenia, and hypergammaglobulinemia [7]. In experimental VL, there is development of organ specific immunity in the two main target tissues of infection i.e. liver and spleen. The liver is the site for resolving acute infection associated with the development of granulomas around infected Kupffer cells and resistance to re-infection. Paradoxically, the spleen is an initial site for the generation of cell mediated immunity (CMI), but ultimately becomes a site of parasite persistence with associated immunopathological changes [8]. In canine VL, natural infection by *L. chagasi* showed splenic architecture disruption, disorganization of normal lymphoid tissue, loss of normal spleen leucocyte diversity via replacement of leucocytes by plasma cells and atrophy of the lymphoid tissue [8].

The Leishmania parasites have digenetic life cycle and exist in two distinct forms: the promastigote in sand fly vector and the amastigote in human host [9]. The infection to human is initiated by injection of metacyclics promastigote, which are then phagocytosed by phagocytes [10]. Internalized promastigotes differentiate into non motile aflagellar amastigotes and replicate inside these cells [9].

Macrophages are specialized for the destruction of invading pathogens and priming of the host adaptive immune response. However, Leishmania parasite surface molecules such as lipophosphoglycan, proteophosphoglycan, glycosylinositol phospholipids and glycoprotein gp63 sub-versioning the host cell signaling pathways and modulating expression of various cytokines, production of microbicidal molecules and antigen presentation. This enables the parasite to evade the innate immune response, multiply within the phagolysome of the infected macrophage and elicit disease progression [11].

The pathology of VL is associated with depressed CMI, characterized by the failure of peripheral blood mononuclear cells to proliferate or to produce appropriate cytokines respond to Leishmania antigen [12]. Recently there is inconclusive statement that microbial translocation (MT) contributes to systemic immune activation and disease progression [6]. Whereas the control of Leishmania infection is

dependent on CMI, involving activated macrophages, T cells, and type 1 cytokines [12].

Cytokines are protein messengers that convey information between and within the immune system via specific cell surface receptor molecules. Cytokines can be divided into pro-inflammatory cytokines and anti-inflammatory cytokines [13]. Pro- and anti-inflammatory cytokines play key roles in protecting from the pathogenesis of Leishmania infection, and their balance and dynamic changes may control or predict clinical outcome [14,15].

Therefore, the aim of this review is to assess the role of pro-and anti-inflammatory cytokines in VL, VL/HIV co-infection and PKDL either in protection and recovery from the disease or susceptibility and progressive nature of the disease, which have a potential use in prevention and control of leishmaniasis. This review also provides information on MT associated immunopathegenesis of VL and/or VL/HIV co-infection, which helps for understanding the pathogenesis of VL beside immunosuppression.

Pro- and Anti-inflammatory Cytokines in Visceral Leishmaniasis

Cytokines are the major orchestrators of host defense processes and playing pivotal roles in modulating the host immune response against Leishmania parasite infection; and determine the resistance or susceptibility nature of the disease. These cytokines can be either pro-inflammatory cytokines or anti-inflammatory cytokines [13-16].

Upon entry of promastigotes into the body of the host, it will be engulfed by dendritic cells (DC) and macrophages. This cell plays a pivotal role in Leishmania infection: participate in innate immunity and act as the first line of defense, secreting pro-inflammatory cytokines and acting as antigen presenting cells to initiate the adaptive immunity [16].

Resolution of Leishmanial infection is dependent on coordinated interactions between components of CMI; activation of targeted T-cell populations for appropriate cytokine production and activation of infected macrophage [12,14]. Studies in human infection and murine model Leishmaniasis have demonstrated induction of a predominant T helper (Th)1 response and interferon gamma (IFN-γ) induced macrophage activation to associate with immunity and healing as it is shown in figure 1 [16-18]. Classically activated macrophages (CAMϕ), induced by stimulation with Th1 cell derived IFN-γ and microbial products like lipopolysaccharide (LPS), produce several pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-12 and IL-1β. Further, it produces co-stimulatory molecules like CD 86, CD40 required for T-cell activation, CD40, and toxic mediators such as reactive oxygen species (ROS) and nitric oxide (NO), through the expression of inducible nitric oxide synthase (iNOS). These molecules play critical roles in the killing of *Leishmania* parasites [19-21].

On the other hand, induction of anti-inflammatory cytokines such as IL-10 and ⁄or IL-4 producing T cells and poor CMI have been shown to correlate with disease dissemination as it is shown figure 1 [14,16,18]. For instance, IL-4 from TH2 cells has been implicated in the generation of alternatively activated macrophages (AAMϕ) that display anti-inflammatory activities. Alternatively activated macrophages produce moderate levels of IL-10 and transforming growth factor (TGF)-β and low or null levels of the pro-inflammatory cytokines, results in susceptibility to Leishmania infection. Additionally, AAMϕs induce high expression of arginase, which competes for its common substrate L-arginine with iNOS and metabolizes L-arginine into urea and ornithine, thereby lowering the levels of NO secretion and also Ornithine is the main intracellular source for the synthesis of polyamines necessary for parasite growth [19-21].

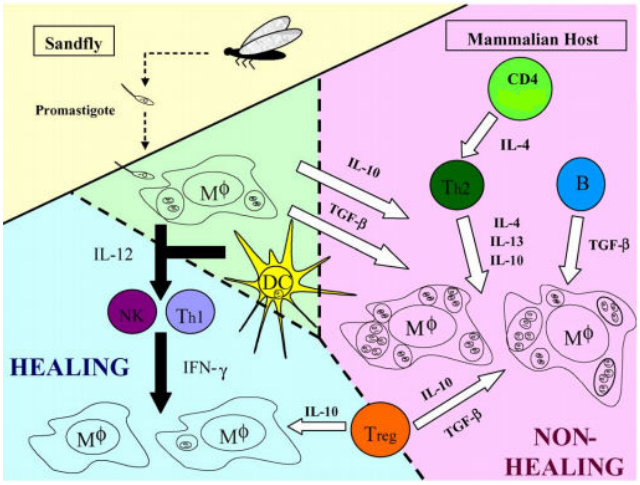

Figure 1: Cytokines associated with healing and non-healing response to Leishmania infection [18].

