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Review

Visceral leishmaniasis: host–parasite interactions and clinical presentation in the immunocompetent and in the immunocompromised host

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SUMMARY

Visceral leishmaniasis are vector-borne parasitic diseases caused by protozoa belonging to the genus *Leishmania*. The heterogeneity of clinical manifestations and epidemiological characteristics of the disease reflect the complex interplay between the infecting *Leishmania* species and the genetic and immunologic characteristics of the infected host. The clinical presentation of visceral leishmaniasis depends strictly on the immunocompetency of the host and ranges from asymptomatic to severe forms. Conditions of depression of the immune system, such as HIV infection or immunosuppressive treatments, impair the capability of the immune response to resolve the infection and allow reactivation and relapses of the disease.

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1. Introduction

Leishmaniasis are a group of vector-borne parasitic diseases caused by protozoa belonging to the genus *Leishmania*. Generally, *Leishmania* infection is transmitted to humans and to other mammals by the bite of an infected sand fly vector. Rarely, the infection can be transmitted by means of blood transfusions,^{1–4} by needle sharing,⁵ or from mother to child during pregnancy.^{6–9}

The World Health Organization (WHO) has stated that leishmaniasis is one of the most neglected diseases, with 350 million people considered at risk of contracting the disease, a burden of about 12 million people currently infected in 98 countries, and two million new cases estimated to occur annually. Among these, visceral leishmaniasis (VL) accounts for about 500 000 cases each year.^{10,11}

The clinical spectrum includes cutaneous, mucocutaneous, and visceral forms. Asymptomatic infections have also been demonstrated, but their role has yet to be clarified.

Here we review the role of the parasite, the vector, and the host, and their complex interplay, in determining the different clinical forms of VL.

2. The parasite

The genus *Leishmania* comprises two subgenera, *Leishmania* and *Viannia*, and each subgenus includes many species. Culture-isolated strains can be typed by isoenzyme electrophoresis for identification by comparison with international reference strains.¹² Parasite populations with common isoenzyme patterns are called zymodemes. Molecular techniques based on DNA amplification and sequencing, or restriction fragment length polymorphism analysis, can be used directly on clinical samples to characterize the isolated strain, but a standardized method of classification based on these techniques is still missing.^{13–15}

The infecting strain of *Leishmania* is very important in determining the clinical manifestations and epidemiological characteristics of the disease. Some *Leishmania* species present a preferential tropism for the viscera: *Leishmania infantum* and *Leishmania donovani*. However, a few cases of VL have been caused by *Leishmania tropica*,¹⁶ which generally causes the cutaneous form in the Old World. Moreover, different strains belonging to a viscerotropic species can cause cutaneous lesions (e.g., some *L. infantum* zymodemes).¹⁷ Within the classical distinction of Old World and New World leishmaniasis, different etiological agents are associated with visceral forms, with *L. donovani* only involved in the Old World, while *L. infantum* circulates worldwide.

The means of transmission of the two *Leishmania* species mainly involved in VL differs depending on the parasite's preferred

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reservoir. Leishmaniasis by *L. infantum* is typically a zoonosis, since the vector becomes infected after biting an animal reservoir. In contrast, *L. donovani* transmission is anthroponotic, as persistent and abundant parasitemia facilitates sand fly infection from humans; post kala-azar dermal lesions are also a source of infection for the vector. In some geographical foci the two *Leishmania* species coexist and clinical forms occur in different epidemiological cycles (e.g., anthroponotic *L. donovani* and zoonotic *L. infantum* in the Arabian Peninsula).¹⁸

3. The vector

Leishmania is a dioxenous parasite since it can infect two species and it carries out part of its lifecycle in each host. Vectors of Leishmania parasites are phlebotomine sand flies. Different genera are responsible for the natural transmission of VL in different geographical areas; vectors belonging to the genus *Phlebotomus* spread Leishmania infection in the Old World (Europe, Asia, and Africa), while sand flies of the genus *Lutzomyia* are involved in the New World (America). Some species of sand fly are specific vectors, meaning that they can support the growth of only one species of *Leishmania*, while other species are permissive vectors, as they can support the growth of more than one species.^{19,20} This specificity is caused by the need for appropriate binding sites on the sand fly gut cells for the specific ligand expressed by the promastigotes, and by the possibility of completing the development of the parasite to produce metacyclic promastigotes, the only infecting form for the vertebrate host. Moreover, the parasite must be able to survive the digestive enzymes produced in the sand fly gut.^{20–22} The epidemiology of leishmaniasis depends on the environmental characteristics that allow the survival of vectors (temperature, humidity, altitude, etc.). Climate changes causing increases in temperature would probably expand the distribution of leishmaniasis and its sand fly vectors to regions that are currently free from both.^{23–26}

