Pathogens and Disease, 76, 2018, fty025

doi: 10.1093/femspd/fty025
Advance Access Publication Date: 21 March 2018
Minireview

MINIREVIEW

Leishmania (Viannia) guyanensis in tegumentary leishmaniasis

Arissa Felipe Borges, Rodrigo Saar Gomes and Fátima Ribeiro-Dias[*,†]

Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiás, Brazil

[*]**Corresponding author:** Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Rua 235 S/N, Setor Leste Universitário, Goiânia – GO, 74605-050, Brazil. Tel: +55 62 3209 6116; Fax: +55 62 3209 6363; E-mail: fatimardias@gmail.com

One sentence summary: The parasite *Leishmania guyanensis* causes cutaneous and mucosal leishmaniasis presenting some differences from other *Leishmania* spp. especially concerning clinical therapeutic outcome and more frequent complications due to *Leishmania* virus infection.

Editor: Jacques Schrenzel

[†]Fátima Ribeiro-Dias, http://orcid.org/0000-0002-7950-3414

ABSTRACT

Leishmania (*Viannia*) *guyanensis* is a causal agent of American tegumentary leishmaniasis (ATL). This protozoan has been poorly investigated; however, it can cause different clinical forms of ATL, ranging from a single cutaneous lesion to severe lesions that can lead to destruction of the nasopharyngeal mucosa. *L.* (*V.*) *guyanensis* and the disease caused by this species can present unique aspects revealing the need to better characterize this parasite species to improve our knowledge of the immunopathological mechanisms and treatment options for ATL. The mechanisms by which some patients develop a more severe form of ATL remain unclear. It is known that the host immune profile and parasite factors may influence the clinical manifestations of the disease. Besides intrinsic parasite factors, *Leishmaniavirus* RNA 1 (LRV1) infecting *L. guyanensis* can contribute to ATL immunopathogenesis. In this review, general aspects of *L. guyanensis* infection in humans and mouse models are presented.

Keywords: *Leishmania guyanensis*; tegumentary leishmaniasis; *Leishmania* virus; immunity; murine models

INTRODUCTION

Leishmaniasis is a public health problem in 98 countries, distributed across four continents (Americas, Europe, Africa and Asia), with compulsory notification in 32 countries. The World Health Organization (WHO) estimates that 350 million people are at risk of contracting the infection, accounting for approximately 2 million new cases of different clinical forms per year. Leishmaniasis is considered one of the six most important infectious diseases due to its high detection coefficient and capacity to produce deformities (WHO 2016).

American tegumentary leishmaniasis (ATL) occurs in the Americas from the southern United States to northern Argentina, with the exception of Uruguay and Chile. In Brazil, around 20 000 cases/year are registered with an incidence coefficient of 10.3 cases/100 000 inhabitants in the last 5 years. In 2015, the North region had the highest coefficient (51.1 cases/100 000 inhabitants), followed by the Midwest (19.0 cases/10 000 inhabitants) and Northeast (9.1 cases/100 000 inhabitants) (Brasil 2017). ATL is a parasitic disease affecting the skin, nasal and pharyngeal mucosa that is caused by different species of protozoa of the genus *Leishmania* (Ross 1903; Gontijo and de Carvalho 2003).

In Brazil, the main species responsible for ATL that belong to the subgenus *Viannia* include *Leishmania* (*V.*) *braziliensis* and *L.* (*V.*) *guyanensis*, which are associated with cutaneous (localized cutaneous leishmaniasis, LCL; disseminated cutaneous leishmaniasis, DCL) and mucosal lesions (mucosal leishmaniasis, ML) (Goto and Lauletta Lindoso 2012). *Leishmania braziliensis* presents the highest geographical distribution in Brazil. Both species can cause severe ML with destructive lesions of nasopharyngeal

Received: 16 July 2017; **Accepted:** 20 March 2018

mucosal tissue, which can be disfiguring and also disabling (Barbosa *et al.* 1976; Grimaldi and McMahan-Pratt 1991; Nunes *et al.* 1995).

ATL lesions are caused by parasites transmitted to the hosts by a blood-feeding phlebotomine vector, which delivers promastigote forms into the skin. The parasites grow inside cells of the mononuclear phagocytic system, especially macrophages, as amastigote forms. The establishment of infection depends on the interaction between the parasite, the vector and the host immune response (Ashford 2000). Although they belong to the subgenus *Viannia*, *L. braziliensis* and *L. guyanensis* present biological differences and different epidemiological distribution, transmission, clinical manifestation and immune response, topics that will be addressed in this review.

Leishmania guyanensis: MOLECULAR CHARACTERIZATION AND GENERAL ASPECTS OF THE TEGUMENTARY LEISHMANIASIS

To determine the species causing ATL is essential not only to evaluate the clinical prognosis, but also to choose the best therapeutic scheme. As parasites sometimes are not easily detected in patient lesions, most of the time the diagnosis is based on clinical data associated with a positive Montenegro skin test (MST). It is difficult for routine diagnosis to identify parasite species. The polymerase chain reaction (PCR)-based method has a high sensitivity for the diagnosis (71%) at the parasite genus level in patients with ML. This method combined with restriction fragment length polymorphism (RFLP) can identify the parasite at the species level (Prestes *et al.* 2015). Montalvo Alvarez *et al.* (2010) used the RFLP method to identify the gene encoding the heat shock protein 70 (hsp70), which has been shown to be efficient in identifying *Leishmania* species when the HaeIII restriction enzyme is used. Another alternative for the differential diagnosis between *L. braziliensis* and *L. guyanensis* is the use of kDNA (kinetoplast DNA) PCR, which has been shown to be highly sensitive (de Benicio et al. 2011).

