Mechanisms of interaction between protozoan parasites and HIV

Guadalupe Andreani¹, Robert Lodge¹,², Dave Richard¹,², and Michel J. Tremblay¹,²

Purpose of review
This review summarizes the current knowledge on human immunodeficiency virus type 1 (hereafter called HIV)/protozoan co-infections in the case of three important, although neglected, tropical diseases: malaria, trypanosomiasis (Chagas disease) and leishmaniasis. The HIV pandemic has modified the immunopathogenic, epidemiological and therapeutic aspects of these human diseases.

Recent findings
In-vitro data suggests that HIV favors Leishmania infection, whereas different parasites have contrasting effects on HIV. However, many of the previous models are a limited representation of the complex interactions within the host; this situation is particularly the case when microbial products are used in place of live parasites.

Summary
In the host, protozoan parasites generally enhance HIV replication and accelerate AIDS progression. HIV alters parasite pathogenesis, often worsening disease outcome. These aspects bring significant complications for the treatment of co-infected individuals.

Keywords
Chagas disease, HIV, leishmaniasis, malaria, protozoan parasite

INTRODUCTION
HIV infection in the developing world has risen dramatically over the last two decades, and in doing so has profoundly modified the epidemiology, therapy and pathology of several established diseases. Although at first not considered as HIV opportunistic diseases, and as such much underestimated because of poor diagnosis, malaria, trypanosomiasis and leishmaniasis are increasingly brought at the frontline of HIV co-infections (Fig. 1). This review focuses on the current status of these important vector-borne protozoan diseases, with particular emphasis on immunopathogenesis and development of in-vitro experimental models.

TRYPANOSOMA AND HIV
Chagas disease, caused by the protozoan parasite Trypanosoma cruzi, represents one of the most important protozoan infections in Latin America. Its distribution extends from the southern USA to southern Latin America and it is estimated that 16–18 million people are infected and 100 million at risk of infection [1]. In recent years, Chagas disease has been considered an opportunistic infection for AIDS and has become a public health concern in countries where the disease is not endemic because of immigration of infected individuals from endemic areas [2,3,4].

Patients with acute T. cruzi infection achieve high levels of parasitemia, regardless of their immune status. In immunocompetent patients, the parasite load decreases after the acute infection but persists at low levels during the chronic phase [5,6] and patients might develop visceral pathologies, possibly as a result of persistent infection and immune reactivation [7,8].

¹Centre de Recherche en Infectiologie, Centre Hospitalier Universitaire de Québec – CHUL and ²Département de Microbiologie-Infectiologie et Immunologie, Faculté de médecine, Université Laval, Québec, Quebec, Canada

Correspondence to Dr Michel J. Tremblay, Centre de Recherche en Infectiologie, RC709, Centre Hospitalier Universitaire de Québec – CHUL, 2705 boul. Laurier, Québec, QC, Canada G1V 4G2. Tel: +1 418 654 2705; e-mail: michel.j.tremblay@crchul.ulaval.ca

¹These authors contributed equally to this work.

DOI:10.1097/COH.0b013e32835211e9
In rural areas, *T. cruzi* is mainly transmitted to humans by vector insects; however, the urban form of the disease is associated with transmission through transfusion of contaminated blood products, intravenous drug use or transplantation of infected organs. Mother-to-child transmission (MTCT) can occur in both rural and urban settings after transplacental infection. As a consequence of the geographic overlap of *T. cruzi* infection and HIV distribution and global migration, the incidence of the co-infection is gaining importance in areas of nonendemicity for the parasitic infection \[4^{*},9^{*}\]. It is generally assumed that persons chronically infected with *T. cruzi* subsequently acquire HIV as a consequence of travel and migration to urban centers. Most cases of manifestations of Chagas disease in HIV-infected patients are the result of immunosuppression leading to parasitic reactivation [10]. Acute meningoencephalitis develops in nearly 80% of co-infected patients, followed by myocarditis [11,12]. In co-infected patients, Chagas disease has been mostly described in patients with

### KEY POINTS

- Protozoan pathogens and HIV interact in their host, modifying the immunopathology of disease and complicating therapeutic intervention.
- Disease prevalence and distribution and population movements impact greatly on HIV/protozoan parasite co-infections.
- Treatment inefficiency, greater drug resistance, misdiagnoses and the exacerbation of both parasitemia and AIDS progression in co-infected individuals all call for a greater focus on these emerging HIV opportunistic diseases.

