Leshmaniose Cutânea e Visceral: Revisão

Cutaneous and Visceral Leishmaniasis: a Review

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RESUMO

Protozoários do gênero Leishmania são parasitos transmitidos por insetos flebotomíneos e causam um espectro de doenças coletivamente conhecidas como leishmanioses. Estes doenças são consideras um sério problema de saúde pública em muitas partes do mundo, resultando em uma estimativa de 12 milhões de casos a cada ano. Apesar dos avanços no diagnóstico, tratamento e nas pesquisa científica de base, as leishmanioses são negligenciadas e correlacionadas com a pobreza. Assim, neste artigo, baseado em informações oriundas de uma pesquisa bibliográfica, são descritos a epidemiologia e imunologia da doença, ciclo de vida do parasito Leishmania, tratamento e vacinas contra as leishmanioses. Conclui-se que os obstáculos atuais estão ligados a um inadequado controle do vetor, a inexistência de uma
vacina eficaz e o inusco no desenvolvimento de novas drogas contra a doença.

**Palavras-chaves:** *Leishmania*; leishmaniose cutânea; leishmaniose visceral; imunologia; flebotomíneo; vacina

**ABSTRACT**

Protozoan parasites from the genus *Leishmania* are spread by a sandfly insect vector and cause a variety of diseases collectively known as leishmaniasis. The disease is a significant health problem in many parts of the world resulting in an estimated 12 million new cases each year. Despite tangible advances in diagnosis, treatment, and basic scientific research, leishmaniasis is neglected and embedded in poverty. Thus, in this article, the epidemiology, immunology, life cycle of *Leishmania*, treatment and vaccine against leishmaniasis are described according to the information found during bibliographic research. The study concludes that current obstacles to prevention and proper management of the disease include inadequate vector (sandfly) control, no vaccine available, and insufficient access to or failure in developing affordable new drugs.

**Key-words:** Leishmania; cutaneous leishmaniasis; visceral leishmaniasis; immunology; sandfly; vaccine

1) **INTRODUCTION**

Leishmaniasis has several diverse clinical manifestations: ulcerative skin lesions, destructive mucosal inflammation, and disseminated visceral infection (kalazar) (Brasil, 2007). Epidemiology, immunopathology, and outcome are similarly diverse, since infection occurs in multiple endemic regions, in both children and adults, and is caused by nearly two-dozen distinct *Leishmania* species (Desjeux, 2001; Desjeux, 2004). According to Alvar *et al.* (2004) and McMahon-Pratt & Alexander (2004), variable disease expression has also been shown in naturally infected animals and, especially, experimentally infected animals. Nevertheless, all forms of this protozoal infection share three pathogenetic features: resident tissue macrophages are targeted and support intracellular parasite replication; the host immunoinflammatory response
regulates expression and outcome of disease; and persistent tissue infection is characteristic.

The World Health Organization (WHO) estimates approximately 12 million affected individuals with an estimated annual incidence of 1.5-2 million, among a susceptible population of approximately 350 million in 88 different countries (WHO, 2008). According to Murray (2002), leishmaniasis is one such infection which rarely shares this limelight and thus remains largely a neglected disease.

Despite this, several issues regarding leishmaniasis merit discussion: resistance to conventional drug treatment has developed in certain areas of the world, necessitating a change of first-line agents; rapid, less invasive diagnostic procedures have been developed which are most useful in poorly resourced parts of the world; despite advances in the understanding of the immunology of the disease and the unraveling of the Leishmania genome, a vaccine has not yet been developed; and the extent of disease in different individuals stresses the complex immunology of leishmaniasis, brought to the fore more recently with the advent of HIV–Leishmania coinfection and Leishmania to infection associated to malnutrition protein-caloric (Malafaia et al., 2008a,b), and its difficult eradication in this scenario.

Thus, this review is based on information from bibliographic research, Entrez-Pubmed searches on leishmaniasis, review articles and papers in their reference lists, and from the authors’ personal archives.