The precise role of major pro-and anti-inflammatory cytokines in modulating host immune response against visceral leishmaniasis outlined below.

Pro- inflammatory Cytokines in Visceral Leishmaniasis

Pro-inflammatory cytokines are produced primarily to amplifying inflammatory reactions; triggers the immune response to Leishmania infection. The major pro-inflammatory cytokines are TNF-α, IFN-γ, IL-1, IL-2, IL-8, IL-12, IL-15, IL-18 and IL-17 [22].

Tumor Necrosis Factor-α:

In another murine model, when severe combined immunodeficiency mice administered moderate levels of TNF-α, lead to resolution of hepatic infection [26]. Most of the studies in human VL demonstrated that the plasma concentrations TNF-α is higher in active VL relative to asymptomatic infection, cured infection and uninfected controls [27-31]. However, when TNF-α secreted at higher levels, it can have deleterious systemic effects and responsible for the typical manifestation of the disease, such as fever, anorexia, weight loss, increased energy expenditure and cutaneous and mucosal pallor, spleen pathology and hypergammaglobulinemia [32]. Tumor necrosis factor-α mediates the destruction of marginal zone macrophages and stromal cells [8]. On the contrast, low level of TNF-α is found in active VL cases of Indian patients [33] and increased level found in patients cured from VL, it has been taken as a reliable marker of VL that has been cured [34]. Still, there is a discrepancy on the exact level of TNF-α in patient with active VL, so further investigation is required.

Interleukin-1: Interleukin-1 exerts many of the same biological activities as TNF-α; induces the production of vascular cell adhesion molecules (CAM) as well as chemokines. These chemokines and CAM attract and assist leukocytes to enter the inflamed area by a process

known as diapedesis. It also induces the production of colony stimulating factors in the bone marrow, thereby increasing the available number of phagocytic cells that can respond against leishmania parasite [13,23,25].

In a study conducted on mouse and human macrophages infected with *L. donovani* demonstrated, a reduced production of IL1 and/or TNFα following stimulation with LPS. A comparable response was also seen with lipophosphoglycan purified from *L. donovani*, which inhibits the expression of IL1β gene [36]. This suggested that suppression of TNFα and IL1 by Leishmania, support its in vivo survival through the inhibition of macrophage and/or T cell activating effects.

Interleukin-12: Interleukin-12 is a multifunctional cytokine: bridge innate and adaptive immunity, induction of Th1 differentiation (CMI) and suppressing Th2 polarization and IL-4 production. IL-12 induces cell mediated protection against Leishmania infection by promoting IFN- γ production and proliferation and activation of natural killer (NK) and T cells [37].

The critical role of IL-12 in controlling VL has been clearly demonstrated in experimental models of *L. donovani* infection: animals genetically deficient for IL-12 gene or genetically resistant mice treated with anti-IL-12 antibodies fail to control parasite replication and are unable to resolve the infection [38]. Treatment of genetically IL-12 deficient mice by exogenous administration of IL-12 confers resistance to the infection [39]. IL12 is effective in reducing the liver parasite burden by 34–45% in mice that had been infected 2 weeks earlier [40]. In human VL, the concentration of IL-12 was higher in patients with the active disease; this may indicate a strong immune response [27,28,41].

Interferon-γ: The major biological function of IFN-γ is to activate macrophages and enhance the microbicidal activity of these cells to kill intracellular pathogens through the generation of ROS and RNS. In addition, IFN-γ acts on B cells to inhibit switching to IL-4 dependent antibody isotypes, promotes the differentiation of CD4+ T cells to the Th1 subset and inhibits the differentiation of Th2 and Th17 cells and enhances MHC associated antigen presentation [35].

The development of cell mediated immune responses capable of controlling L. donovani complex infection and resolving disease are critically dependent upon IFN-γ [42]. In experimental VL, IFN-γ gene-deficient mice infected with *L. donovani* showed decreased expression of iNOS and finally unable to kill the parasite [43]. In patients with active VL, the production of IFN-γ is not depressed [27,28,31,33,41] but there appears to be unresponsiveness to *L. donovani* antigen. The underlying mechanism of immunologic unresponsiveness remains unknown, but it may be due to elevated level of immunesuppressive cytokines in active VL patients [12,41]. Higher plasmatic levels IFN- γ also associated with clinical manifestation observed in active VL [44].

Interleukin-2: Interleukin-2 is a pro-inflammatory cytokines produced by activated $CD4^+$ Th cells, which has important roles in the immune response and in the killing of Leishmania [45]. IL-2 is a growth factor that drives the growth and differentiation of both T and B cells and induces lytic activity in NK cells. IL-2 and IFN-γ induce the development of Th1 cells, which, in turn, induces macrophage activation and the production of Immunoglobulin (Ig) G1 and IgG3 [35], which may assist cell-mediated immune responses and important for the resolution of Lishmania infection. But IL-2 alone can activate proliferation of Th2 cells and helps to generate IgG1 and IgE producing cells, this may play in non-healing activity of VL [13,35].