4. The host

The immunological response to Leishmania infection is complex and differs depending on the infecting species. Significant differences in host–parasite interactions have been found for cutaneous and visceral leishmaniasis. Distinct virulence factors have been identified in the species involved, and differences in the mechanisms of susceptibility/resistance to the infection have been demonstrated depending on the species of the parasite involved in the infection.²⁷

In VL, resistance and susceptibility to the infection have been related to a number of genetic factors influencing both the severity of the disease and its prognosis. Genetic susceptibility to the disease is probably related to polymorphism in genes associated with the pathways that contribute to the pathogenesis of disease, for example, by influencing trafficking or survival of the parasite in host macrophages, or delaying the development of a protective immune response.^{28,29} The host immune response is of crucial importance in determining the clinical outcome of infection. Both innate and adaptive immunity play a role in defense against the Leishmania parasite. Among protective innate mechanisms, the complement system is very rapidly activated once promastigotes penetrate the dermis and react with serum, resulting in efficient killing of more than 90% of all inoculated parasites within a few minutes.^{30,31} Thus only a small percentage of inoculations of Leishmania parasites would lead to the establishment of an infection. Cytokines and cells of the innate immune system strongly participate to early protection against leishmaniasis; interleukin (IL)-12, produced by dendritic cells, triggers natural killer (NK) cell activation; NK cells are the initial source of

interferon gamma (IFN- γ) production and so they are able to limit parasite spread until a specific T-cell response has been mounted. In fact, IFN- γ is important to enhance killing mechanisms in macrophages, which are the primary target cells of Leishmania.³² It is possible that a robust first-line cytokine response is enough to prevent further spread and growth of the parasites, while in symptomatic individuals, this aspecific response is overcome by high parasite inocula, or is weak for genetic reasons.³³

Tumor necrosis factor (TNF) plays a critical role in both parasite clearance in the liver and tissue damage in the spleen. An effective response of the liver to Leishmania infection requires the fine regulation of TNF production, because this cytokine is essential for granuloma formation and the control of parasite growth. In contrast, an excess of TNF in the spleen is responsible for progressive cell damage and immunological dysfunction.³⁴ Chemokines also modulate the immune response to Leishmania. Chemokine profiles of the host are modified by Leishmania parasites. In the spleen, high levels of TNF and dysfunctions in the production of chemokines are responsible for parasite persistence.^{34,35}

The specific immune response to Leishmania infection is cell-mediated. The development of clinical symptoms is related to the subset of CD4+ T helper (Th) lymphocytes primarily activated. Many experimental models and clinical studies have suggested that the Th1 response with IFN- γ production correlates with resistance or resolution of infection by inducing a potent leishmanicidal mechanism in phagocytes. In contrast, the Th2 response with IL-4 and IL-10 production results in susceptibility to infection and the development of severe disease, due to the inhibition of macrophage activation with consequent intracellular replication of the parasite. However, when experimental models representative of the genetic heterogeneity of the human population have been adopted, more complex immune responses have been observed, showing that several patterns of the immune response may be associated with the same clinical outcome.³⁶ Accordingly, in human disease, a mixed picture of Th1 and Th2 cytokines is often observed.^{37–41}

CD8+ T-cells also participate in the specific immune response to Leishmania through IFN- γ production and cytotoxic activity on Leishmania-infected macrophages.⁴²

So, clinical manifestations of VL are largely dependent on the host's immune competence with respect to those mechanisms involved in granuloma formation and subsequent control of infection, and this is also observed in cutaneous and mucocutaneous leishmaniasis.^{43–45}