2. EPIDEMIOLOGY AND LIFE CYCLE OF LEISHMANIA

According to WHO (2008), leishmaniasis is endemic in more than 60 countries worldwide including Southern Europe, North Africa, the Middle East, Central and South America and the Indian subcontinent. The burden of disease
(90% of cases) is borne by Afghanistan, Pakistan, Syria, Saudi Arabia, Algeria, Iran, Peru and Brazil in the case of cutaneous leishmaniasis (CL), and by India, Bangladesh, Nepal, Sudan, and Brazil in the case of visceral leishmaniasis (VL) (Desjeux, 2004). In view of this geography, leishmaniasis remains embedded in poverty as a neglected disease (Yamey, 2002). Recently the number of reported cases and geographical areas has increased (Arias et al., 1996) and this has sparked concern regarding the contribution that global warming might have on this observation (Desjeux, 2001; Kuhn, 1999).

One of the causative organisms of leishmaniasis, *Leishmania donovani*, was first described in 1903 by Leishman and Donovan almost simultaneously (Desjeux, 2004). *Leishmania* is a protozoon, able to infect animals, humans and sandflies. Nine major species of *Leishmania* involved in human disease are grouped into old world and new world species (*Table 1*). Each may cause a disease specific to the species and the host response. Every year, an estimated 1.5 to 2 million children and adults develop symptomatic disease (CL 1 to 1.5 million and VL 0.5 million), and the incidence of infection is substantial when subclinical infections are included (Desjeux, 2004). Leishmaniasis is associated with about 2.4 million disability-adjusted life years and around 70 000 deaths per year (Desjeux, 2004).

*Table 1.* Major species of *Leishmania* causing human disease and their geographic distribution.

<table>
<thead>
<tr>
<th>Species</th>
<th>Region</th>
<th>Distribution</th>
<th>Manifestation</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. major</em></td>
<td>Old World</td>
<td>Middle East, Africa and Asia</td>
<td>Cutaneous</td>
</tr>
<tr>
<td><em>L. tropica</em></td>
<td>Mediterranean basin</td>
<td>Mediterranean basin</td>
<td>Cutaneous</td>
</tr>
<tr>
<td><em>L. aethiopica</em></td>
<td>Ethiopia and Kenya</td>
<td>Ethiopia and Kenya</td>
<td>Cutaneous/Mucocutaneous</td>
</tr>
<tr>
<td><em>L. infantum</em></td>
<td>Mediterranean basin</td>
<td>Mediterranean basin</td>
<td>Visceral</td>
</tr>
<tr>
<td><em>L. donovani</em></td>
<td>Africa, India, Arabian Peninsula</td>
<td>Africa, India, Arabian Peninsula</td>
<td>Visceral</td>
</tr>
</tbody>
</table>
Table 1. (continuation)

<table>
<thead>
<tr>
<th>Species</th>
<th>Region</th>
<th>Distribution</th>
<th>Manifestation</th>
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<tbody>
<tr>
<td><em>L. amazonensis</em></td>
<td>New World</td>
<td>North, Central and South America</td>
<td>Cutaneous</td>
</tr>
<tr>
<td><em>L. mexicana</em></td>
<td>South America</td>
<td>South America</td>
<td>Cutaneous</td>
</tr>
<tr>
<td><em>L. braziliensis</em></td>
<td>South America</td>
<td>South America</td>
<td>Cutaneous/Mucocutaneous</td>
</tr>
<tr>
<td><em>L. chagasi</em></td>
<td>South America</td>
<td>Central and South America</td>
<td>Visceral</td>
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About 70 different species of sandfly can transmit leishmaniasis (Murray, 2005). The species are mainly *Lutzomyia* in the Americas and *Phlebotomous* elsewhere (Mandell *et al.*, 2005). The sandfly characteristically feeds at dusk, and being a weak flier, tends to remain close to its breeding area, not too high from the ground. Different species have different feeding and resting patterns.