![FIGURE 1](https://www.co-hivandaids.com/figures/1.png)

**FIGURE 1.** World maps showing (a) HIV prevalence in 2010; (b) populations infected by *T. cruzi*, the agent of Chagas disease, per country, in 2009; (c) malaria cases per country and per 100,000 people, in 2004; and (d) the distribution of leishmaniasis as compared with HIV/Leishmania co-infections (1990–1998). Data in (a) is from http://www.unaids.org/globaReport/hiv_prevalence_map.htm. Data in (b) is from http://www.treatchagas.org/imagens/mapchagasjun09_large.jpg. Data in (c) is from http://www.bu.edu/themovement/files/2010/10/malaria.jpg. Data in (d) is from ‘The Leishmaniases and Leishmania/HIV Co-Infection’ WHO Fact Sheet No. 116. May 2000.
advanced AIDS, having CD4⁺ T-cell counts below 200 cells/μl, and is associated with high mortality. Moreover, reactivation and subsequent parasitemia are associated with increased plasma viral loads and decreased CD4⁺/CD8⁺ T-cell ratios [13**,14–16]. On the other hand, HIV infection favors higher levels of parasitemia [14], not only inducing tissue damage but also increasing the likelihood of further parasitic transmission [17]. Although most current methods are not sufficiently sensitive to detect Chagas disease reactivation, De Freitas et al. [13**] have recently proposed quantitative RT-PCR to monitor parasitemia, detecting reactivation even in patients having not yet presented clinical symptoms. Finally, in the case of co-infected mothers, MTCT of both pathogens with severe outcome for the children [18] and simultaneous congenital transmission of T. cruzi and HIV have been reported [19**].

Although the mechanisms involved in increased parasite load are not yet well understood, studies have suggested that an imbalanced, skewed Th2 response favors parasite replication [20,21]. In this regard, it has been shown that IL-6, IL-8, IP-10 and MCP-1, which downmodulate regard, it has been shown that IL-6, IL-8, IP-10 and MCP-1, which downmodulate T. cruzi replication [22], are diminished in human placental histocultures co-infected with HIV and T. cruzi, as well as in those stimulated with parasite-shed/-secreted factors. However, T. cruzi inhibits HIV replication at the placental level [23]. Similar results were observed in primary human monocyte-derived macrophages (MDMs), where HIV replication was inhibited by both free trypomastigotes and trypomastigote soluble factors. The highly antigenic parasitic cysteine protease cruzipain had the same effect [24]. However, it is important to underline that these in vitro observations are reduced to limited systems that do not take into account the complex interactions found in the co-infected host, explaining the apparent conflicting opposite effects of T. cruzi on HIV-infected cells.

Better T. cruzi diagnosis in HIV-positive immigrants from endemic areas to urban centers, both of endemic and nonendemic countries, is needed. Furthermore, Chagas disease is rarely suspected by the professionals assisting HIV-infected patients [25,26*,27*], thus highlighting that the incidence of co-infection is probably much underestimated.

A related deadly parasitic infection, endemic in sub-Saharan Africa, is sleeping sickness or African trypanosomiasis, caused by infection by Trypanosoma brucei. Although co-infection with HIV is possible, the interaction between both causative agents is still unclear. Some studies suggest that HIV-positive patients are at higher risk of unfavorable outcome and treatment failure [28–30].

**MALARIA AND HIV**

Malaria is a major cause of death among infectious diseases, with 300 million cases and 1–3 million deaths per year in the tropical world [31]. Although other pathogenic Plasmodium spp. exist, Plasmodium falciparum causes the majority of severe cases. Considering that HIV infection also entails numerous immunological defects and that the control of malaria parasitemia requires a functional immune response, it is not surprising that HIV-infected individuals have increased incidence and severity of malaria episodes ([32–34] and reviewed in [35*]). However, the impact of malaria infection on HIV transmission and disease progression is less clear. Field studies have revealed that P. falciparum infection transiently increases HIV replication and enhances plasma viral load in co-infected individuals for several weeks after treatment with antimalarials, suggesting that malaria could have an impact on HIV disease progression and transmission [36]. Furthermore, mathematical modeling using data collected from Kisumu (Kenya) between 1990 and 2005 has estimated that 4.8% of HIV infections were attributable to malaria [37]. Intriguingly, it was recently shown that individuals who live in areas with high prevalences of P. falciparum and HIV (eastern sub-Saharan Africa) have about twice the risk of being positive for HIV compared with people living in areas of low levels of malaria [38**]. However, when populations with low HIV prevalence were studied (western sub-Saharan Africa), there was no evidence of association between HIV and malaria [39**]. These two studies clearly highlight the fact that future investigations on the clinical impact of malaria on HIV progression should also take into account the external factors such as disease prevalence and distribution in the population.

The mechanisms by which the malaria parasite and its associated pathophysiological effects influence the course of HIV infection also remain unclear. During a malaria episode, red blood cells infected with P. falciparum ring stages circulate in the bloodstream and then sequester in the microvasculature as the parasite matures to the trophozoite and schizont stages. In addition to the merozoites that then go on to infect new erythrocytes, rupture of the parasite-infected red blood cells (iRBCs) also releases malarial products such as hemozoin. All these will thus likely come in contact with cells of the immune system present in the circulation. It is well documented that exposure of immune cells to malarial antigens has major effects in vitro. For example, peripheral blood mononuclear cells (PBMCs) exposed to iRBCs produce TNFα and IFNγ [40,41]. Moreover, iRBCs are recognized and internalized by human macrophages [42–44].
and dendritic cells, but the impact of this exposure on the activation state of the phagocytes is somewhat conflicting. The few in-vitro studies investigating the impact of malaria on HIV have demonstrated that exposure to soluble malarial antigens and hemozoin induced HIV replication or reactivation via CD4+ T-cell activation and the production of proinflammatory cytokines (e.g. IL-1β, IL-6 and TNFα) [45,46]. However, these studies used whole-cell extracts of P. falciparum schizont-stage parasites and PBMCs, making it hard to decipher which malarial component(s) was responsible for the observed effects and what the target host cells were [45,46]. Exposure of an HIV-infected human placental BeWo cell line to a recombinant domain of P. falciparum iRBC surface adhesin able to bind chondroitin sulfate A on the trophoblast surface leads to TNF secretion and stimulation of HIV replication [47]. Hemozoin has recently been shown to increase the ability of dendritic cells to transfer HIV to CD4+ T cells [48,49] but also to decrease HIV infection of macrophages [50]. It is thus obvious that further investigations are needed to get a clearer portrait of the multifaceted interactions between malaria and HIV. Perhaps, a bottom-up approach needs to be taken in which the interactions between the different stages of the erythrocytic cycle of malaria and HIV are systematically analyzed in different relevant primary human cell populations.

**LEISHMANIA AND HIV**

Leishmaniasis affects the population of 88 countries, with more than 12 million infected people and 350 million at risk [51]. The two basic forms of the disease are cutaneous leishmaniasis (caused by *Leishmania major*, among others) and visceral leishmaniasis (caused by *Leishmania infantum* and *Leishmania donovani*), the last being potentially fatal if not treated. Although numerous cases with both HIV and cutaneous leishmaniasis have been reported [52], the seriousness and the impact of HIV infection on visceral leishmaniasis explain the greater focus on this form of leishmaniasis in HIV co-infections. Indeed, HIV infection increases the risk of developing visceral leishmaniasis several hundred-fold (from 100 to 2300 times) [51], increasing possible relapse, whereas visceral leishmaniasis enhances HIV infection to AIDS-defining conditions.