Rougeron *et al.* (2011) evaluated the genetic structure of 153 strains of *L. guyanensis* obtained from patients with LCL from French Guiana and 120 reference samples. They have demonstrated that this species is characterized by a mixture in the reproduction mode, alternating the cloning in both the vertebrate and vector, and inbreeding within the vector. Previously, it was demonstrated that *L. guyanensis* isolates from Colombia present chromosome-size polymorphisms and variability in chromosomal localization of mini-exon gene sequences (Pacheco *et al.* 2000). In French Guiana, two distinct populations of *L. guyanensis* have been identified using small subunit ribosomal RNA (SSU rDNA) analysis (Rotureau *et al.* 2006). Our group identified *L. guyanensis* using three strategies: (i) SSU rDNA sequenced using primers S12/S4; (ii) sets of primers (G6PDISVC and G6PD-ISVB) for PCR assays to discriminate *L. braziliensis* from other parasites of the *Viannia* subgenus (G6PD-ISVG and G6PD-LVF); and (iii) ribosomal internal transcribed spacer (ITS) using IR1/IR2 primers, followed by HaeIII treatment; the product was cloned and the nucleotide sequence was determined, followed by phylogenetic analysis (Pires *et al.* 2015). These methods are expensive to be used in endemic areas because they require sophisticated equipment and reagents. Simplification of these techniques is necessary for their clinical use in regions where several species of *Leishmania* can coexist.

The differential diagnosis between species is important since they have different sensitivities to the drugs. Patients diagnosed with *L. guyanensis* could be treated with pentamidine in some places or with other drugs, since this species has been shown to be less sensitive to pentavalent antimonials (Arevalo *et al.* 2007).

Leishmania guyanensis is a species found in Brazil, Bolivia, Colombia, French Guiana, Peru, Suriname and Venezuela. In Brazil, it has a high distribution mainly in the Amazon region, including the states of Acre, Amapá, Roraima, Amazonas and Pará (Grimaldi and McMahan-Pratt 1991). In 2015, our group described the first case of a patient infected with the *L. guyanensis* in Goiás, a state in which autochthonous cases associated with this species had not been recorded. This circulation of parasites in different parts of the country can create new foci of leishmaniasis in other Brazilian regions. The major species of vectors that transmit *L. guyanensis* are *Lutzomyia umbratilis*, *Lu. anduzei* and *Lu. whitmani*, which are distributed in several countries of South America (Scarpassa and Alencar 2012; de Souza Freitas *et al.* 2015). In Brazil, *Lu. umbratilis* (Amazon) and *Lu. anduzei* (Pará) are present in the northern region of the country and *Lu. whitmani* in the northeastern region (Killick-Kendrick 1990).

The transmission cycle of *L. guyanensis* is mainly associated with the presence of the human beings in forest regions. There are several wild mammals as natural reservoirs for this species such as sloths (*Choloepus didactylus*), anteater (*Tamandua tetradactyla*), marsupials and rodents, all responsible for maintaining the parasite in a given environment (Lainson *et al.* 1981).

In general, patients with LCL caused by *L. guyanensis* present the primary involvement of the skin. The lesion is an ulcer with a tendency to spontaneously heal. It can appear as only one lesion or multiple lesions. In the North Region of Brazil, multiple lesions are frequently caused by *L. guyanensis* and appear to be related to multiple *Lu. umbratilis* bites. LCL may be accompanied by regional lymphadenopathy and nodular lymphangitis, and usually the patients present a positive MST (Mellors *et al.* 1997; Trujillo *et al.* 1999; Guerra *et al.* 2011; Pires *et al.* 2015).

In *L. guyanensis* infection, the involvement of nasal or oral mucosa is more frequent in males than in females. The disease is severe and there is a high incidence of lesion with perforation of the nasal septum.

In the state of Amazonas, 46 cases of ML were reported and 30 cases were associated with *L. braziliensis* and 16 cases with *L. guyanensis* in 16 different municipalities of the state (Guerra *et al.* 2011). Comparing with the frequency of mucosal involvement in patients infected with *L. braziliensis* ML caused by *L. guyanensis* is rare (Naiff, Talhari and Barrett 1988; Santrich *et al.* 1990).

The DCL was reported after *L. guyanensis* infection. This clinical form is characterized by the presence of numerous small and papular lesions at different anatomical sites, appearing simultaneously or secondarily to one or more ulcerated cutaneous lesions (Carvalho *et al.* 1994). A *L. guyanensis* DCL case of a woman, who tested negative for acquired immunodeficiency virus (HIV), and who started the disease with the onset of three ulcerated lesions, was reported in French Guiana. One week after the appearance of the first lesions, there were 425 papulo-nodular lesions in several parts of the body (Couppié *et al.* 2004). Another case of a patient with HIV and *L. guyanensis* coinfection in Ecuador was reported. The patient presented pleomorphic and disseminated ulcers, papules and cutaneous lesions throughout the body. In the fragment biopsies of the lesions numerous amastigotes were observed and the analysis of the *cytochrome b* gene sequence confirmed the presence of *L. guyanensis*. The patient did not respond to the treatment with pentavalent antimonial and amphotericin B, and died 6 months later with bacterial septic shock, demonstrating that immunosuppression is a risk

factor for the development of severe forms of ATL (Calvopina *et al.* 2017). In a second case of DCL also reported in Ecuador, the patient first manifested a papular lesion on the forearm and then other lesions appeared on the right ear and nose. After 2 years, the lesions appeared at different extremities, followed by multiple and confluent lesions in the abdomen, thorax, side of the body and thigh, and the superficial involvement of the mucosa of the nose and upper lip. *Leishmania guyanensis* was identified by a PCR assay in fragment biopsy of the lesions. The patient was an alcoholic and complications due to liver cirrhosis led to his death after DCL healing (Hashiguchi *et al.* 2016).