Interleukin-8, 15 and 18: VL patients have been found to have elevated plasma levels of IL-8 [33], a pro-inflammatory cytokine produced by tissue resident macrophages in response to Leishmania infections. Interleukin-8 plays a key role in the initial stages of infection or tissue damage; it serves as a chemo attractant for neutrophils, recruiting them to sites of infection [35,44]. On the other hand, IL-15 activates NK cells to produce IFN-γ, induces IL-12 production and stimulates the leishmaniacidal activity of the macrophages. Furthermore, the increased level of IL-15 in acute VL patients can suppress Th2 cytokines such as IL-4 [46]. Interleukin-18 is also a pro-inflammatory cytokine produced by macrophages triggers IFNγ synthesis in NK and T cell cells. It supports IL12 dependent NK cell response to control the disease progression in *L. infantum* infected mice [47].

Interleukin-17: Interleukin-17 is a signature cytokine of Th17. Th17 cells secrete not only IL-17 but also IL-21, and IL-22. IL-21 together with TGF-β is able to induce Th17 differentiation and provides as a positive feedback loop to amplify the precursor of Th17 cells. IL-23 stabilizes differentiated Th17 cells and helps further maturation of Th17 cells by inducing IL-22 [48].

Interleukin-17 is a potent inflammatory cytokines capable of inducing expression of chemokines that promote cell recruitment and granuloma organization throughout infection. In Human VL, infection with *L. donovani* stimulates the differentiation of Th17 cells, which produce IL-17, IL-22, and IFN-γ and protection to VL is strongly associated IL-17 and IL-22 [49]. However, a shift of the response towards excessive IL-17 production may responsible for tissue destruction by matrix metalloproteinase enzyme with massive inflammation and influx of neutrophils [50]. In IL-10 deficient mice infected with *L. major* demonstrated elevated level of IL-17 levels and it was responsible for the severe immunopathology [51]. IL-27 is produced by macrophages and known to be an important regulator of the expression of Th17 cell lineage. In human VL, IL-27 along with IL-21 from T cell sources are suggested to be disease promoting cytokines by promoting the differentiation and expansion of antigen specific, IL-10 producing T cells [52].

Anti-inflammatory Cytokines in Visceral Leishmaniasis

Anti-inflammatory cytokines are a series of immunoregulatory molecules that cytokines counteract the effects of pro-inflammatory cytokines to limit the inflammation present. The major Anti-inflammatory cytokines are IL-6, IL-4, IL-10, IL-13 and TGF-β [22].

Interleukin-6: Interleukin-6 is a pleiotropic cytokine elaborated in response to a wide range of inflammatory stimuli, is ordinarily considered as a pro-inflammatory cytokine [13]. However, it has also inflammatory suppressive activity and impair macrophage activation via inhibit its production ability of TNF-α and IL-1 [53]. A study in human infection demonstrated that production IL-6 associated with the progressive nature of visceral leishmaniasis [33] and responsible for polyclonal activation of B cells and hypergamaglobulinaemia with increased levels of circulating immune complexes [44]. In another study, IL-6 deficient mice infected by *L. donovani* parasite demonstrated that an earlier and rapid parasite killing along with increased levels of circulating IFN-γ, accelerated granuloma assembly and heightened responsiveness to chemotherapy [54].

Interleukin-4: Interleukin-4 is a signature cytokine of Th2: which helps proliferation of Th2 cell population, down regulating of Th1 cell response and inhibiting macrophage function [55]. Cytokine analysis in VL revealed enhanced induction of IL-4 mRNA in tissues and enhanced presence of IL-4 in the circulation of patients with progressive disease [17,31]. However, in vitro addition of mAb against IL-4 did not restore the lymphocyte proliferative response or IFN-γ production in *L. chagasi* stimulated peripheral blood mononuclear cells (PBMC) [56].

In experimental VL, increased parasite load observed in IL-4 and IL-4 receptor α deficient mice correlates with retarded granuloma maturation and antileishmanial activity [57]. IL-4 is also required for the resolution of hepatic infection [57]; this indicated that its role is not well defined. For instance, in murine model of VL the parasite burden of *L. donovani* in IL-4 deficient mice and wild strain was similar and IL-4 deficient mice were more susceptible to VL than their counter parts wild type after chemotherapy, suggesting that IL-4 is necessary for effective chemotherapy in VL [58].

Interleukin-13: Interleukin-13 is a Th2 cytokine which promote Th2 differentiation, correlated with establishment of Leishmania pathogenesis. Susceptibility to infection with several species of Leishmania involves the production of IL-13. On the contrast, IL-13 gene knockout mice infected with *L. donovani* showed impaired IFNγ secretion and incomplete granuloma assembly [57,59].

Interleukin-10: Interleukin-10 is a regulatory cytokine produced by many cell types, including B cells, macrophages, and $CD4^+$T cells; presumed to keep homeostatic network and protect tissue from collateral damage caused by excessive inflammation [22,35]. IL-10 promotes intracellular infection including human VL, by disabling Th1 cell type responses and/or deactivating parasitized tissue macrophages [44,60]. In murine VL model, IL-10 knockout and normal mice treated prophylactically with anti-IL-10R demonstrated accelerated granuloma assembly and rapid parasite killing without tissue inflammation. In these models, there was enhanced expression of IL-12, IFN-γ mRNA and iNOS reactivity, and responsiveness to chemotherapy [61]. In human study, increased plasma levels of IL-10 during active VL have been reported [12,27,31,41] and cure from disease is associated with a fall in IL-10 mRNA levels [62]. The above evidences suggested that IL-10 plays a major role for failure to control the growth and systemic spread of Leishmania parasites in human VL.

The progressive development of splenic pathology is largely associated with high levels of IL-10. Interleukin-10 promotes impaired DC migration into T-cell areas with consequent ineffective T-cell priming. The splenic stromal cell function is also altered, promoting the selective development of IL-10 producing DC with immunoregulatory properties [8].

Importantly, the source of IL-10 in human VL has not clearly defined. Monocytes/macrophages were implicated as the main source of IL-10 in VL, and examination of lymphoid organs of VL patients has revealed an increase in the number of these cells [63]. However, another study demonstrated that macrophages represented only a small percentage of spleen aspirates and were not a major source of IL-10. Natural Treg cells also did not accumulate in the spleen and were not a major source of IL-10, and their removal did not rescue antigen-specific IFN-γ responses and expression of IL-10 mRNA not depressed, rather $CD4^+CD25^-Foxp3^-$ T cells were implicated as a major source of IL-10 [64].

Transforming Growth Factor-β: It is an immune regulatory cytokine produced by macrophage and Treg cells; and plays role in down regulating host protective responses. The production of TGF-β by infected macrophages is also associated with inhibition of IFN-γ production, suppression of macrophage activation, and progressive nature of the disease. Like IL-10 and IL-4, TGF-β is also implicated in the pathology of both experimental and human leishmanial disease progression [65]. The role of TGF-β in VL is not clearly confirmed, but anticipated to act in synergy with IL-10 as suggested by administration of anti-TGF-β mAB decreases IL4 production, but enhances IFN-γ level and promotes resistance to Leishmania species in normally susceptible mice [66].

Pro-and Anti-inflammatory Cytokines in VL/HIV Co-infection

HIV has been identified as one of the major threats to control VL and VL has emerged as an important opportunistic infection associated with HIV. This is because of the increasing overlap between urban regions of high HIV-1 transmission and areas where Leishmania is endemic. Cases of HIV and visceral leishmaniasis co-infection have been reported in 35 countries worldwide [3,4]. In Ethiopia, more than one in three patients with VL is HIV positive, the world's highest co-infection rate [67].

The two diseases are mutually reinforcing: VL accelerates the onset of AIDS by encouraging further opportunistic infections, and thus it reduces the life expectancy of people with HIV infection. Meanwhile, HIV related immune-suppression increases the risk of acquiring VL by 100 and 2320 times in endemic areas and encourages the development of relapsing and drug resistant leishmaniasis [3,4,67].

Both HIV and *Leishmania* parasite are able to infect and multiply efficiently cells of the macrophage-dendritic cell lineage, resulting in a cumulative deficiency of the immune response [68,69]. Both pathogens are mutually promoting their replication in these host cells: Leishmania parasites promote HIV replication, likewise HIV increases parasite replication. In vitro study demonstrated that when human DC are co-cultured together with autologous CD4+ T cells, *L. infantum* amastigotes increase HIV-1 production in both DCs and lymphocytes, due to a parasite-mediated production of soluble factors by DCs. *L. infantum* amastigotes infection induces a higher secretion of several cytokines (including IL-1α, IL-2, IL-6, IL-10 and TNF-α) in these cells; and use of specific neutralizing antibodies revealed that the Leishmania induced increase in HIV-1 replication is due to IL-6 and TNF-α [69] .

Pro-inflammatory cytokines have been implicated in the pathogenesis of Leishmania/HIV co-infection. In a human study, serum levels of TNF-α was high in Leishmania/HIV co-infected patient compared to HIV patients without Leishmania infection. This was associated with increased viremia, decreased $CD4^+$T cell numbers; suggested that opportunistic infections during HIV infection could lead to the production of pro-inflammatory cytokines, thereby accelerating disease progression [70].

HIV infection alters the physiologic response of macrophage and DC leading to production of immunomodulatory cytokines like TGF-β, it may responsible for the increased replication of Leishmania [71]. In addition, HIV infection also alters the responsiveness of T cells to Leishmania. For example, it has been shown that stimulation PBMC from HIV-1 infected patients with Leishmania antigen results in decreased production of macrophage activating cytokine i.e. IFN-γ,

IL-12 and IL-18 (key cytokines for inducing robust Th1 response), while IL-4 and IL-10 production increased [72].

Another cytokine that appears to be modulated by VL/HIV co-infection is IL-15, a cytokine that enhances the Th1 response and potentiates the immune response to intracellular human pathogens. During VL/HIV co-infection, serum circulating levels of IL-15 is decreased in VL/HIV co-infection; interestingly increased levels of IL-15 were detected in VL/HIV co-infected patients with clinical and parasitological response to therapy. These observations indicate that there is a relationship between IL-15 and clinical disease outcome and response to therapy, suggesting that this cytokine could play a critical role in VL/HIV co-infection [73].

Pro-and Anti-inflammatory Cytokines Associated With Microbial Translocation

Microbial translocation defined as the passage of viable and nonviable microbes and microbial products such as LPS through the epithelial mucosa into the lamina propria and then to the mesenteric lymph nodes, and possibly other tissues [74]. Microbial products like LPS stimulate mononuclear cells via TLR-4 and promote the secretion of a variety of inflammatory soluble factors such as IFN-γ, IL-1, IL-6, and TNF-α [75], which in turn activate lymphocyte. Continuous and exaggerated stimulation causes exhaustion of the T-cell compartment and leading to immunosuppression [6].

Normally the lumen of the gastrointestinal tract (GIT) is a complex ecosystem harboring dense and diverse microorganisms termed as microbiota, establishes a generally symbiotic relationship with host immune system and epithelial cells [76]. The correct interaction of the components of the GIT permits the normal function of this ecosystem, reduces the risk of an excess of MT from the lumen of GIT to the systemic circulation, and prevents its systemic consequences such as immune activation [74].

In peoples with HIV/AIDS damage to gut-associated lymphocyte tissues occurs; enabling luminal bacteria to enter into the circulation. Microbial translocation from the lumen of GIT to the systemic circulation contributes to systemic immune activation and plays a causative role in the progression of the disease. This phenomenon may also contribute to immunopathogenesis of other infectious diseases such as leishmaniasis [77].

Recently a human study in patients with VL demonstrated an increment of plasma LPS. It has been correlated with lower $CD4^+$ and $CD8^+$ T cell count in the blood, T-cell activation, and higher plasma level of pro-inflammatory cytokine. Lipopolysaccharide along with leishmanial antigens can also attribute for elevated cellular immune activation, indirectly lead to impairment of T cell counts by a mechanism of cell death due to intense stimulation. This T-cell depletion may affect the mucosal immune system, along with intestinal parasitization by amastigotes, may attribute to gut barrier damage and consequent microbial translocation. This suggested that LPS is associated with immunopathogenesis of visceral leishmaniasis [6].

In people with VL/HIV co-infection, MT induces an intense pro-inflammatory response. These patients present with low $CD4^+$ T cell counts, higher proportion of activated T lymphocytes despite low HIV viral load and highly elevated plasma levels of pro-inflammatory cytokines, finally it results in immunesuppresion and leads to death [78,79].

Contrarily, in another human study conducted on cytokine expression in the duodenal mucosa of VL patients to determine organ specific immunity, demonstrated higher level of IFN-γ, but no difference in expression TNF-α, IL-10 and IL-4 compared to healthy controls. This pattern was not found in other organs affected by the disease and suggested that the presence of Leishmania did not change this microsystem [80].

Pro-and Anti-inflammatory Cytokines in PKDL

Post kala-azar dermal leishmaniasis is a skin disorder seen in patients treated for visceral leishmaniasis. It is an unusual dermatosis develops in 5–15% of apparently cured visceral leishmaniasis cases in Indian subcontinent and in about 60% of cases in Sudan. Patients with PKDL do not feel sick and it has only cosmetic significance for the individual and treatment is rarely sought. However, PKDL lesions harbor parasites and therefore could represent a source of transmission, through the bite of female sand flies [5].

Factors predispose to PKDL are inadequate treatment of VL, genetics, nutrition and immunesuppresion that allow renewed multiplication of latent parasites or re-infection. The immunopathogenesis of PKDL is still poorly understood [81].

Currently, PKDL considered as a form of paradoxical immune reconstitution syndrome that emerges as a new disease entity following successful VL treatment and immune recovery. Post kala-azar dermal leishmaniasis lesions are immune inflammatory in nature with granuloma, adequate response to immunochemotherapy, and an ensuing hypersensitivity reaction, the leishmanin skin test (LST). The cytokine pattern of PKDL in an active disease state is dominated by IL-10, later healing response is followed by spontaneous or treatment induced IL-12 priming, IL-2 stimulation, and INF- production. INF-activated macrophages eliminate the Leishmania parasites to be followed by LST conversion [82].

Interleukin-10 is widely accepted as an immunosuppressive cytokine involved in the pathogenesis of PKDL [83]. Another study had also demonstrated that accumulation of nTreg cells in infected tissue and a correlation of both IL-10 and nTreg levels with parasite burden; suggesting that their role in parasite persistence and disease severity [81]. Post kala-azar dermal leishmaniasis can be predicted before treatment of VL; Leishmania parasite present in skin of all VL patients, but IL-10 were detected in keratinocytes and sweat glands who later developed PKDL and not in patients who did not develop PKDL [83].

Moreover, up regulation of Th17 markers like IL-17 and IL-23 both in the system and at the site of lesion and expression of high level of IFN-γ, IL-6, TNF-α, IL-10 and TGF-β was indicated in PKDL patients [84]. T helper-17 cells secrete pre-dominantly IL-17, which act on fibroblasts, endothelial cells, epithelial cells, keratinocytes and macrophages [48]. Thus, IL-17 responses contribute significantly to disease pathogenesis by inducing TNF-α and NO production in those cells [84].

Conclusions

The protective immunity for VL depends on pro-inflammatory like TNF-α, IL-12, IFN-γ. However, in patients with active VL the production of these cytokines is not depressed, but there appears to be unresponsiveness. The underlying mechanism of immunologic unresponsiveness remains unknown, but it has been correlated with

increased production of immunosuppressive cytokines. Anti-inflammatory cytokines are also implicated in the pathogenesis of PKDL. In contrast, HIV patients infected with Leishmania parasite demonstrated decreased production of macrophage activating cytokine. Recently, in patients with VL and/ or VL/HIV co-infection plasma LPS levels were increased and it has been associated with progression of the disease. This is an indicative of microbial translocation involved in the pathogenesis. The increment of pro-inflammatory cytokines in VL also may not necessarily from *L. donovani* antigen response, rather LPS associated. Therefore, understanding such mechanisms will lead to more accurate prognosis, therapeutic intervention and effective vaccine production.

References

1. Badaro R, Jones TC, Carvalho EM, Sampaio D, Reed SG, et al. (1986) New perspectives on a subclinical form of visceral leishmaniasis. J Infect Dis 154: 1003-1011.
2. Alvar J, Vélez ID, Bern C, Herrero M, Desjeux P, et al. (2012) Leishmaniasis worldwide and global estimates of its incidence. PLoS One 7: e35671.
3. Alvar J, Aparicio P, Aseffa A, Den Boer M, Cañavate C, et al. (2008) The relationship between leishmaniasis and AIDS: the second 10 years. Clin Microbiol Rev 21: 334-359, table of contents.
4. WHO/CDS (2007) Report of the Fifth Consultative Meeting on Leishmania/HIV Co-infection. Addis Ababa, Ethiopia.
5. Uranw S, Ostyn B, Rijal A, Devkota S, Khanal B, et al. (2011) Post-kala-azar dermal leishmaniasis in Nepal: a retrospective cohort study (2000-2010). PLoS Negl Trop Dis 5: e1433.
6. Santos-Oliveira JR, Regis EG, Leal CR, Cunha RV, Bozza PT, et al. (2011) Evidence that lipopolisaccharide may contribute to the cytokine storm and cellular activation in patients with visceral leishmaniasis. PLoS Negl Trop Dis 5: e1198.
7. WHO (2010) Control of the leishmaniases: report of a meeting of the WHO Expert Committee on the Control of Leishmaniases, Geneva, Switzerland.
8. Stanley AC, Engwerda CR (2007) Balancing immunity and pathology in visceral leishmaniasis. Immunol Cell Biol 85: 138-147.
9. Dostálová A, Volf P (2012) Leishmania development in sand flies: parasite-vector interactions overview. Parasit Vectors 5: 276.
10. Pearson RD, Jeronimo SMB, souse AQ (2001) Lishmaniasis. In: Gillespie SH, Pearson RD. principles and practice of clinical parasitology. John Wiley & Sons Ltd, USA.
11. Gregory DJ, Godbout M, Contreras I, Forget G, Olivier M (2008) A novel form of NF-kappaB is induced by Leishmania infection: involvement in macrophage gene expression. Eur J Immunol 38: 1071-1081.
12. Singh OP, Gidwani K, Kumar R, Nylén S, Jones SL, et al. (2012) Reassessment of immune correlates in human visceral leishmaniasis as defined by cytokine release in whole blood. Clinical and Vaccine Immunology 19: 961-966.
13. Stegall T (2010) Cytokines, Clinical Immunology & Serology. (4thedn) F.A. Davis Company, Philadelphia.
14. Mutiso JM, Macharia JC, Gicheru MM, Ozwara H, Moodi B, et al. (2013) Immunology of leishmaniasis. Sci Parasitol 14: 51-61.
15. Kumar R, Nylén S (2012) Immunobiology of visceral leishmaniasis. Front Immunol 3: 251.
16. Selvapandiyan A, Dey R, Gannavaram S, Lakhal-Naouar I, Duncan R, et al. (2012) Immunity to visceral leishmaniasis using genetically defined live-attenuated parasites. J Trop Med 2012: 631460.
17. Mutiso JM, Macharia JC, Gicheru MM, Ozwara H (2013) Immunology of leishmaniasis. Sci Parasitol 14: 51-61.
18. Alexander J, Bryson K (2005) T helper (h)1/Th2 and Leishmania: paradox rather than paradigm. Immunol Lett 99: 17-23.
19. Gordon S (2003) Alternative activation of macrophages. Nat Rev Immunol 3: 23-35.
20. Gordon S, Martinez FO (2010) Alternative activation of macrophages: mechanism and functions. Immunity 32: 593-604.
21. Classen A, Lloberas J, Celada A (2009) Macrophage activation: classical vs. alternative, Macrophages and Dendritic Cells. Springer: 29-43.
22. Owen JA, Punt J, Stanford SA, Jones PP (2013) Kuby Immunology. (7thedn) W. H. Freeman and Company, New York.
23. Liew FY, Li Y, Millott S (1990) Tumor necrosis factor-alpha synergizes with IFN-gamma in mediating killing of Leishmania major through the induction of nitric oxide. J Immunol 145: 4306-4310.
24. Murray HW, Jungbluth A, Ritter E, Montelibano C, Marino MW (2000) Visceral leishmaniasis in mice devoid of tumor necrosis factor and response to treatment. Infect Immun 68: 6289-6293.
25. Engwerda CR, Smelt SC, Kaye PM (1996) An in vivo analysis of cytokine production during Leishmania donovani infection in scid mice. Exp Parasitol 84: 195-202.
26. Costa ASA, Costa GC, Aquino DMCd, Mendonça VRRd, Barral A, et al. (2012) Cytokines and visceral leishmaniasis: a comparison of plasma cytokine profiles between the clinical forms of visceral leishmaniasis. Memórias do Instituto Oswaldo Cruz 107: 735-739.
27. Hailu A, van Baarle D, Knol GJ, Berhe N, Miedema F, et al. (2005) T cell subset and cytokine profiles in human visceral leishmaniasis during active and asymptomatic or sub-clinical infection with Leishmania donovani. Clin Immunol 117: 182-191.
28. Hailu A, van der Poll T, Berhe N, Kager PA (2004) Elevated plasma levels of interferon (IFN)-gamma, IFN-gamma inducing cytokines, and IFN-gamma inducible CXC chemokines in visceral leishmaniasis. Am J Trop Med Hyg 71: 561-567.
29. Kurkjian KM, Mahmutovic AJ, Kellar KL, Haque R, Bern C, et al. (2006) Multiplex analysis of circulating cytokines in the sera of patients with different clinical forms of visceral leishmaniasis. Cytometry A 69: 353-358.
30. Peruhype-Magalhães V M-FO, Prataz A, Silva LA, , Rabello A T-CA, Figueiredo RM, et al. (2005) Immune response in human visceral leishmaniasis: analysis of the correlation between innate immunity cytokine profile and disease outcome. Scand J Immunol 62: 487-495.
31. Engwerda CR, Ato M, Stäger S, Alexander CE, Stanley AC, et al. (2004) Distinct roles for lymphotoxin-alpha and tumor necrosis factor in the control of Leishmania donovani infection. Am J Pathol 165: 2123-2133.
32. Ansari NA, Saluja S, Salotra P (2006) Elevated levels of interferon-gamma, interleukin-10, and interleukin-6 during active disease in Indian kala azar. Clin Immunol 119: 339-345.
33. Barral-Netto M, Badaró R, Barral A, Almeida RP, Santos SB, et al. (1991) Tumor necrosis factor (cachectin) in human visceral leishmaniasis. J Infect Dis 163: 853-857.
34. Abbas AK, Lichtman AH, Pillai S (2012) Cellular and Molecular Immunology. (7thedn) Elsivier Inc, Philadelphia.
35. Alexander J, Satoskar AR, Russell DG (1999) Leishmania species: models of intracellular parasitism. J Cell Sci 112 Pt 18: 2993-3002.
36. Opal SM, DePalo VA (2000) Anti-inflammatory cytokines. Chest 117: 1162-1172.
37. Murray HW (2008) Accelerated control of visceral Leishmania donovani infection in interleukin-6-deficient mice. Infect Immun 76: 4088-4091.
38. Peruhype-Magalhães V, Martins-Filho OA, Prata A, Silva Lde A, Rabello A, et al. (2006) Mixed inflammatory/regulatory cytokine profile marked by simultaneous raise of interferon-g and interleukin-10 and low frequency of tumour necrosis factor-a +onocytes are hallmarks of active human visceral Leishmaniasis due to Leishmania chagasi infection. Clin Exp Immunol 146: 124–132.
39. Del Vecchio M, Bajetta E, Canova S, Lotze MT, Wesa A, et al. (2007) Interleukin-12: biological properties and clinical application. Clin Cancer Res 13: 4677-4685.

40. Cummings HE, Tuladhar R, Satoskar AR (2010) Cytokines and their STATs in cutaneous and visceral leishmaniasis. J Biomed Biotechnol 2010: 294389.
41. Murray HW, Hariprashad J (1995) Interleukin 12 is effective treatment for an established systemic intracellular infection: experimental visceral leishmaniasis. J ExpMed 181: 387–391.
42. Bhattacharjee S, Bhattacharya A (2013) The host pathogen interaction and immunomodulation during leishmaniasis. Microbial Pathogenesis: Infection and Immunity: 203-232.
43. Khoshdel A, Alborzi A, Rosouli M, Taheri E, Kiany S, et al. (2009) Increased levels of IL-10, IL-1, and IFN- in patients with visceral leishmaniasis. Braz J Infect Dis 13: 44-46.
44. Squires KE, Schreiber RD, McElrath MJ, Rubin BY, Anderson SL, et al. (1989) Experimental visceral leishmaniasis: role of endogenous IFN-gamma in host defense and tissue granulomatous response. J Immunol 143: 4244-4249.
45. Murray HW, Delph-Etienne S (2000) Roles of endogenous gamma interferon and macrophage microbicidal mechanisms in host response to chemotherapy in experimental visceral leishmaniasis. Infection and immunity 68: 288-293.
46. Carvalho EM, Badaró R, Reed SG, Jones TC, Johnson WD Jr (1985) Absence of gamma interferon and interleukin 2 production during active visceral leishmaniasis. J Clin Invest 76: 2066-2069.
47. Rasouli M, Kalani M, Kiany S (2013) The role of IL15 gene variants in visceral leishmaniasis among Iranian patients. Mol Biol Rep 40: 5151-5157.
48. Haeberlein S, Sebald H, Bogdan C, Schleicher U (2010) IL-18, but not IL-15, contributes to the IL-12-dependent induction of NK-cell effector functions by Leishmania infantum in vivo. Eur J Immunol 40: 1708-1717.
49. Korn T, Bettelli E, Oukka M, Kuchroo VK (2009) IL-17 and Th17 Cells. Annu Rev Immunol 27: 485-517.
50. Pitta MG, Romano A, Cabantous S, Henri S, Hammad A, et al. (2009) IL-17 and IL-22 are associated with protection against human kala azar caused by Leishmania donovani. J Clin Invest 119: 2379-2387.
51. Romagnani S (2008) Human Th17 cells. Arthritis Res Ther 10: 206.
52. Gonzalez-Lombana C, Gimblet C, Bacellar O, Oliveira WW, Passos S, et al. (2013) IL-17 mediates immunopathology in the absence of IL-10 following Leishmania major infection. PLoS Pathog 9: e1003243.
53. Ansari NA, Kumar R, Gautam S, Nylén S, Singh OP, et al. (2011) IL-27 and IL-21 are associated with T cell IL-10 responses in human visceral leishmaniasis. J Immunol 186: 3977-3985.
54. Zhu J, Yamane H, Paul WE (2010) Differentiation of effector CD4 T cell populations (*). Annu Rev Immunol 28: 445-489.
55. Carvalho EM, Bacellar O, Brownell C, Regis T, Coffman RL, et al. (1994) Restoration of IFN-gamma production and lymphocyte proliferation in visceral leishmaniasis. J Immunol 152: 5949-5956.
56. Stäger S, Alexander J, Carter KC, Brombacher F, Kaye PM (2003) Both interleukin-4 (IL-4) and IL-4 receptor a signaling contribute to the development of hepatic granulomas with optimal antileishmanial activity. Infection and immunity 71: 4804-4807.
57. Alexander J, Carter KC, Al-Fasi N, Satoskar A, Brombacher F (2000) Endogenous IL-4 is necessary for effective drug therapy against visceral leishmaniasis. Eur J Immunol 30: 2935-2943.
58. Murray HW, Tsai CW, Liu J, Ma X (2006) Visceral Leishmania donovani infection in interleukin-13-/- mice. Infect Immun 74: 2487-2490.
59. Murphy K (2012) Janway's Immunobiology. (8thedn) Garland Science, Taylor & Francis Group, LLC, London, UK.
60. Murray HW, Flanders KC, Donaldson DD, Sypek JP, Gotwals PJ, et al. (2005) Antagonizing deactivating cytokines to enhance host defense and chemotherapy in experimental visceral leishmaniasis. Infect Immun 73: 3903-3911.
61. Murray HW, Lu CM, Mauze S, Freeman S, Moreira AL, et al. (2002) Interleukin-10 (IL-10) in experimental visceral leishmaniasis and IL-10 receptor blockade as immunotherapy. Infect Immun 70: 6284-6293.
62. Ghalib HW, Piuvezam MR, Skeiky YA, Siddig M, Hashim FA, et al. (1993) Interleukin 10 production correlates with pathology in human Leishmania donovani infections. J Clin Invest 92: 324-329.
63. Haque I, Haque MZ, Krishnani N, Srivastava SP, Khan EM (1993) Fine needle aspiration cytology of the spleen in visceral leishmaniasis. Acta Cytol 37: 73-76.