The fact that 2–12% of all visceral leishmaniasis cases involve HIV co-infections underlines the synergic aspect of both diseases; such proportions may reach 40%, as in Humera, northwest Ethiopia [53], where co-infections have increased two-fold in the last decade. However, much focus is on Southern Asia, which currently accounts for two-thirds of visceral leishmaniasis cases worldwide [53] and where the HIV epidemic is expanding. As in many regions, the geographical overlap of both diseases raises the risk of co-infection. Thus, economic migrants who acquire HIV in urban areas return to rural regions endemic for visceral leishmaniasis. Furthermore, the decline in immunity following HIV infection enhances leishmaniasis reactivation [54], or establishment of unusual clinical manifestations and complications [55]. This is particularly alarming given that, in Africa, the fatality rate for co-infected patients can be as much as four times higher than for HIV-negative visceral leishmaniasis cases [56]. In Brazil, another area of co-infection, both HIV and visceral leishmaniasis are expanding; visceral leishmaniasis is endemic in the Northeast: about 2% of these individuals are co-infected with HIV, compared with less than 0.1% for cutaneous leishmaniasis [51]. However, the prevalent clinical pattern that further develops in visceral leishmaniasis and HIV-positive individuals is mucocutaneous leishmaniasis, which is particular to South America and is only very rarely seen on other continents. This is an example of visceral-associated or cutaneous-associated *Leishmania* species developing atypical manifestations in HIV co-infected patients. Indeed, viscerotropic variants may be isolated in cutaneous lesions, whereas dermotropic species may be identified as causes of visceral leishmaniasis in HIV-positive individuals [57].

Both HIV and *Leishmania* infect cells of the monocyte lineage. The presence of the pathogens in the same cell type (macrophages or dendritic cells) puts in perspective the particular effects of HIV infection on *Leishmania* or, conversely, the parasite’s impact on HIV/AIDS progression. Indeed, the enhanced reciprocal effect of each pathogen’s multiplication is now well documented [58,59]. Parasite infection concurrently to HIV induces chronic immune activation, and thus increased HIV load and accelerated progression toward AIDS, whereas immunosuppressed conditions caused by HIV favor *Leishmania* multiplication.

Early reports showed that the promastigote lipophosphoglycan (LPG), which is an abundant cell surface glycolipid, enhanced HIV replication in lymphocytes [60], but also inhibited virus entry [61]. LPG being downmodulated in amastigotes, the major stage of the parasite in humans, these observations relate to early events in *Leishmania* infection. Models having examined the impact of HIV/*Leishmania* co-infection using primary human MDMs [62,63] or dendritic cells [64,65], or human tonsillar tissue cultured ex vivo [66], show that parasites modulate the viral cycle of HIV through...
the induction of TNFα and IL-1α, leading to upregulation of HIV gene expression by NF-κB. Cytokines released by Leishmania-infected cells also impact on lymphocytes [65]; indeed, co-infected patients have higher numbers of CCR5+CD3+ T cells than individuals with visceral leishmaniasis or HIV-positive individuals without visceral leishmaniasis [67]. The human leukocyte antigen G has also been reported upregulated in co-infected individuals [68].

The HIV enhancement of Leishmania multiplication has been determined both clinically and experimentally. Higher levels of peripheral parasitemia in co-infected patients correlate well with the in-vitro observations of similarly co-infected MDMs [69]. It is perhaps conceivable that impairment of intracellular killing and altered cytokine production by HIV in the infected host’s immune cell population should enhance parasite growth. Such concepts are supported by the striking Th2-type, as compared with the absence or even downregulated Th1-type, responses reported in HIV-infected cultured human PBMCs, and greater IL-4 and IL-10 secretion from such cells obtained from co-infected individuals than patients infected solely by HIV [70]. In addition, co-infected individuals have decreased IL-15, a cytokine linked to Th1 enhanced responses [71]. Some in-vitro studies have suggested that the HIV Tat protein, which is secreted by HIV-infected cells, may trigger IL-1β that the HIV Tat protein, which is secreted by HIV-infected cells, may trigger IL-1β, IL-4, IL-6, IL-8, TNFα/β and TGFβ secretion, eventually leading to favorable conditions for Leishmania replication and growth in macrophages [72]. Interestingly, our group has shown that macrophage cultures infected with HIV will have greater Leishmania uptake and thus harbor more parasites and enhancing infection, a unique situation given that phagocytosis is usually inhibited by HIV infection [62].