Clinical findings and diagnostic methods were compared among patients with LCL who were infected with *L. braziliensis* in the state of Bahia with patients infected with *L. guyanensis* in the state of Amazonas, Brazil. The group of patients infected with *L. guyanensis* presented a higher number of lesions, of smaller and localized sizes, mainly above the hip, compared to those infected with *L. braziliensis*. In *L. braziliensis*-infected patients the lesions were mainly localized in the lower limbs. Lymphatic involvement was present in 83.3% of patients infected with *L. braziliensis* and in 61.8% of patients infected with *L. guyanensis*. In addition, the positivity of direct examination and cultures of cutaneous lesions was higher in patients infected with *L. guyanensis* than with *L. braziliensis*. The sensitivity for parasite isolation in *in vitro* cultures of lesion fragments was higher in the group of patients infected with *L. guyanensis* (91.2%) than with *L. braziliensis* (47.0%) (Romero *et al.* 2001). The data suggest that *L. guyanensis* parasites show better adaptation to *in vitro* culture conditions than those of *L. braziliensis*.

The early diagnosis and treatment of LCL are important to avoid the development of the mucosal form of ATL and the complications of this clinical form (Guerra *et al.* 2011). Fast parasitological diagnosis is done by direct examination or culture, using material collected for biopsy or aspirated from the lesions. The microscopic identification of parasites in the smears requires experienced technicians, and there can be a delay for cultures to be positive and contaminated since the ulcerated lesions may suffer bacterial or fungal secondary contamination, making diagnosis difficult (Dorta *et al.* 2012). In the indirect methods, MST has the limitation of only becoming positive 3–6 weeks after the onset of the disease with variable sensitivity in endemic areas. In addition, the standardization of the antigens used in its production is missing. PCR assays, as reported above, and serological tests have been used as diagnostic options (Neves *et al.* 2011; Brasil 2017). In the state of Amazonas, between 2010 and 2011, different diagnostic methods, such as MST, enzyme-linked immunosorbent assay (ELISA), direct examination, culture isolation and identification of *Leishmania* spp. were performed for ATL diagnosis. A total of 38 suspected cases of ATL were diagnosed showing 71% positive by direct examination and 75.6% positive cultures. Fifty-four per cent of the cases were associated with *L. guyanensis*. Seventy-seven per cent of the cases presented positive MST and serology detected IgG and IgM antibodies in 100% of the cases. Together, the results indicated that the diagnosis must be made with the association of different methods (Espir *et al.* 2016).

The treatment of ATL is classically performed with pentavalent antimonial as a first-choice drug, followed by amphotericin B, regardless of the infecting species. There is still need for clinical trials to compare the efficacy of the drugs used in patients infected with different species of *Leishmania* as this may interfere with therapeutic responses. Despite its various side effects, pentavalent antimonials remain in use as the percentage of apparent clinical cure after correct treatment is high. The pentavalent antimonials are considered leishmanicidal since they interfere with the energetic metabolism of the amastigote forms inside macrophages (Azeredo-Coutinho *et al.* 2007).

The amphotericin B deoxycholate is considered the first-choice drug for the treatment of pregnant women and the second choice when it has not obtained a positive response during treatment with pentavalent antimonials or when it is not possible for their use in patients with ATL. However, amphotericin B has several reported side effects and therefore requires careful monitoring of patients (Thakur *et al.* 1999). Amphotericin B in liposomes has shown fewer side effects, can be used in lower doses than amphotericin B, and also show a longer half-life within the macrophages leading to a better action against parasites living in the vacuoles (Berman *et al.* 1998). Nevertheless, the high cost of liposomal formulation makes routine use unfeasible.

The use of pentamidines for the treatment of ATL in Brazil has been suspended at the present time, but they are still first-line drugs in Suriname and French Guiana. Pentamidines have an action on glucose metabolism and the effect appears to be cumulative and dose dependent; thus, diabetes mellitus can manifest itself (Azeredo-Coutinho *et al.* 2007; van der Meide *et al.* 2009). In ATL caused by *L. guyanensis* the response to pentamidine treatment was evaluated by Mans *et al.* (2016). This group demonstrated treatment failure in 22 patients out of a total of 92 with LCL in Suriname. On the other hand, Gadelha *et al.* (2015) evaluated the use of pentamidine in a single dose (7 mg kg^{-1}) in the treatment of LCL caused by *L. guyanensis* in 20 patients from Manaus, Brazil. After 6 months of follow-up, 55% of the patients were considered cured with mild side effects reported.

Neves *et al.* (2011) compared the efficacy and safety of N-methylglucamine antimonyate, amphotericin B deoxycholate and pentamidine isethionate in standard doses, times and routes of administration in patients infected with *L. guyanensis* in Manaus, Brazil. When evaluating 163 patients with LCL, pentamidine and pentavalent antimonial showed similar efficacies, with the advantage that pentamidine was used for a shorter time and had lower side effects. Due to the low number of patients treated with amphotericin B it was not possible to obtain satisfactory data for analysis.

Recently, it was demonstrated that the modulation of *ornithine decarboxylase* (ODC) and *γ-glutamylcysteine synthetase* (GSH1) genes, which encode molecules that are precursors of trypanothione is responsible for a mechanism of peroxide detoxification present in trypanosomatids. The ODC and GSH1 molecules were overexpressed in different *L. guyanensis* strains leading to antimonial resistance. In contrast, when these molecules were inhibited there was an increase in the anti-leishmanicidal effect. The modulation of ODC and GSH1 levels and activity is sufficient to affect *L. guyanensis* susceptibility to antimonial and suggests that a combined therapy with inhibitors of polyamine, glutathione and trypanothione biosynthesis could be a strategy to minimize drug resistance, reduce drug toxicity and increase treatment efficacy (Fonseca, Comini and Resende 2017).