*Leishmania* to Infection is more common in men than in women, but this may reflect increased exposure to sandflies. Although disease occurs irrespective of age, children aged 1 to 4 years are particularly at risk of infection and childhood infection may account for more than half of all cases in some of these countries (Grech *et al.*, 2000). Untreated VL carries a mortality of 75–95%, while CL can disseminate to involve the mucosa, resulting in death from secondary infection (Laison & Shaw, 1987).

As reviewed by Killinck-Kendrick (1990) and Gossage *et al.* (2003), the life cycle of *Leishmania* involves alternation between a mammalian host and a phlebotomine sand fly host. In the mammalian host the developmental biology of the parasite is relatively simple and consistent between species: metacyclic promastigotes (infective forms) are introduced into the skin by the bite of the sand fly. These are resistant to complement attack and they enter local phagocytes rapidly. Transformation into aflagellate amastigotes then occurs and
the life history of the infection in humans is perpetuated by this life cycle stage (Handman & Bullen, 2002; Engweda et al., 2004). In contrast the developmental biology of the parasite in the sand fly host is more complex and less well understood.

According to Kamhawi (2006), outside the mammalian host, the Leishmania life cycle is confined to the digestive tract of sand flies. Most Leishmania species (subgenus Leishmania) are suprapylarian parasites; that is, their development is restricted to the midgut. Members of the New World Viannia subgenus, such as L. braziliensis, are peripylarian parasites: they enter the hindgut before migrating forward into the midgut.

3. IMMUNOLOGY

Most of the experimental immunological data come from mouse models and less is known about the immunology of human leishmaniasis. Although mouse models have been used for the study of both CL and VL, they more closely reflect the situation in human cutaneous leishmaniasis than visceral disease. One knows that the immune response to Leishmania infection is cell mediated (Table 2). The organism lies exclusively intracellular, mainly inside macrophages as replicating amastigotes. The outcome of infection will depend on whether the host mounts primarily a T-helper (Th1 or Th2 response) (Heinzel et al., 1989).

In the case of CL, studies in animal models have indicated that effective protection against infection is attributed to the development of a potent CD4+ Th1 – type immune response, characterized by the production of IL-12 and IFN-c, which subsequently mediates macrophage activation, nitric oxide production and parasite killing (Rogers et al. 2002; Alexander & Bryson, 2005). In contrast, as demonstrated for von Stebut & Udey (2004), clear-cut polarization of T helper cell responses is not evident in human leishmaniasis which shows a mixed Th1 and Th2 immune response. Studies in animals models suggest that
the same parasite epitope can induce a Th1 response in animals with resolving infection or a Th2 response in others with disease progression (Reiner et al., 1993). Other animal studies have shown that Th1 and natural-killer (NK) cells produce interferon gamma (IFN-$\gamma$), which mediates resistance, whilst interleukin (IL) 4-producing Th2 cells confer susceptibility to infection (Reed & Scoot, 1993). Human studies have also shown that IL-4, a component of the Th2 response, may also be associated with disease progression (Sundar et al., 1997).

Table 2. Types of immunity mediated by cells in leishmaniasis.

<table>
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<tr>
<th>Th1 immune response</th>
<th>Th2 immune response</th>
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<tr>
<td>• T-helper CD4 cells producing IL-2, IL-3 and IFN-$\gamma$;</td>
<td>• Th2 cells produce IL-4, IL-5, IL-6, IL-10 which favour induction of antibody responses by B cells;</td>
</tr>
<tr>
<td>• Will promote immune responses that are primarily cell mediated/inflammatory by activating cytotoxic T cells, NK cells and macrophages;</td>
<td>• In leishmaniasis, associated with disease progression.</td>
</tr>
<tr>
<td>• In leishmaniasis, associated with disease resolution.</td>
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In the Th1 response, promastigotes attach to reticuloendothelial cells and T helper CD4 cells produce IL-2, IL-3 and IFN-$\gamma$ which activate macrophages. IL-12 and tumour necrosis factor (TNF) are also important in this type of response. The promastigotes are then phagocytosed by the activated macrophages into vacuoles which then fuse with lysosomes. Host genetics inevitably influence the type of immune response. Studies in mice and humans have shown that genes such as those coding for natural resistance associated macrophage protein 1 (NRAMP1), TNF or the major histocompatibility complex are thought to play a major role in the outcome of infection (Roberts et al., 2000).
The parasite itself can affect the macrophage and dendritic cell responses. Specific gene loci such as the A2 gene can code for products which promote *L. donovani* infectivity (Zijlstra & El-Hassan, 2001). Thus the interplay between the host-determined delayed-type hypersensitivity, antigen-specific T-cell reactivity, and cytokine secretion, and the type and virulence of the particular infecting *Leishmania* species determine what type of disease expression develops in the host.