64. Nylén S, Maurya R, Eidsmo L, Manandhar KD, Sundar S, et al. (2007) Splenic accumulation of IL-10 mRNA in T cells distinct from CD4+CD25+ (Foxp3) regulatory T cells in human visceral leishmaniasis. J Exp Med 204: 805-817.
65. Saha S, Mondal S, Ravindran R, Bhowmick S, Modak D, et al. (2007) IL-10- and TGF-beta-mediated susceptibility in kala-azar and post-kala-azar dermal leishmaniasis: the significance of amphotericin B in the control of Leishmania donovani infection in India. J Immunol 179: 5592-5603.
66. Barral A, Barral-Netto M, Yong EC, Brownell CE, Twardzik DR, et al. (1993) Transforming growth factor beta as a virulence mechanism for Leishmania braziliensis. Proc Natl Acad Sci U S A 90: 3442-3446.
67. http://www.who.int/leishmaniasis/burden/hiv_coinfection/burden_hiv_coinfection/en/
68. Okwor I, Uzonna JE (2013) The immunology of Leishmania/HIV co-infection. Immunol Res 56: 163-171.
69. Garg R, Barat C, Ouellet M, Lodge R, Tremblay MJ. Leishmania infantum Amastigotes Enhance HIV-1 Production in Cocultures of Human Dendritic Cells and CD4+ T Cells by Inducing Secretion of IL-6 and TNF-a. PLoS Neglected Tropical Diseases. 2009; 3(5): e441.
70. Olivier M, Badaró R, Medrano FJ, Moreno J (2003) The pathogenesis of Leishmania/HIV co-infection: cellular and immunological mechanisms. Ann Trop Med Parasitol 97 Suppl 1: 79-98.
71. Barreto-de-Souza V, Pacheco GJ, Silva AR, Castro-Faria-Neto HC, Bozza PT, et al. (2006) Increased Leishmania replication in HIV-1–infected macrophages is mediated by tat protein through cyclooxygenase-2 expression and prostaglandin E2 synthesis. Journal of infectious diseases 194: 846-854.
72. Cruz I, Nieto J, Moreno J, Cañavate C, Desjeux P, et al. (2006) Leishmania/HIV co-infections in the second decade. Indian J Med Res 123: 357-388.
73. d'Ettorre G, Ceccarelli G, Carnevalini M, Forcina G, Zaffiri L, et al. (2006) Central role of interleukin-15 in human immunodeficiency virus (HIV)-infected patients with visceral leishmaniasis. Acta Trop 99: 83-87.
74. d'Ettorre G, Douek D, Paiardini M, Ceccarelli G, Vullo V (2012) Microbial translocation and infectious diseases: what is the link? International journal of microbiology: 1-2.
75. Lu YC, Yeh WC, Ohashi PS (2008) LPS/TLR4 signal transduction pathway. Cytokine 42: 145-151.
76. Purchiaroni F, Tortora A, Gabrielli M, Bertucci F, Gigante G, et al. (2013) The role of intestinal microbiota and the immune system. Eur Rev Med Pharmacol Sci 17: 323-333.
77. Santos-Oliveira JR, Da-Cruz AM (2012) Lipopolysaccharide-Induced Cellular Activation May Participate in the Immunopathogenesis of Visceral Leishmaniasis Alone or in HIV Coinfection. International journal of microbiology: 1-4.
78. Santos-Oliveira JR, Giacoia-Gripp CB, Alexandrino de Oliveira P, Amato VS, Lindoso JÂ, et al. (2010) High levels of T lymphocyte activation in Leishmania-HIV-1 co-infected individuals despite low HIV viral load. BMC Infect Dis 10: 358.
79. Santos-Oliveira JR, Regis EG, Giacoia-Gripp CB, Valverde JG, Alexandrino-de-Oliveira P, et al. (2013) Microbial translocation induces an intense proinflammatory response in patients with visceral leishmaniasis and HIV type 1 coinfection. J Infect Dis 208: 57-66.
80. Luz KG, Tuon FF, Duarte MI, Maia GM, Matos P, et al. (2010) Cytokine expression in the duodenal mucosa of patients with visceral leishmaniasis. Rev Soc Bras Med Trop 43: 393-395.
81. Katara GK, Ansari NA, Verma S, Ramesh V, Salotra P (2011) Foxp3 and IL-10 expression correlates with parasite burden in lesional tissues of post

kala azar dermal leishmaniasis (PKDL) patients. PLoS Negl Trop Dis 5: e1171.

82. Khalil EA, Khidir SA, Musa AM, Musa BY, Elfaki ME, et al. (2013) Post-Kala-Azar Dermal Leishmaniasis: A Paradigm of Paradoxical Immune Reconstitution Syndrome in Non-HIV/AIDS Patients. J Trop Med 2013: 275253.

83. Gasim S, Elhassan A, Khalil E, Ismail A, Kadaru A, et al. (1998) High levels of plasma IL-10 and expression of IL-10 by keratinocytes during viscera leishmaniasis predict subsequent development of post-kala-azar dermal leishmaniasis. Clinical and experimental immunology 111: 64-69.

84. Katara GK, Ansari NA, Singh A, Ramesh V, Salotra P (2012) Evidence for involvement of Th17 type responses in post kala azar dermal leishmaniasis (PKDL). PLoS Negl Trop Dis 6: e1703.