Some mutant strains of *L. guyanensis* that are resistant to pentavalent antimonials had the genome sequenced; different chromosomal alterations were found such as aneuploidy, amplification of intrachromosomal genes and gene exclusion. All mutant strains showed a reduction in antimonial accumulation due to genomic changes at chromosome 31, which contains the gene coding for aquaglycerporine-1 (LgAQP1). This LgAQP1 is responsible for the absorption of antimonial in *Leishmania*. Thus, mutation in LgAQP1 was associated with pentavalent antimonial

resistance (Monte-Neto *et al.* 2015). In addition, the expression of the mRNA of six genes encoding proteins important for antimonial metabolism (AQP1, MRPA, GSH1, GSH2, TRYR and TDR1) was analysed in 25 isolates of *L. guyanensis*. These isolates were obtained from patients who had different outcomes after antimonial treatment. The results demonstrated that GSH1 was overexpressed in isolates from patients who had failed therapy, thus suggesting the involvement of this molecule in antimonial resistance (Torres *et al.* 2010).

In patients with ML caused by *L. guyanensis* it was demonstrated that the treatment needs to be longer than in those patients infected with *L. braziliensis* (Dietze *et al.* 2011). The authors suggested that the low therapeutic efficacy can be explained by the genetic variability among the strains of *L. guyanensis*, resulting in different susceptibilities to the treatment. In contrast to these findings, Arevalo *et al.* (2007) evaluated the influence of the parasite species on the outcome of therapy with pentavalent antimonials in patients with LCL in Peru. The authors showed that there is an association between the infecting species and the outcome of the treatment. The percentage of treatment failure after 12 months of follow-up of patients infected with *L. braziliensis* was much higher (30.4%) when compared to patients infected with *L. guyanensis* (8.3%).

Miltefosine is a salt, hexadecylphosphocholine, which has been synthesized and investigated as an anti-neoplastic agent due to its cytostatic allyl phospholipid activity (Croft and Coombs 2003). However, it was discarded as an anti-carcinogenic agent due to side effects and low efficacy. Miltefosine was registered in India in March 2002 for oral treatment of visceral leishmaniasis; and in 2005–06 it was registered for treatment of ATL in Colombia, Guatemala, Honduras and Ecuador (Soto and Berman 2006; Sundar *et al.* 2006; Soto *et al.* 2007). Efficacy and clinical safety of miltefosine in the treatment of LCL caused by *L. guyanensis* was evaluated in patients in Manaus, Brazil. It was demonstrated that miltefosine was more effective (71.4% of cases with clinical cure) than the pentavalent antimonial (53.6% of the cases) to obtain the healing of the LCL lesions (Dietze *et al.* 2011).

INFECTION WITH *Leishmania guyanensis*: IMMUNOLOGICAL ASPECTS IN HUMANS AND MICE

Human infection with *Leishmania* spp. begins after the bite of naturally infected sandfly species. In this parasitic entrance microenvironment, blood components, such as complement system proteins probably cause the destruction of some parasites. After activation, complement proteins bind to the parasite membrane leading to the formation of the molecular attack complex that leads to killing of the parasite (Sacks and Perkins 1984). However, some parasites survive the lytic action of the complement and use complement components to establish the infection. Metacyclic promastigotes carry lipophosphoglycans (LPG) on their surface, which hinders the penetration of the C5b-C9 lytic complex in the plasma membrane. Alterations in LPG content take place during metacyclogenesis to increase the resistance of metacyclic forms to complement (Puentes *et al.* 1988). The biochemical analysis of LPG expressed in parasites of the subgenus *Viannia* showed the presence of mannose and galactose similar to *Leishmania donovani* LPG (Muskus *et al.* 1997). Our group has evaluated the metacyclogenesis of clinical isolates of *Leishmania* of the subgenus *Viannia*, demonstrating alterations in *Bauhinia purpurea* lectin binding capacity and increased resistance to the complement system in metacyclic forms (da Silva *et al.* 2015). Parasites evade lysis mediated by the complement and are phagocytosed by phagocytic mononuclear cells to establish the infection.

For the phagocytosis of *Leishmania* spp. promastigote forms, in addition to receptors for complement proteins (CR1, CR3) the macrophage uses the mannose receptor (MR) and fibronectin receptor (FnR). On the other hand, for the phagocytosis of amastigote forms, the receptor for the Fc portion of immunoglobulin G (FcγR) is also used (Ueno and Wilson 2012).

In addition to the interaction of parasites with phagocytosis receptors, toll-like receptors (TLRs) expressed on monocytes/macrophages are also activated by parasite molecules and are responsible for connecting the innate immunity with the adaptive immune response. Murine models have allowed the study of the role of TLRs in *Leishmania* infection. *Leishmania guyanensis* infection in mice is discussed below.

Genetic variations in TLR signaling pathways may be associated with susceptibility or resistance to disease (Schröder and Schumann 2005). It has been demonstrated that genetic polymorphisms in the Toll-interacting protein (TOLLIP), which regulates TLR signaling pathways in humans, may down-regulate the production of proinflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor in patients with tuberculosis (Shah *et al.* 2012). In ATL patients, a study showed that two polymorphisms in the *TOLLIP* gene, the rs5743899 associated with increased IL-6, decreased IL-10 and TOLLIP mRNA expression, and the rs3750920 responsible for high TOLLIP mRNA expression, but no correlation with the levels of either IL-6 or IL-10 are associated with the development of LCL caused by *L. guyanensis* in the state of Amazonas, Brazil. These results favor the assumption that TLRs are important innate receptors in ATL responses (de Araujo *et al.* 2015).

The development of an acquired cellular immune response with the participation of T helper lymphocytes type 1 (Th1) and the production of reactive oxygen and nitrogen species is necessary for controlling parasites and for the healing of ATL lesions (Ribeiro-de-Jesus *et al.* 1998; Trinchieri 2007). Espir *et al.* (2014) evaluated the production of cytokines in patients with LCL infected with different *Leishmania* spp. They observed high levels of IL-2 and interferon gamma (IFNγ) in the group of patients infected with *L. guyanensis*. However, it has been demonstrated that lesions caused by *L. guyanensis* can also present high amounts of Th2 cytokines, especially IL-13. This cytokine can inhibit the expression of the IL-12 receptor in T cells leading to a deficient Th1 immune response (Bourreau *et al.* 2001b). A predominantly Th2 profile has been found in patients infected with *L. guyanensis* in an endemic area to the north of the Amazon River. In these patients, reduced or non-detectable antigen recognition was associated with high levels of IL-10 and IL-5 as well as with low antibody titers (Matta *et al.* 2009). Infection with *L. guyanensis* affects the expansion of antigen specific-T lymphocyte clones, causing low lymphocyte proliferation and decreased IFNγ production. The induction of high levels of Th2-type cytokines alters Th1-type responses, allowing the parasite replication. The low levels of cellular and humoral immune responses during infection caused by *L. guyanensis* can explain the high tissue parasitic load and the recurrence of the disease when compared to the infection caused by *L. braziliensis* (Matta *et al.* 2009). The presence of Th17 lymphocytes in LCL and ML lesions was also reported and it is associated with the presence of neutrophils and tissue destruction. There were higher numbers of Th17 lymphocytes in lesions caused by *L. guyanensis* than in those caused by *Leishmania amazonensis* or *Leishmania naiffi* (Bacellar *et al.* 2009; Espir *et al.* 2014). Then, together the data

suggest a Th1/Th2/Th17 mixed response profile during *L. guyanensis* infection.

Considering the regulation of immune responses during *L. guyanensis* infection, expression of suppressor cytokines has been described, especially in patients with treatment failure. High expression of IL-10 in LCL lesions caused by *L. guyanensis* was associated with failure to pentamidine treatment (Bourreau *et al.* 2001a). In addition, *L. guyanensis*-infected untreated LCL patients or patients presenting treatment failure showed unresponsiveness of specific T cells to IL-12. These results suggested that the lack of IL-12 response contributes to the persistence of parasites (Bourreau *et al.* 2001c). In patients not responding to pentamidine treatment, the presence of natural regulatory T cells has been demonstrated by Bourreau *et al.* (2009). The association between treatment failure and presence of regulatory T cells was dependent on the duration of lesions, and the suppressive activity of regulatory cells was independent of IL-10. The regulatory T cells accumulate in the lesions during the early phase of LCL (<1 month). These cells in acute or chronic LCL present suppressive activity inhibiting IFNγ production by $CD4^+$ T cells and express 2,3-indoleamine dioxygenase (IDO). Suppressive activity of intralesional regulatory T cells can be mediated by IDO and IL-10 mainly during chronic LCL (Bourreau *et al.* 2009).

The IL-10 cytokine has a regulatory role during immune responses involving inhibiting inflammatory mediators and the activation of monocytes/macrophages needed for controlling immunopathology in LCL. In addition, IL-10 production by regulatory T cells is associated with parasite persistence and reactivation of the disease (Mendez *et al.* 2004). Kariminia *et al.* (2005) demonstrated that mononuclear cells from healthy individuals infected with *L. guyanensis in vitro* produce the transforming growth factor β (TGFβ), a cytokine that promotes the escape of parasites from protective mechanisms during human and murine infection by inhibiting macrophages. These TGFβ-producing cells were phenotypically and functionally identified as $CD4^+CD25^+$ regulatory T cells with suppressive activity *in vitro*. When stimulated with *L. guyanensis* they produced TGFβ but not IL-10. On the other side, after stimulation with anti-CD3 antibodies they produced TGFβ and IL-10. These results suggested that *L. guyanensis* can activate a subpopulation of regulatory T cells to favor the establishment of the infection. In addition to these regulatory cells, it has been demonstrated that *L. guyanensis* antigens can induce IL-10 in $CD8^+$ memory T cells of LCL patients (Bourreau *et al.* 2007). It was observed that patients infected with *L. guyanensis* presented higher IL-10 production in comparison with patients infected with *L. amazonensis* (Espir *et al.* 2014). Thus, together these regulatory cells can facilitate the establishment and perpetuation of the infection.

Concerning humoral immune responses, patients diagnosed with LCL caused by *L. guyanensis* produced lower levels of antibodies in comparison with those infected with *L. braziliensis*. In addition, in *L. guyanensis*-infected patients the reduction of antibody titers occurs at the end of the therapy regardless of cure (Romero *et al.* 2005). These data were corroborated with those of Matta *et al.* (2009) who also detected low antibody titers in patients infected with *L. guyanensis*.

Leishmania guyanensis (MHOM/BR/75/M4147 strain) causes a small lesion in the footpad of C57BL/6 mice that is completed cured a few weeks after infection The profile of lesion development was similar in C57BL/6 and BALB/c mice: both strains were resistant to infection (DaMata *et al.* 2015). We observed a similar profile of lesion development in C57BL/6 mouse infected with a clinical isolate of *L. guyanensis* (MHOM/BR/2006/PLR6). In this mouse strain, *L. guyanensis* was better controlled than *L. braziliensis* infection (Pires *et al.* 2015). In contrast, susceptibility to infection was observed in C57BL/6 IFNγ knockout mice, which after infection with *L. guyanensis* developed lesions rapidly and presented metastatic lesions to the non-infected contralateral paw. In these mice, lesions were persistent and progressive demonstrating that *L. guyanensis* is susceptible to leishmanicidal mechanisms induced by IFNγ (Pires *et al.* 2015). The development of metastatic lesions has been previously observed in hamsters during *L. guyanensis* infection (Martinez *et al.* 1991). Similar to the results of DaMata *et al.* (2015), BALB/c mice were more susceptible to *L. guyanensis* than C57BL/6 mice after infection with the PLR6 *L. guyanensis* strain (Pires *et al.* 2015).

In vitro, peritoneal macrophages from BALB/c mice infected with *L. guyanensis* promastigotes present microbicidal mechanisms that are effective in eliminating the amastigotes. The promastigotes of *L. guyanensis* induced a stronger production of reactive oxygen species, mainly hydrogen peroxide, when compared to *L. amazonensis*. These results demonstrated that products generated by the NADPH oxidase pathway are responsible for eliminating parasites. *Leishmania guyanensis* amastigote forms were also susceptible to the toxic effects of reactive oxygen species, which induced apoptosis by DNA fragmentation of the parasite (Sousa-Franco *et al.* 2006). On the other hand, *L. guyanensis* is able to cause loss of murine macrophage membrane integrity leading to a massive macrophage death. This can favor dissemination of the parasite to other macrophages during infection (DaMata *et al.* 2015).

Parasite and host factors are responsible for the immunopathogenesis of ATL. It has been shown that *Leishmania* of *Viannia* subgenus can be infected by virus, which may be responsible for alterations of the immune responses and, consequently, the outcome of the *L. guyanensis* infection. This aspect will be addressed in the final part of this review.

Some studies have indicated that besides the parasite genetic characteristics, the vector saliva components, and host immune status, the presence of *Leishmania* virus (LRV) may predispose to the development of severe tegumentary leishmaniasis (Ives *et al.* 2011; Hartley *et al.* 2012). Virus-like particles were described in 1974 in a *Leishmania hertigi* isolate (Molyneux 1974), and at the end of the 1980s the first molecular descriptions of an RNA virus in *L. guyanensis* were published (Tarr *et al.* 1988; Widmer *et al.* 1989). LRVs are classified into the Totiviridae family, which comprise non-enveloped viruses composed of icosahedral particles. The viruses in this family are composed of 40 nm particles with a non-segmented double-stranded RNA (dsRNA) of 4–8 kb size, encoding a viral capsid protein and an RNA-dependent RNA polymerase (RDRP) that is essential for replication of the dsRNA virus and is fused to the viral capsid protein. The LRV1 (with 14 subtypes) is common in South American parasites whereas LRV2 is present in parasites of the Old World. The LRV was detected in *L. braziliensis* (LRV1), *L. guyanensis* (LRV1), *Leishmania major* (LRV2) and *Leishmania aethiopica* (LRV2) isolates (Tarr *et al.* 1988; Stuart *et al.* 1992; Scheffter *et al.* 1995; Zangger *et al.* 2014).

The role of LRV in parasite virulence and gene expression has not been studied in detail and there is no information about why some *Leishmania* strains are capable of maintaining LRV1 infection and others are not. In addition, it is not known how the virus is transmitted in the *L.* (*Viannia*) subgenus (Hartley *et al.* 2012). The prevalence of the virus and its clinical significance are still controversial. In French Guiana, Ginouvès *et al.* (2016) demonstrated the presence of LRV1 in 74% of the *L.* (*Viannia*) subgenus clinical isolates; 55% were *L. braziliensis* and 80% *L. guyanensis* isolates. According to geographical distribution the virus was mainly present in isolates from more internal areas of the

country. Searching for the virus in lesions of ATL patients the prevalence of LRV1 has ranged from 4.2% to 70.3% of *L.* (*Viannia*)-infected patients with higher prevalence in patients with ML (Salinas *et al.* 1996; Saiz *et al.* 1998; Ogg *et al.* 2003; Pereira *et al.* 2013; Cantanhêde *et al.* 2015; Ito *et al.* 2015; Bourreau *et al.* 2016). In one of these studies, it was not possible to detect LRV1 in 40 patients from the Southeast Region of Brazil (Pereira *et al.* 2013). Thus, the prevalence of LRV1 seems to be associated with geographical distribution and clinical form of ATL.

In patients from the northern region of Brazil presenting ML, Ito *et al.* (2015) detected LRV1 in 70.3% (26/37) of the cases; most of them were caused by *L. braziliensis* (23), followed by *L. guyanensis* (2) and one by a mixed infection with both species. The predominant species in cases of ML was *L. braziliensis*, accounting for 81% of the cases, whereas 13.5% of the cases were associated with *L. guyanensis*. At the same time, Cantanhêde *et al.* (2015) evaluated lesions of 147 ATL patients (109 LCL; 38 ML) from the Amazonian region of Brazil. They detected LRV1 in 36.7% of the LCL cases and in 71.1% of the ML cases. The virus was detected in *L. braziliensis*, *L. guyanensis*, *L. amazonensis* and *Leishmania lainsoni*. These results favored the hypothesis that the presence of LRV1 can increase the risk of developing mucosal lesions.

The concept of infectious metastasis caused by *Leishmania* has been used to describe the dissemination of the parasites from cutaneous lesions to other sites. In line with this, ML is a metastatic infectious disease (Martínez *et al.* 2000; Hartley *et al.* 2012). Although the studies above revealed a high frequency of virus detection in mucosal lesions caused by *L. braziliensis* or *L. guyanensis*, one-third of patients who had ML did not present the virus. These studies suggest that factors other than virus presence are responsible for metastatic lesions in ML. Host factors such as immunocompetence, malnutrition and coinfections, or parasite factors such as genetic polymorphisms or the presence of LRV1, besides environmental factors are potential risk factors for metastasis of *Leishmania* spp. (Martínez *et al.* 2000; Smith, Peacock and Cruz 2007; Ives *et al.* 2011; Gomes *et al.* 2014; Hartley *et al.* 2014). Metastatic clones of *L. guyanensis* isolated from ML patients are more resistant to oxidative stress than non-metastatic ones, suggesting this mechanism as a factor for persistence and dissemination of the parasites (Acestor *et al.* 2006; Walker *et al.* 2006).

In murine models, it has been observed that metastasizing clones of *L. guyanensis* present a high LRV1 burden and its viral dsRNA is recognized by TLR3 to induce pro-inflammatory cytokine and chemokine production. This increased inflammatory process contributes to exacerbate the disease. In fact, murine macrophages treated with LRV1 dsRNA *in vitro* showed a similar response of macrophages infected with metastatic LRV1-infected parasites, which was dependent on TLR3. These macrophages showed an increased expression of chemokines (CXCL10, CCL5) and cytokines such as tumor necrosis factor α (TNFα) and IL-6, thus demonstrating that LRV1 is able to induce amplification of the inflammatory response to *Leishmania* antigens (Ives *et al.* 2011).

The recognition of LRV1 occurs during the first hours after the infection with LRV1-infected *L. guyanensis*. Then, it is expected that after the death of some parasites viral dsRNA is released and binds to TLR3 leading to the production of type I IFN, which triggers an inflammatory cascade that leads to worsening disease (Ives *et al.* 2011). In fact, the role of type I IFN in the exacerbation of *L. guyanensis* infection has been confirmed by Rossi *et al.* (2017). LRV1 infecting *L. guyanensis* or coinfection with non-infected *L. guyanensis* and other external type I IFN-inducing virus increased parasite dissemination in C57BL/6 mice. Mechanistically, these results can be explained by a down-regulation of IFNγ receptor expression caused by type I IFN reducing macrophage activation and increasing the persistence of parasites (Rossi *et al.* 2017). That LRV1-containing *L. guyanensis* affects the innate immune response to survive and persist in macrophages has been demonstrated by Eren *et al.* (2016). LRV1-infected *L. guyanensis* up-regulated the expression of microRNA-155 in a TLR3-dependent manner. This signaling pathway led to Akt (Protein kinase B) activation, which promoted macrophage survival, persistence of the parasites and disease severity.

Ives *et al.* (2014) demonstrated the role of TLR9 and MyD88-dependent signaling pathways in the development of resistance to *L. guyanensis* infection. Independent of viral burden, the resistance to *L. guyanensis* infection was shown to be dependent on TLR9 and MyD88 activation. This activation pathway leads to a protective Th1 immune response. In the absence of these molecules, *L. guyanensis* induced a Th2 immune response that accounted for an increased susceptibility (Ives *et al.* 2014).

Yet, in the murine model LRV1-infected *L. guyanensis* induced a Th17 immune response, which contributed to parasite virulence and dissemination in IFNγ-deficient mice. In accordance, in *L. guyanensis*-infected patients (with cutaneous leishmaniasis) there is an association between the presence of LRV1, the levels of IL-17A, and disease chronicity. In patient carriers of LRV1-infected *L. guyanensis* there is a high ratio of IL-17A/IFNγ in the lesions. In addition, blood cultures from these patients produced higher IL-17A levels after stimulation with live parasites than those infected with LRV1-free *L. guyanensis* (Hartley *et al.* 2016). These results suggest that besides the risk of developing ML being increased by LRV1, the presence of the virus in the parasites can lead to other complications in cutaneous leishmaniasis. Recently, Bourreau *et al.* (2016) investigated the treatment efficacy of patients with LCL caused by *L. guyanensis* infected or non-infected with LRV1. The presence of LRV1 was associated with persistent infection or relapses as well as a significant increase in inflammatory molecules in the lesions. Thus, the presence of the virus in the parasites can be predictive of therapeutic failure or relapses in patients infected with *L. guyanensis*. These observations raise the need to think about new therapeutic strategies for *L. guyanensis* infection.

Based on the studies above, various methods of direct detection of LRV1 in lesions, including immunofluorescence, immunoenzymatic assay (ELISA) and dot blot have been proposed and may be useful tools in driving the treatment of *Leishmania* infections (Zangger *et al.* 2013). Alternatively, it has been proposed to eliminate LRV1 from parasites thereby reducing the risks associated with LRV1 during *Leishmania* infection. In this sense, 2′-C-methyladenosine analogs and RNA interference (RNAi) have been described to be efficient in eliminating the *Leishmania* virus and thus reducing the immunopathology associated with infections caused by both *L. guyanensis* and *L. braziliensis* (Brettmann *et al.* 2016; Kuhlmann *et al.* 2017). In addition, a vaccine targeted to prevent viral-induced complications during *L. guyanensis* infection has been tested. The vaccine comprises LRV1 capsid polypeptide and CpG ODN-1826, an adjuvant to Th1 polarization. The immunization conferred protection of C57BL/6 mice against deleterious effects caused by LRV1 within *L. guyanensis*, decreasing lesion size and parasite burden. This protection was mediated by IFNγ produced by a strong Th1 immune response against LRV1 capsid (Castiglioni *et al.* 2017). This could be a strategy to avoid LRV1 complications during ATL.

Figure 1 summarizes the aspects of immune responses to *L. guyanensis*, including the consequences of LRV1 parasite infection.

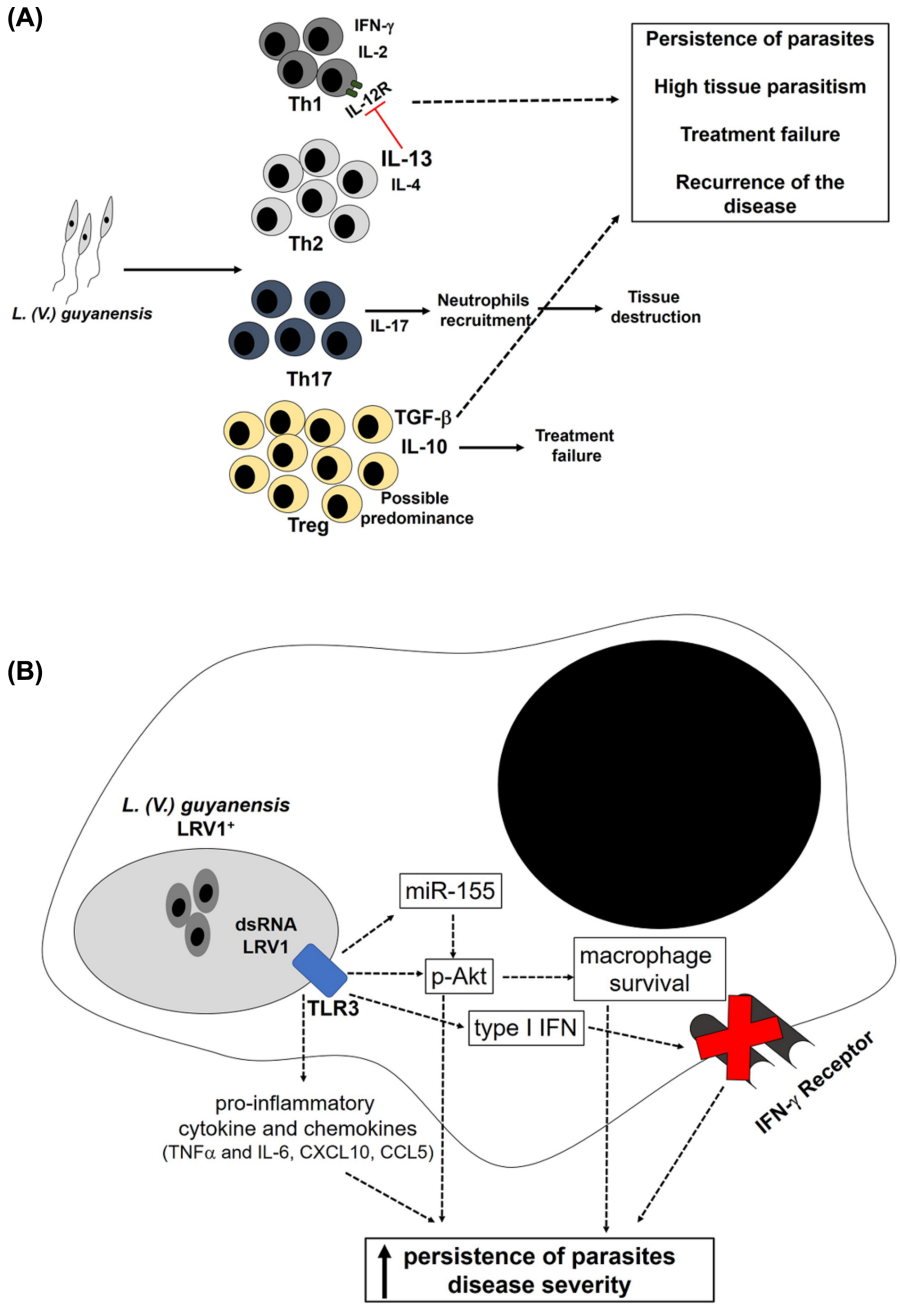

Figure 1. (**A**) General aspects of the human immune response against *Leishmania guyanensis*. *L. guyanensis* infection induces a mixed Th1/Th2/Th17 immune response. The high production of IL-13 by Th2 cells reduces the expression IL-12 receptor in Th1 cells, disfavoring the Th1 protective immune response. Th17-produced IL-17 induces neutrophil recruitment and tissue destruction. Regulatory T (Treg) cells appear to predominate in the active phase of the disease. TGF-beta is essential for the establishment of infection and, together with IL-10, for the persistence of the parasite, leading to therapeutic failures and recurrence of the disease. (**B**) Consequences of Leishmania guyanensis infection by dsRNA virus (LRV1). After the parasite death, dsRNA from LRV1 can bind to TLR3 and together parasite molecules promote the production of proinflammatory molecules. The stimulation of TLR3 results in type I IFN production, which triggers an inflammatory cascade that leads to worsening disease. Besides, type I interferon downregulates IFNgamma receptor expression reducing macrophage activation and increasing the persistence of parasites. Indeed, TLR3 activation by LRV1 induces *L. guyanensis* persistence inside macrophages via Akt signaling.

CONCLUSIONS

In conclusion, the present review summarizes the knowledge about *L. guyanensis*, one of the most prevalent species in Latin America. ATL is a serious public health problem and the absence of efficient prophylactic and therapeutic measures justifies studies on different *Leishmania* species; differences among *Leishmania* spp. can lead to different clinical manifestations, therapeutic responses and complications. In this way, *L. guyanensis* shows unique characteristics that need to be investigated in more detail to understand the mechanisms that govern immune responses and control inflammatory reactions against this parasite. The presence of LRV1 in *L. guyanensis* isolates has revealed that virus infection can exacerbate the ATL caused by this species. Control of this process is essential to obtain better therapeutic success than we have at present.

FUNDING

The authors thank CNPq for financial support, grant no. 465771/2014-9 – INCT – National Institute of Science and Technology of the strategies in host–pathogen interaction, Brazil. FRD is research fellow of CNPq; AFB is a PhD fellow of CAPES; RSG is a post-doctoral fellow of CNPq.

Conflict of interest. None declared.

REFERENCES

Acestor N, Masina S, Ives A *et al.* Resistance to oxidative stress is associated with metastasis in mucocutaneous leishmaniasis. *J Infect Dis* 2006;**194**:1160–7.

Arevalo J, Ramirez L, Adaui V *et al.* Influence of *Leishmania (Viannia)* species on the response to antimonial treatment in patients with American tegumentary leishmaniasis. *J Infect Dis* 2007;**195**:1846–51.

Ashford RW. The leishmaniases as emerging and reemerging zoonoses. *Int J Parasitol* 2000;**30**:1269–81.

Azeredo-Coutinho RBG, Mendonça SCF, Callahan H *et al.* Sensitivity of *Leishmania braziliensis* promastigotes to meglumine antimoniate (Glucantime) is higher than that of other *Leishmania* species and correlates with response to therapy in American tegumentary leishmaniasis. *J Parasitol* 2007;**93**:688–93.

Bacellar O, Faria D, Nascimento M *et al.* