

The immunology of *Leishmania*/HIV co-infection

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Abstract Leishmaniasis are emerging as an important disease in human immunodeficiency virus (HIV)-infected persons living in several sub-tropical and tropical regions around the world, including the Mediterranean. The HIV/AIDS pandemic is spreading at an alarming rate in Africa and the Indian subcontinent, areas with very high prevalence of leishmaniasis. The spread of HIV into rural areas and the concomitant spread of leishmaniasis to suburban/urban areas have helped maintain the occurrence of *Leishmania*/HIV co-infection in many parts of the world. The number of cases of *Leishmania*/HIV co-infection is expected to rise owing to the overlapping geographical distribution of the two infections. In South-western Europe, there is also an increasing incidence of *Leishmania*/HIV co-infection (particularly visceral leishmaniasis) in such countries as France, Italy, Spain and Portugal. Studies suggest that in humans, very complex mechanisms involving dysregulation of host immune responses contribute to *Leishmania*-mediated immune activation and pathogenesis of HIV. In addition, both HIV-1 and *Leishmania* infect and multiply within cells of myeloid or lymphoid origin, thereby presenting a perfect recipe for reciprocal modulation of *Leishmania* and HIV-1-related disease pathogenesis. Importantly, because recovery from leishmaniasis is associated with

long-term persistence of parasites at the primary infection sites and their draining lymph nodes, there is very real possibility that HIV-mediated immunosuppression (due to CD4⁺ T cell depletion) could lead to reactivation of latent infections (reactivation leishmaniasis) in immunocompromised patients. Here, we present an overview of the immunopathogenesis of *Leishmania*/HIV co-infection and the implications of this interaction on *Leishmania* and HIV disease outcome.

Keywords *Leishmania* · Leishmaniasis · Human immunodeficiency virus (HIV) · Acquired immunodeficiency syndrome (AIDS) · Co-infection · Reactivation leishmaniasis

Introduction

Leishmania infection

Leishmaniasis are endemic in 98 countries in 5 continents; most of which are in the developing parts of the world. Currently, about 12 million people are infected, with an annual incidence of about 1.7–2 million, and the disease mostly occurs in the tropics and subtropics [1]. The disease in both humans and animals is caused by different species of *Leishmania* that are transmitted by the bite of an infected female sandfly [2]. The *Phlebotomine spp.* and the *Lutzomyia spp.* are vectors of the disease in the Old and New Worlds, respectively [3]. The sandfly vectors become infected when taking blood meal from infected mammalian hosts, which include humans, wild rodents and domestic dogs. Once the parasites are injected along with saliva into the host [2], they are taken up by cells of myeloid origin including macrophages, monocytes, neutrophils and dendritic cells (DC) [4]. Inside these cells, the parasites morph into the

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non-flagellated, non-motile amastigote form in the phagolysosome. By actively subverting the host cellular defenses, *Leishmania* parasites are able to survive in the macrophages where they proliferate and replicate extensively. This is followed by cell rupture and release of the amastigotes, which are taken up by uninfected macrophages. Uninfected sandflies ingest the amastigote forms when they feed on infected hosts [5, 6]. Leishmaniasis occurs as a spectrum of clinical syndromes divided into cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL) and visceral leishmaniasis (VL). The epidemiology and clinical features are highly variable due to interplay of many factors such as parasite species, vectors, host and environment [7].

HIV infection and transmission

The human immunodeficiency virus (HIV) is a double-stranded RNA virus belonging to the family *retroviridae*. Members of this family infect cells of the human immune system, destroying them, and/or impairing their function. HIV-1 primarily infects and replicates within CD4⁺ T cells leading to their depletion and resultant immunodeficiency. In the early stages of infection, infected individuals show no obvious symptoms but as the infection progresses, the immune system becomes overwhelmed, in most part due to CD4⁺ T cell depletion, and the infected individual becomes immunocompromised leading to increased susceptibility to opportunistic infections. The most advanced stage of HIV infection is acquired immunodeficiency syndrome (AIDS), which could take as long as 10–15 years to develop [8]. AIDS was first recognized in 1981 when young gay men began falling ill and dying of opportunistic infections, their immune systems should have been able to take care of [9, 10]. The afflicted individuals became emaciated and developed dark purple lesions on their arms and faces due to a relatively rare and aggressive form of cancer called Kaposi's sarcoma [11]. The infected persons experienced a rapid decline in health, and death due to opportunistic infection was almost always inevitable [12]. By 1982, epidemiological data determined that AIDS was caused by an infectious disease transmitted through the exchange of bodily fluids and/or by exposure to contaminated blood or blood products [10]. Between 1981 (when the first case of AIDS was reported) and 1984, about 15,000 hemophiliacs that received blood transfusion were infected with HIV [12]. Intravenous drug users were at risk of infection due to needle sharing and subsequent blood exchange [12]. Current estimates from the UNAIDS/WHO AIDS epidemic update show that in 2011, 31.4–35.9 million people were living with HIV with 2.2–2.8 million new infections and 1.5–1.9 million deaths due to the infection [13].