5. Asymptomatic infection

It has been estimated that in endemic areas the proportion of asymptomatic infections is 5–10-times greater than the number of clinically apparent VL cases in immunocompetent hosts.^{10,28,46,47} Cryptic infection can be detected in persons without a previous history of clinical VL by serological evidence of anti-Leishmania antibodies, by detection of parasite DNA in blood samples, or by a positive reaction to the leishmanin skin test (LST). The meaning and the possible evolution of asymptomatic infections is still unclear; recent studies suggest that a previous contact with *L. infantum* in asymptomatic subjects from an endemic area does not indicate a risk of progression to VL and may only be temporary.⁴⁸ However, a high rate of disease conversion was observed in previously asymptomatic carriers of *L. donovani* from an endemic area.⁴⁹ Parasite DNA has been detected in blood samples from blood donors,^{4,50–53} and its presence would reflect the very recent presence of viable parasites in the host, since DNA degradation occurs very rapidly after parasite death.⁵⁴ Nevertheless, leishmaniasis transmission by blood transfusion has only very rarely been

reported,^{1–4} and it is preventable by the particular treatment of blood units.^{4,55–57} Finally, the role of asymptotically infected individuals in the transmission cycle is currently unknown; people infected with the zoonotic species *L. infantum* are probably not infectious for the sand fly vector, due to the very low or undetectable parasitemia found in this group, as demonstrated by the lack of infection in sand fly vectors that have fed on asymptomatic subjects infected with *L. infantum*.⁵⁸ However, the large proportion of asymptomatic carriers found in endemic countries does not allow their possible role as a reservoir of the infection to be definitively ruled out.⁵³ On the other hand, *L. donovani* amastigotes have been detected by microscopic examination of blood smears from asymptomatic carriers,⁵⁹ suggesting a higher level of parasitemia in these subjects, given the lower sensitivity of this technique with respect to molecular methods. So, carriers of the anthroponotic species *L. donovani*, for which humans are considered to be the sole proven reservoir, could potentially be infectious for the sand fly vector, with important implications for control strategies.¹⁰

Asymptomatic infection has also been demonstrated in HIV-infected patients. Also in this population, the percentage of asymptomatic infection could be larger than symptomatic cases.⁵⁰ Leishmania parasitemia was found to be significantly higher in patients with higher viral loads.⁵⁰ A high parasitemic burden could possibly be related to a higher risk of developing symptomatic disease, due to the reciprocal effects that enhance the multiplication of both pathogens. Indeed, it has been shown that HIV infection increases the risk of developing VL by 100- to 2300-times in areas of endemicity.⁴¹ Periodic screening for Leishmania by PCR could be useful in HIV-infected subjects to select patients at higher risk of Leishmania reactivation (e.g., those with high Leishmania parasitemia) and to survey the long-term outcome of asymptomatic infection.⁵⁰

6. Visceral leishmaniasis in the immunocompetent host

In the immunocompetent host, VL is caused by a primary infection with Leishmania parasites transmitted by the bite of a phlebotomine sand fly. VL is the result of a chronic infection; the incubation period ranges from 10 days to 1 year.¹⁰ Clinical features of typical forms are fever, weight loss, hepatosplenomegaly, and pancytopenia.^{18,60,61} Fever can be intermittent in the first period and successively continuous. Non-tender splenomegaly and hepatomegaly are caused by infection of the reticuloendothelial system. Pancytopenia caused by parasites invading the bone marrow is responsible for pallor due to anemia, and can subsequently cause hemorrhages due to thrombocytopenia and concurrent infections due to leukopenia. Anorexia and weight loss can lead to wasting syndrome in misdiagnosed cases. Lymphadenopathy may be present in some geographical areas and may be the only clinical manifestation. Darkening of the skin is typically found in India (kala-azar means black fever).^{10,18} PCR on peripheral blood allows a rapid and noninvasive parasitologic diagnosis of VL.⁶² The disease can be fatal if untreated, while appropriate treatment usually leads to a complete cure. Anti-leishmanial treatment is based on the systemic administration of one or a combination of effective drugs. Alongside pentavalent antimonials, which have been the standard first-line medicines for many decades, new anti-leishmanial drugs such as lipid formulations of amphotericin B, miltefosine, and paromomycin are currently used for the treatment of VL. Lipid formulations of amphotericin B, and in particular liposomal amphotericin B, are considered to be the drugs of choice for the treatment of VL.^{10,46} Effective therapy can be different in different geographical areas. While liposomal amphotericin B can be valuable as monotherapy, combination treatment should be considered for other drugs, with the

advantages of shortening the duration of treatment, reducing the overall dose of medicines, and reducing the probability of selection of drug-resistant parasites.^{10,46}

Recurrence of the disease is rare in the immunocompetent host; relapses can occur soon after the end of therapy and are generally responsive to a new course of treatment.⁶³

7. Visceral leishmaniasis in the immunocompromised host

Leishmania parasites are pathogens that establish chronic intracellular parasitism and survive for an infected person's lifetime. Conditions of depression of the immune system, such as HIV infection or immunosuppressive treatments in transplant recipients and in patients with autoimmune diseases, impair the capability of the immune response to resolve the infection and allow the reactivation of the disease from sites of latency of the parasite.^{46,64–66} Reactivation of chronic infection can occur long after primary contact with the parasite.