Dendritic cells are also infected by HIV and Leishmania, and such infected cells may act as reservoirs for the pathogens in the co-infected individual. Indeed, both pathogens bind DC-SIGN to gain and enhance entry into dendritic cells [64]. Thus, the presence of Leishmania prior to virus infection of dendritic cells impacts on HIV transmission to CD4+ T cells by inducing IL-6 and TNFα [65]. Other immune cells, such as CD16+ monocytes, which are permissive to HIV infection [73], probably also harbor Leishmania parasites; yet, their impact in co-infections is not established. Recently, activation of CD8+ T cells was shown to be substantially higher in co-infected patients than those with HIV [74].

CONCLUSION

Given the immunocompromised state of HIV-infected individuals, it is not surprising that their susceptibility to parasitic diseases is enhanced. However, the difficulties incurred in the treatment of these new opportunistic neglected tropical diseases are compounded by the complex interactions with HIV and the immunosuppressed state of the patient. Complications entailed from drug resistance, the inefficiency of treatments, misdiagnosis and the exacerbation of both parasitemia and AIDS progression can only be countered by a better understanding of the complex interconnections between both pathogens with the host immune system, and a better access to highly active antiretroviral therapy (HAART) in co-infection endemic areas.

Acknowledgements

This study was supported by an operating grant to M.J.T. from the Canadian Institutes of Health Research HIV/AIDS Research Program (MOP-84555) and infrastructure support from the FQRNT Centre for Host-Parasite Interactions (RS-87902). M.J.T. is the recipient of the Canada Research Chair in Human Immuno-Retrovirology (Tier 1 level). The authors acknowledge numerous contributions from various laboratories whose references were not cited in the present review because of space limitations.

Conflicts of interest

The authors declare no conflict of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:
- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 289).


Migration of women of childbearing age from Latin America to developed countries and the importance of T. cruzi screening for controlling MTCT in nonendemic areas are discussed in this study.
This study highlights the importance of diagnosis of parasite reactivation in co-infected patients and describes a quantitative method to detect such reactivation.


This study demonstrates that malaria does not play an important role in the spread of HIV in populations where HIV prevalence is low. These results, obtained in western sub-Saharan Africa, contrast with what was observed in eastern sub-Saharan Africa, a region of high HIV prevalence and where malaria was found to be a risk factor for HIV infection.

This study shows that exposure of primary human immature monocyte-derived macrophages by parasite Trypanosoma cruzi, PloS One 2009; 4:e8246.


This is a comprehensive report that summarizes the results from various field studies investigating the impact of malaria on HIV infection.

This study shows that exposure of primary human immature monocyte-derived dendritic cells to hemozoin induces their maturation, leading to a decrease of their susceptibility to HIV infection and increases their ability to transfer the virus to CD4+ T cells.

This study describes that exposure of primary human immature monocyte-derived dendritic cells to hemozoin induces their maturation, leading to a decrease of their susceptibility to HIV infection and increases their ability to transfer the virus to CD4+ T cells.

This study describes that exposure of primary human immature monocyte-derived dendritic cells to hemozoin induces their maturation, leading to a decrease of their susceptibility to HIV infection and increases their ability to transfer the virus to CD4+ T cells.

This study describes that exposure of primary human immature monocyte-derived dendritic cells to hemozoin induces their maturation, leading to a decrease of their susceptibility to HIV infection and increases their ability to transfer the virus to CD4+ T cells.

This study describes that exposure of primary human immature monocyte-derived dendritic cells to hemozoin induces their maturation, leading to a decrease of their susceptibility to HIV infection and increases their ability to transfer the virus to CD4+ T cells.

This study describes that exposure of primary human immature monocyte-derived dendritic cells to hemozoin induces their maturation, leading to a decrease of their susceptibility to HIV infection and increases their ability to transfer the virus to CD4+ T cells.

This study describes that exposure of primary human immature monocyte-derived dendritic cells to hemozoin induces their maturation, leading to a decrease of their susceptibility to HIV infection and increases their ability to transfer the virus to CD4+ T cells.

This study describes that exposure of primary human immature monocyte-derived dendritic cells to hemozoin induces their maturation, leading to a decrease of their susceptibility to HIV infection and increases their ability to transfer the virus to CD4+ T cells.

This study describes that exposure of primary human immature monocyte-derived dendritic cells to hemozoin induces their maturation, leading to a decrease of their susceptibility to HIV infection and increases their ability to transfer the virus to CD4+ T cells.

This study describes that exposure of primary human immature monocyte-derived dendritic cells to hemozoin induces their maturation, leading to a decrease of their susceptibility to HIV infection and increases their ability to transfer the virus to CD4+ T cells.

This study describes that exposure of primary human immature monocyte-derived dendritic cells to hemozoin induces their maturation, leading to a decrease of their susceptibility to HIV infection and increases their ability to transfer the virus to CD4+ T cells.

This study describes that exposure of primary human immature monocyte-derived dendritic cells to hemozoin induces their maturation, leading to a decrease of their susceptibility to HIV infection and increases their ability to transfer the virus to CD4+ T cells.

This study describes that exposure of primary human immature monocyte-derived dendritic cells to hemozoin induces their maturation, leading to a decrease of their susceptibility to HIV infection and increases their ability to transfer the virus to CD4+ T cells.

This study describes that exposure of primary human immature monocyte-derived dendritic cells to hemozoin induces their maturation, leading to a decrease of their susceptibility to HIV infection and increases their ability to transfer the virus to CD4+ T cells.

This study describes that exposure of primary human immature monocyte-derived dendritic cells to hemozoin induces their maturation, leading to a decrease of their susceptibility to HIV infection and increases their ability to transfer the virus to CD4+ T cells.

This study describes that exposure of primary human immature monocyte-derived dendritic cells to hemozoin induces their maturation, leading to a decrease of their susceptibility to HIV infection and increases their ability to transfer the virus to CD4+ T cells.

This study describes that exposure of primary human immature monocyte-derived dendritic cells to hemozoin induces their maturation, leading to a decrease of their susceptibility to HIV infection and increases their ability to transfer the virus to CD4+ T cells.

This study describes that exposure of primary human immature monocyte-derived dendritic cells to hemozoin induces their maturation, leading to a decrease of their susceptibility to HIV infection and increases their ability to transfer the virus to CD4+ T cells.

This study describes that exposure of primary human immature monocyte-derived dendritic cells to hemozoin induces their maturation, leading to a decrease of their susceptibility to HIV infection and increases their ability to transfer the virus to CD4+ T cells.

This study describes that exposure of primary human immature monocyte-derived dendritic cells to hemozoin induces their maturation, leading to a decrease of their susceptibility to HIV infection and increases their ability to transfer the virus to CD4+ T cells.

This study describes that exposure of primary human immature monocyte-derived dendritic cells to hemozoin induces their maturation, leading to a decrease of their susceptibility to HIV infection and increases their ability to transfer the virus to CD4+ T cells.

This study describes that exposure of primary human immature monocyte-derived dendritic cells to hemozoin induces their maturation, leading to a decrease of their susceptibility to HIV infection and increases their ability to transfer the virus to CD4+ T cells.

This study describes that exposure of primary human immature monocyte-derived dendritic cells to hemozoin induces their maturation, leading to a decrease of their susceptibility to HIV infection and increases their ability to transfer the virus to CD4+ T cells.
Co-infection of poverty related diseases with worms


The authors show that, in addition to presenting a more severe immunosuppression than co-infected individuals with tegumentary cutaneous leishmaniasis, HIV-infected patients with visceral leishmaniasis may also present low CD4+ T-cell counts in spite of low or undetectable viral loads following HAART treatment. Furthermore, both cutaneous leishmaniasis and visceral leishmaniasis HIV co-infected patients presented enhanced activation of CD8+ T cells as compared with HIV-infected or healthy individuals.