The HIV life cycle starts with the binding of viral glycoprotein (gp120) to its receptor; the CD4 on T cells and

co-receptors; CXCR4 and CCR5 molecules on macrophages and monocytes [14]. This leads to the fusion of the viral membrane to the target cell membrane through the action of another viral protein gp41, which allows the injection of the viral nucleic acid core through the fusion pore to the cytoplasm [12]. Inside the cytoplasm, the viral reverse transcriptase generates a double-stranded DNA version of the virus [15]. At a point thereafter, the DNA enters the nucleus as a nucleic acid–protein complex (the preintegration complex). Through the action of the HIV integrase, the viral DNA gets inserted into the host DNA and remains there throughout the life of the infected cell [12]. The covalently integrated form of viral DNA, known as the provirus, serves as the template for viral transcription. Through the nuclear pore, the newly created viral DNA gains entry into the nucleus. Contrary to the prior belief, complete sequencing of the human genome showed that the provirus preferentially inserts in actively transcribed genes [16], and this may enhance viral multiplication and spread [12]. Once all the viral components are expressed, new virions assemble at the plasma membrane and bud from the cell surface through the action of a viral enzyme, protease. To achieve complete budding, the virus takes over components of the cell's vesicular transport machinery [17].

Epidemiology of *Leishmania*/HIV co-infection

The increase in the incidence of *Leishmania*/HIV co-infection can be attributed in part to the geographical overlap of the two diseases [18]. In visceral leishmaniasis endemic areas, people who are immunocompromised due to HIV infection are more prone to developing clinical VL compared to those without HIV co-infection. In fact, *L. infantum* co-infection is now the third most frequent infection in HIV-infected individuals in VL endemic areas [19]. HIV infection rates are 5 % in Brazil, 2–5 % in India [20] and range between 25 and 40 % in Ethiopia [21]. Furthermore, even non-pathogenic strains of *Leishmania* and other species of trypanosomatids may cause disease in HIV-infected individuals [22]. Since 1994, the incidence of *Leishmania*/HIV co-infection has been monitored by a surveillance network consisting of 16 institutions [23] in four countries: France, Italy, Portugal and Spain [24]. Spain consistently recorded the highest number of cases, an observation that may be related to a number of factors including reactivation of asymptomatic infection and greater geographical overlap between leishmaniasis and HIV infections compared to other southern European countries [25]. In this region, many asymptomatic individuals are positive for leishmanin skin test (LST) [26], an indication that they have been actively exposed to

Leishmania and may not have developed clinical disease due to effective immune response. Infection with HIV may reactivate latent infections and enhance both anthroponotic (through sandfly bites) and artificial (through needle sharing by intravenous drug users [IVDU]) transmissions. In Spain, 68 % of *Leishmania*/HIV co-infection is seen among IVDUs [19]. It is important to note that at the time of these studies, Spain had five to seven times more IVDU than the European average [25]. However, the proportion of reactivated leishmaniasis cases in asymptomatic co-infected patients compared to symptomatic co-infected patients due to contaminated needles and syringes has not been established [25].

Southeast Asia accounts for 67 % of all leishmaniasis [27] where the disease is spreading fast. The number of reported cases in 2005 was 7000, 32,800 and 3000 in Bangladesh, India and Nepal, respectively [28–30]. These numbers are highly misleading because official house-to-house survey of people living with VL in Bihar state of India showed that only one in eight cases are reported [31]. As with leishmaniasis, HIV/AIDS epidemic is also spreading rapidly in southeast Asia with 4 million people currently living with HIV in 2011 [13]. It is estimated that 2.4 million people were living with HIV infection in India, 6300 people in Bangladesh and 64,000 people in Nepal in 2009 [32, 33]. The first *Leishmania*/HIV co-infection case was reported in 1999 in Kumaon region of India [34]. The problem of co-infection seems to be exacerbated by economic migrants who acquire HIV in urban settings and then return to their rural homes in areas where VL is endemic. They either acquire or reactivate latent leishmaniasis due to HIV infection-induced deteriorating immune system [25]. At the Kala-Azar Medical Research Centre in Muzaffarpur (Bihar), the number of HIV-positive patients increased by 50 % from 2000 to 2006, and this was accompanied by an increase in the VL/HIV co-infection rate, from 0.88 % in 2000 to 2.18 % in 2006. In 2006, a prevalence rate of 2.48 % for VL was reported among HIV-positive patients in India. In a study conducted in 39 hospitals in Nepal, 5.7 % of individuals who had VL were HIV positive [35]. No data on *Leishmania*/HIV co-infection are as yet available from Bangladesh, but judging by the rates in neighboring India and Nepal, the rate is likely to be high [25].

While most *Leishmania*/HIV co-infection in southeast Asia involves VL, both CL and VL are important in Africa due to the geographical spread and distribution of the various *Leishmania* spp. In Burkina Faso, the seroprevalence of HIV is 1.2 % (110,000 people) (http://www.unicef.org/info/country/burkinafaso_statistics.html#89) and 827 cases of CL leishmaniasis (mostly caused by *Leishmania major*) were reported in 2005 although this is likely to have been by far grossly under-reported [25]. About 14 % of CL patients were

co-infected with HIV, and this was characterized by many unusual clinical signs [36]. In French Guyana, co-infected patients had more lesions with a poorer response to treatment and higher recurrence and/or reinfection rates compared to immunocompetent patients [37]. In Ethiopia, both CL/HIV and VL/HIV co-infections have been reported although VL/HIV co-infection appears to be more prevalent. In Humera region of NW Ethiopia, 40 % of VL patients were also co-infected with HIV in 2006, representing an increase of 21.5 % from 1999 [38]. More importantly, the case fatality rate of VL/HIV-infected patients was four times higher than those with VL but not HIV infection [38]. In contrast, the incidence of HIV/*Leishmania* co-infection in Sudan remained basically unchanged for 5 years after the first three cases of *Leishmania*/HIV co-infection were reported in 1998 [39]. In Kenya, only 15 cases of VL/HIV co-infection were reported in 2006, and there is no doubt this is a gross underreporting of the real prevalence rate and that the risk of co-infection is on the rise given the rising rate of HIV infection in that country [25]. Recent report from Cameroon showed a 4.8 % rate of CL/HIV co-infection in the Mokolo Region (Northern Cameroon) where CL is endemic [40]. Given that this was the first of this kind of study in this region, it will be interesting to see what this number will look like over time. Generally, there is poor data collection and analysis in this region of the world. There are also very limited disease surveillance, reporting and control programs in place in many African countries. Hence, it is conceivable that the few available data may be grossly underestimated and misleading. This is supported by the fact that in that part of the world, the potential for *Leishmania*/HIV co-infection is increased by several factors including poverty, political instability (war), famine and hunger.

As in Africa, data on the prevalence of HIV/*Leishmania* co-infection in South America are also scanty and may be misleading. In 2006, the Pan American Health Organization reported 62,000 cases of CL and 5,000 cases of VL for the America's region, and the majority of cases were from Brazil [41]. Nevertheless, linked databases from the National Surveillance Systems in Brazil for the period 2002–2003 generated a corrected estimate of 10,207 VL cases for the same period, indicating that 40–50 % of cases are underreported [41]. Of the 315 estimated cases of VL/HIV co-infection between 2001 and 2005, 78 % were males with a median age of 38 years [41]. This is in agreement with more recent studies, which showed that 86.6 % [42] and 88 % [43] of co-infected individuals were males. A study showed that most (66.6 %) of the individuals co-infected with VL and HIV were from the rural areas and included different occupations like hair dressers, doormen, salesmen, office assistants, maids, farmers and unemployed [42]. Although this was a very small study ($n = 15$), it suffices to say that most of the people were low-income earners. There is a strong geographical overlap