The HIV epidemic has modified the epidemiology and the clinical features of VL. Both pathogens, HIV and Leishmania, infect the same host cell, the macrophage, and establish a vicious circle whereby the parasite Leishmania induces a more robust HIV-1 production and the virus mediates a greater parasitic replication. Moreover, HIV patients are more likely to develop VL due to the depletion of both the cellular and humoral responses to Leishmania.⁶⁷ Symptomatic disease develops more frequently from a reactivation of a dormant infection, but can also occur after primary infection.

The clinical presentation of VL in HIV-infected patients is similar to that observed in HIV-uninfected individuals. The main difference is the lower rate of response to treatment and the consequent high rate of disease relapse, especially in those who are not receiving highly active antiretroviral treatment (HAART).⁶⁸ Once HAART is established, relapses are reduced, but not completely prevented. In fact, low-level continuous persistence of Leishmania DNA in peripheral blood has been demonstrated in treated patients, in spite of an adequate clinical response to specific therapy, leading to a clinical condition characterized by alternating asymptomatic and symptomatic periods.^{69–72} However, low but detectable levels of Leishmania DNA in peripheral blood does not always herald a clinical relapse.

In the last decades, leishmaniasis has been most frequently reported among transplant recipients, mainly in the Mediterranean area.^{46,64,73} The immunosuppressive drugs used in these patients hamper T-cell activation and proliferation, thereby altering defense mechanisms against intracellular microorganisms.⁷⁴ In this group of patients, the disease can be the result of different pathogenic models. The first is the typical route of transmission through the bite of an infected sand fly, which causes a symptomatic disease due to the host immunosuppression; the second is the reactivation of a pre-existing, dormant Leishmania infection induced by the immunosuppressive drugs; the third is iatrogenic acquisition of Leishmania infection by transplant of an infected organ or transfusion of infected blood products.⁷⁵ VL can occur as an early (median delay of 17 days) or late (median delay of 18 months) complication after transplantation, generally depending on the transplanted organ.^{64,75} Transplant recipients develop the classic clinical form of VL, with fever, hepatosplenomegaly, and pancytopenia. In addition, atypical cases of mucosal leishmaniasis caused by viscerotropic strains have been reported. Due to immunosuppression, multiple relapses are frequent.^{64,73} Since the clinical picture may simulate other infections, misdiagnosis and a consequent delay in the establishment of an adequate treatment can occur. Early diagnosis and treatment are crucial in transplant patients – for the severity of the disease itself and because the course of VL can cause graft dysfunction and loss.^{75,76}

Appropriate screening for leishmaniasis before beginning immunosuppressive treatments could be useful for calling to attention the potential risk for VL in the immunocompromised host. The LST (Montenegro test), an intradermal injection of a suspension of killed promastigotes, measures delayed hypersensitivity reactions and appears to be valuable for detecting asymptomatic *Leishmania* infections.⁷⁷ However, some limitations of this test, related to poor antigen standardization, variability in sensitivity and specificity from one antigen to another, and evidence of loss of antigen potency over time,⁷⁸ have prompted the development of new screening tools. Recently, *in vitro* tests based on the measurement of IFN- γ released by activated T-cells stimulated with *Leishmania*-specific antigens have been developed to detect the cell-mediated immune response to *L. infantum*⁷⁹ and *L. donovani*,^{78,80} with promising results.

8. Conclusions

The complex interplay between the infecting *Leishmania* species and the genetic and immunologic characteristics of the infected host causes the heterogeneity of clinical manifestations and epidemiological characteristics of the disease. *Leishmania* establishes chronic intracellular parasitism and survives for an infected person's lifetime, and for this reason leishmaniasis today has to be considered a disease of the immunocompromised host; screening for leishmaniasis before initiation of any immunosuppressive treatment is clearly needed to determine the potential risk for VL.

Conflict of interest: No conflict of interest to declare.

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