However, most studies on experimental VL question about the role of IL-4 in susceptibility to visceral infection by *Leishmania* due to the contradictory results reported (Kaye *et al.*, 1991; Saha *et al.*, 1993; Miralles *et al.*, 1994). Our results point to a less significant role for IL-4 in protection against VL, because IL-4 production was not correlated with reduction of the parasite load in mice vaccinated with *L. chagasi* antigen plus saponin, as reported by Lehmann *et al.* (2000). The authors observed that in VL a Th1 dominated immune response is protective against the *L. donovani* parasites and, furthermore, that the capacity to produce IFN-γ rather than the presence of IL-4 determines the efficacy of the immune response in susceptible mice.

Others studies have shown that protection in the visceral model is associated with a mixed Th1/Th2 pattern. Ghosh *et al.* (2002) showed that immunization with A2 protein protects mice against *L. donovani* infection, and this protein induces a mixed Th1/Th2 and a humoral response. Ramiro *et al.* (2003) showed that LACK immunization in a heterologous prime-boost regime (plasmid DNA and recombinant vector) was able to protect dogs against *L. infantum* infection, and this protection was associated with an increase in the mRNA level of IL-4 and IFN-γ in peripheral blood mononuclear cells. These studies suggest that, although protection in experimental visceral infection demands IFN-γ production response, IL-4 is also necessary in this model. Furthermore, Stager *et al.* (2003) showed that IL-4 may be beneficial against *L. donovani* infection, and that mice knockout for the α chain of IL-4 receptor had a
retardation of granuloma maturation and an increase in parasite load both in the liver and spleen.

4. TREATMENT OF LEISHMANIASIS

The first line of treatment is predominantly based on pentavalent antimonials, a classic treatment in most of the endemic areas. However, increasing resistance to antimonials is a major problem, and this is most evident in North Bihar, India, where the failure rate for this treatment is more than 50% (Murray, 2005; Sundar et al., 2003).

The second line treatment includes drugs such as amphotericin B and pentamidine, which are characterized by high efficacy, but are relatively expensive and have severe side effects (Berman, 2003; Davis & Kedzierski, 2005). Amphotericin B is an effective treatment used in Sb(V)-resistant cases. It is toxic and needs to be given for a prolonged period on an inpatient basis.

In deciding the best mode of treatment of CL, some facts need to be considered. Old World CL is not a life threatening disease and 90% of patients heal spontaneously within 3–18 months. The outcome of CL in the New World depends on the infecting species and may vary from benign to more severe manifestations. It is thus important to try to identify the infecting species, either by knowing the endemic species of the specific geographical area, or by means of diagnostic procedures. This can throw light on the prognosis and management options.

Treatment of CL will accelerate cure and reduce scarring. This is especially important at cosmetically important sites. Options in the treatment of CL include local or systemic treatment. Criteria in favour of local treatment (Blum et al., 2004) include: Old world CL: small, single lesions; lack of risk of development of mucocutaneous leishmaniasis, lack of lymph node metastases; and L. mexicana lesions. New world lesions except L. mexicana, mucosal or
lymph node involvement and lesions refractory to local treatment would be indications for systemic treatment.

5. VACCINE AGAINST LEISHMANIASIS

According to Kedzierski et al. (2006), leishmaniasis in general, but particularly CL, is probably one of a few parasitic diseases that is most likely to be controlled by vaccines. The relatively uncomplicated leishmanial life cycle and the fact that recovery from a primary infection renders the host resistant to subsequent infections indicate that a successful vaccine is feasible. Extensive evidence from studies in animal models, mainly mice, indicates that solid protection can be achieved upon immunization with defined protein or DNA vaccines.

During the past several decades, extensive efforts have been made to search for an effective Leishmania vaccine. Vaccine formulations including killed, live attenuated parasites, recombinant Leishmania proteins or DNA encoding leishmanial proteins, as well as immunomodulators from sandfly saliva have been examined. Although to date, there is no vaccine against Leishmania, several of the vaccine preparations are at advanced stages of clinical testing.

In this in case, as discussed for Kedzierski et al., (2006), an ideal anti-Leishmania vaccine would need to possess several attributes, but not all of them may be easily achievable. These include: i) safety; ii) affordability to the populations in need; iii) induction of CD4+ and CD8+ T cell responses and long-term immunological memory that can be boosted by natural infections, thus minimizing the number of immunizations; iv) effectiveness against species causing CL and VL; v) stability at room temperature eliminating the need for a cold chain to preserve potency and; vi) effectiveness as a prophylactic as well as a therapeutic vaccine.
There is no effective vaccine for prevention of leishmaniasis. The closest effective alternative to vaccination follows from the traditional “leishmanization” technique adopted in the Middle East and Eastern Europe. This involved the encouragement of sandfly bites on traditionally unexposed areas of skin, such as the buttocks. The resulting lesion would heal spontaneously, in the process providing immunity against CL in less acceptable areas, such as the face. Early studies recommended using \textit{L. tropica} inoculations to induce immunity (Berberian, 1939). This technique has been further developed in Iran, where scientists have produced standardised and stable \textit{L. major} populations which can produce more consistent and acceptable iatrogenically-induced lesions (Davies \textit{et al.}, 2003).

The safety and efficacy of live-attenuated and killed vaccines has been debated and shifts in favour of development some of these has been recorded in recent years. Killed vaccines were favored in the 1990s because of safety problems with live-attenuated vaccines; however, recent advances in manipulation of the \textit{Leishmania} genome may make development of a live attenuated vaccine more feasible (Handman, 2001). Work on recombinant DNA derived antigen vaccines and protein or peptide-based vaccines is a more recent approach, made possible by the \textit{Leishmania} Genome Project (www.sanger.ac.uk/Projects/L_major/). The realization that CD8 cells are as important as CD4 cells in inducing resistance (Belkaid \textit{et al.}, 2002) and maintaining immunity has led to a shift in vaccine research (Rhee \textit{et al.}, 2002). Most vaccine research is targeted against CL; any effectiveness against VL is uncertain. Work on a vaccine against human VL has been less successful, (Khalil \textit{et al.}, 2000) but should be boosted following success with a CL vaccine.

While the cost-effectiveness and safety issues can be relatively straightforward to resolve, the induction and maintenance of the required immune responses are much more difficult to solve and cross-species protection may not be achieved by the same vaccine.
6. CONCLUSION

Based in what it was displayed, leishmaniasis remains a problematic infection requiring either potentially toxic treatments or less toxic, but expensive drugs. However, the availability of newer oral agents may change the way this disease is managed. Relapse may occur, especially in situations where immunosuppression is present; secondary prophylaxis needs to be given in this setting.

The combination of *Leishmania*, HIV and anthroponotic transmission between injecting drug users heralds a potential for higher incidence rates in endemic countries with severe drug abuse problems. In the absence of an effective vaccine, and with extension of endemicity, possibly due to climate change, these problems may become worse. Current obstacles to realistic prevention and proper management include inadequate vector (sandfly) control, no vaccine, and insufficient access to or impetus for developing affordable new drugs.

7. ACKNOWLEDGEMENTS

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8. REFERENCES


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