between VL and HIV in Brazil. This can be attributed to the spread of HIV from the urban to rural areas and the spread of leishmaniasis from rural to urban areas [44]. According to the Brazilian Ministry of Health, the rate of HIV/VL co-infection is currently estimated to be in the order of 6.5 % [45]. Despite the reduction in the number of VL and HIV/VL cases in 2009 and 2010, the proportion of HIV/VL continues to increase and in 2010 was 17.4 % [45]. Because 57 % of patients in Brazil have HIV diagnosis after VL manifestations, the Ministry of Health has recommended that all patients with VL be tested for HIV [45].

Immunopathogenesis of *Leishmania*/HIV co-infection

The existence of both *Leishmania* and HIV in the same cell has been shown to influence the multiplication and expression of either one or both organisms [46]. It is believed that in co-infected patients, there is a symbiotic relationship between *Leishmania* parasites and HIV. *Leishmania* parasites stimulate chronic immune activation, leading to an increased HIV viral load with faster progression to AIDS [47]. However, a recent study showed that the immune system can be highly activated without a concomitant increase in HIV viral load in co-infected patients [48]. The immunosuppression caused by HIV is particularly favorable for the uncontrolled multiplication of the *Leishmania* parasite [25]. Over the years, several efforts have been made to determine the mechanism behind the pathogenesis of *Leishmania*/HIV co-infection and also to confirm whether there are interactions between the two pathogens at both cellular and molecular levels [25]. *Leishmania* parasites and HIV infect and interact with both dendritic cells and macrophages [49, 50]. The consequences of this interaction are yet to be fully determined. However, it has been suggested that *Leishmania* and HIV-1 can exploit DCs by modulating several cell surface molecules, inhibition of DC function, production of soluble factors, delayed lysosomal fusion and intracellular killing activities [51]. When DCs and macrophages encounter antigens, they process and display their peptides on their surfaces and migrate to the nearest draining lymphoid organs where they present these peptides to naïve T cells. Infection of dendritic cells and macrophages with *Leishmania* and HIV has been shown to alter the physiologic functions of these cells, particularly their antigen processing and presentation capacities [52]. HIV-1 infection has also been shown to affect phagocytosis and replication of *Leishmania* parasites by macrophages [53, 54]. In addition to altering the activation states of infected macrophages and dendritic cells, HIV infection also alters their physiologic response leading to production of immunomodulatory

molecules. For example, the HIV-1 transactivation (Tat) protein increases the expression of prostaglandin E2, cyclooxygenase 2 and the transforming growth factor-beta (TGF- β) by macrophages and DCs, and this may be responsible for the increased replication of *Leishmania* parasites in these cells [55].

Lipophosphoglycan (LPG) is a major glycopospholipid expressed on the surface of *Leishmania* promastigotes. Following phagocytosis, the promastigotes are engulfed within the phagolysosomes in antigen-presenting cells (APCs), especially macrophages, where they transform to the intracellular (amastigote) forms. In the phagolysosome, the LPG molecule is shed leaving only the phosphatidylinositol anchor. The LPG has been thought to be critical for the survival and establishment of the parasite in macrophages [56] and plays important role in modulating the functions of macrophages. Bernier et al. [46] using two monocytoid cells lines latently infected with HIV-1 showed that *Leishmania donovani* LPG can upregulate virus expression. In a subsequent study, they reported that *L. donovani* LPG and its core-PI moiety trigger HIV-1 long terminal repeat (LTR) transcription in T cells through the NF- κ B motif, thus suggesting that exposure of HIV-1 bearing T cells to *Leishmania*-infected phagocytes may be sufficient to initiate the activation of latent provirus DNA. In vitro studies show that *Leishmania infantum* amastigotes within DC/T cell conjugates cause enhanced production of HIV-1 virus, and this was attributed to the production of proinflammatory cytokines by the infected DCs [57]. In line with this, the induction of HIV-1 replication by *Leishmania* parasite in CD4⁺ T cells latently infected with HIV-1 was shown to be dependent on TNF- α as blockade of TNF- α by the injection of thalidomide prevented HIV-1 replication [58]. Indeed, studies suggest that *Leishmania* may directly modulate virus life cycle through NF- κ B-dependent induction of TNF- α and IL-1 α [59]. Collectively, these findings suggest that co-infection with *Leishmania* might enhance the progression to clinical HIV disease [60].

Human immunodeficiency virus infection also negatively impacts on the outcome of *Leishmania* infection. In vitro studies with monocyte-derived macrophages co-infected with HIV-1 and *L. donovani* [61] or *L. infantum* [62] promastigotes led to increased parasite growth in co-infected cells compared to cells infected with only *Leishmania* parasites. This enhanced parasite replication was attributed to impairment in macrophage effector functions that usually result in parasite control [52]. Furthermore, human studies show increased disseminated leishmaniasis and high parasitemia in AIDS patients in Spain [63, 64]. Although not yet demonstrated in vivo, it is conceivable that the high parasitemia in AIDS patients may be related to the observed impairment in macrophage effector

functions in vitro [52]. This could also account for the increased disease recurrence and reactivation seen in HIV patients co-infected with *Leishmania*. In addition, patients co-infected with CL and HIV and with moderate immunosuppression have higher rate of re-infection, higher number of lesion and poor response to treatment compared to the HIV-negative individuals [37]. A 2009 study reported unusual lesions in unusual locations in patients coinfecting with tegumentary leishmaniasis and AIDS [65]. This included erythematous plaques and extensive ulcers in lips and genital areas, and these unusual lesions were attributed to severe immunosuppression in 27 % of these patients [65]. Interestingly, in addition to immunosuppression caused by HIV, VL also leads to immunosuppression on its own which has been attributed to the immunoregulatory role of IL-10 usually seen in high levels in sera of infected patients [66]. However, recent findings in mice suggest that mechanisms other than IL-10 may be responsible for the immunopathology in experimental visceral leishmaniasis [67]. The fact that HIV and *Leishmania* (VL) cause immunosuppression on their own suggests that there could be more pronounced immunosuppression in *Leishmania*/HIV co-infected individuals. Collectively, these observations suggest a reciprocal regulation of disease pathogenesis by HIV and *Leishmania* in patients co-infected with these pathogens.

Effect of cytokines on pathogenesis of *Leishmania*/HIV co-infection:

Resolution of both cutaneous and visceral leishmaniases is usually associated with a strong Th1 response because it ensures the production of macrophage-activating cytokines (especially IFN- γ) that prevent parasite replication. Conversely, susceptibility has been attributed to the over production of Th2-associated cytokines (including IL-4, IL-5 and IL-10), which inhibit Th1 cytokines and deactivate macrophages leading to parasite proliferation and survival [68]. Thus, in cutaneous leishmaniasis (caused by *L. major*), healing in resistant mice is associated with the development of CD4⁺ Th1 cells that produce IFN- γ [68–72]. In contrast, susceptible mice produce early IL-4 that promotes the development and expansion of Th2 cells [68, 70, 71, 73]. However, in VL, the role of Th1 or Th2 cytokines in the resistance and susceptibility, respectively, is not very obvious because both Th1 and Th2 cytokines have been detected in VL patients showing different clinical disease manifestations [74].

Studies suggest that HIV infection could alter the responsiveness of T cells to *Leishmania*. For example, it has been shown that HIV influences the responsiveness of

peripheral blood mononuclear cells (PBMC) to *Leishmania* antigens. Treating PBMCs from healthy individuals with HIV antigens diminished *L. donovani*-induced proliferation as well as purified protein derivative (PPD)-induced proliferation in a dose-dependent fashion [75]. In addition, stimulation of PBMCs from HIV-infected patients with *Leishmania* antigen results in the production of low levels of IFN- γ but high levels of IL-10 [76]. Similarly, the production of IL-12 and/or IL-18 (key cytokines for inducing robust Th1 response) by PBMCs is lower in VL patients, HIV-infected or VL/HIV co-infected patients when compared to those of healthy donors [77]. Collectively, these observations suggest that the inhibitory effect of HIV and VL on IFN- γ production may not be solely dependent on the anti-inflammatory effect of IL-10, but may be related to the effect of HIV in regulating IFN- γ -inducing factors, such as IL-12 and IL-18 [77]. In line with this, Nigro et al. [78] showed that patients infected with HIV alone produced more IL-10 and IL-4 than VL/HIV co-infected patients.

Another cytokine that appears to be modulated by *Leishmania*/HIV co-infection is IL-15, a critical cytokine that enhances CD8⁺ T cell response and the production of cytokines that enhance protective immune response to intracellular parasites [79]. Low level of IL-15 has been reported in serum of HIV-infected immunocompromised patients [80]. Interestingly, increased levels of IL-15 were detected in VL/HIV[−] and VL/HIV⁺ patients with clinical and parasitological response to therapy [80]. 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 *Leishmania*/HIV co-infection [80].

Proinflammatory cytokines have also been implicated in the pathogenesis of *Leishmania*/HIV co-infection. A study in Spain compared the serum levels of proinflammatory cytokines in *Leishmania*/HIV co-infected patients to HIV patients without *Leishmania* infection. Serum TNF- α level was higher (and remained high after recovery from VL) in VL⁺/HIV⁺ patients compared to the VL⁺/HIV[−] patients [81]. In line with this observation, monocytes latently infected with HIV-1 produced high amounts of TNF- α when restimulated with *Leishmania* antigen in vitro [46] and in vivo [82], and this was associated with increased viral replication. The pathologic effect of TNF- α in co-infected patients could provide explanation for increased viremia, decreased CD4⁺ T cell numbers and enhanced seroconversion observed in these patients [82]. Olivier et al. [81] suggested that opportunistic infections during HIV infection could lead to the production of proinflammatory cytokines during the stage of immunodeficiency, thereby accelerating disease progression.

Role of highly active antiretroviral treatment in *Leishmania*/HIV co-infection

Highly active antiretroviral treatment (HAART) was introduced in 1997 for the treatment of HIV infection, and this has led to a decline in the incidence of *Leishmania*/HIV co-infection particularly in most of Southern Europe [25]. Between 1997 and 2006, there was a decrease in the average number of cases recorded at the WHO network centers for monitoring *Leishmania*/HIV co-infection in Madrid, Rome and Montpellier from 35 cases in 1997 to 12 in 2006 [25]. In 2000, a prospective study designed with the aim of determining the impact of HAART in VL/HIV co-infected patients found that HAART could neither prevent relapses nor modify the clinical outcome of VL patients co-infected with HIV [83]. However, another study carried out in Spain found that although HAART reduced the incidence of new VL cases, it failed to prevent relapses in co-infected patients and had little positive effect on the clinical manifestations of VL [84]. Furthermore, the study found that the rate of VL relapses in HIV-infected patients receiving HAART was very high although these relapses were seen in participants with poor control of viral load and/or a low CD4⁺ T cell count [84]. In contrast, other studies found that HAART reduces the relapse and/or incidence of VL in VL/HIV co-infected patients [85–88]. Similar reports have also been shown for HIV patients with bacterial infection [89, 90] and in HIV-associated multifocal leukoencephalopathy, where a better prognosis was associated with HAART [91]. In Europe, HAART has greatly reduced the incidence of symptomatic first episode of VL [92]. However, this has not been the case in Asia and Africa where free HAART is very scarce [25]. The differences in the role of HAART in VL/HIV co-infection suggest that more studies are required to fully elucidate the effect of HAART on HIV patients co-infected with *Leishmania*. Although some studies clearly show that HAART reduces the incidence of *Leishmania*/HIV co-infection (new infection or reactivation of latent infection), no study is available to suggest potential mechanism(s) of this effect. It is conceivable that in addition to reducing the viral load, HAART may alter cytokine and monokine milieu of infected cells and/or environment leading to more effective parasite control. Alternatively, the reduction in viral load following HAART might lead to recovery of T cell functions, (such as recovery from exhaustion), leading to a more effective effector activities [93].

Persistent parasites and reactivation leishmaniasis in HIV/*Leishmania* co-infection

Following recovery from natural or deliberate infection with *Leishmania major*, a small number of viable parasites persist

at the primary site of infection and its draining lymph node [94, 95]. This persistence is mostly seen following healing of cutaneous leishmaniasis, but there is also evidence to suggest that *Leishmania* spp that cause visceral leishmaniasis (particularly *L. donovani*, *L. infantum*) also persist in infected patients for life [96]. This has led to the dogma that *Leishmania* organisms are never completely eliminated from infected host. It is speculated that under certain physiologic conditions including malnutrition and immunosuppression, recrudescence (reactivation leishmaniasis) can occur from persisting parasites. Indeed, reactivation leishmaniasis arising from latent (persistent) parasites is a common occurrence in AIDS patients in the sub-Saharan Africa and India [97–99].

It has been debated whether leishmaniasis seen in HIV/AIDS patients or in other immunocompromised individuals in *Leishmania* endemic areas is due to new infection or reactivation of persistent parasites (latent infections). The same strain of parasite that caused initial disease was isolated from 50 % of individuals with recurrent cutaneous leishmaniasis due to *L. braziliensis* [100]. However, one might argue that this may have been from a new infection with the same strain. The strongest evidence suggesting that reactivation can occur from persistent parasites comes from murine studies. Healed mice treated with L-NIL, the competitive inhibitor of nitric oxide synthase (the enzyme which catalyzes the synthesis of nitric oxide), develop recrudescence and progressive disease [101]. Similarly, administration of rIL-12- to IL-12-deficient mice promotes control of *L. major* but recrudescence occurs following cessation of cytokine treatment [102]. We have found that administration of anti-IFN- γ or weekly injection of cyclophosphamide to healed mice results in reactivation disease (Uzonna, unpublished data). Collectively, these murine studies suggest that some cases of reactivation disease in humans could arise from persistent parasites but whether this actually occurs in individuals co-infected with *Leishmania* and HIV remains to be conclusively determined.

Implications of *Leishmania*/HIV co-infection

According to Nascimento et al. [43], VL/HIV co-infection is an emerging threat in Northeast Brazil. Forty-one percent (41 %) of co-infected patients in this region had a previous history of VL, suggesting that the current increase in the prevalence of VL may be due to relapses in the face of increasing HIV infection. VL relapse is a major challenge in the care of *Leishmania*/HIV co-infected individuals [103]. Several studies have been carried out to establish the predictors of VL relapse in co-infected patients (reviewed in [103]). Some of these predictors include persistent decrease in numbers of CD4⁺ cells at follow-up, absence of secondary prophylaxis, previous history of VL relapse

and CD4⁺ T cell counts less than 100 cells/ml at the time of primary VL diagnosis.

Human immunodeficiency virus infection is associated with a bias toward the production of Th2 cytokine milieu (91) and concomitant decline in the population of CD4⁺ T cells resulting in a decline in IFN- γ production. This presents a major problem in parasite control in patients co-infected with *Leishmania* where a strong Th1 response by the CD4⁺ T cells is required for effective anti-*Leishmania* immunity. Furthermore, in leishmaniasis, recovery from primary infection is associated with the persistence of a small number of parasites at the site of primary infection and its draining lymph nodes [104]. This persistence is important for the maintenance of anti-*Leishmania* immunity [105, 106]. The maintenance of these parasites and, by implication maintenance of immunity, is dependent on the host striking a fine balance between effector (Th1) and regulatory T cell (Treg) responses [105]. HIV infection has been shown to skew the cytokine responsiveness of peripheral blood mononuclear cells to *Leishmania* antigens toward a Th2 profile. Hence, tipping the balance toward a Th2 environment could lead to failure to optimally control persistent infection leading to disease recrudescence, uncontrolled parasite proliferation and delayed resolution (healing) of cutaneous lesions. In line with this, co-infection with HIV-1 has been shown to prolong healing time as well as to present more severe clinical signs in individuals co-infected with *Leishmania major* [40]. Also, a recent study in rhesus macaques showed that infection with simian immunodeficiency virus (SIV) led to dysfunction in primary and secondary CD4⁺ T cell and B cell responses to *Leishmania major* leading to a more progressive cutaneous disease and prolonged healing time [107].

In conclusion, the depletion of CD4⁺ T cells with its resultant immunosuppression that heralds the onset of AIDS could lead to disease recrudescence as seen in experimental infections. On the other hand, *Leishmania* infection in HIV-infected patients leads to profound activation of immune cells and the production of proinflammatory cytokines, thereby making these cells ready targets for HIV infection, which could accelerate progression to AIDS. More research is needed to clearly determine the impact of the interaction between *Leishmania* and HIV in order to better understand the disease pathogenesis in co-infected individuals. This would permit better treatment and management strategies of both diseases in these individuals.

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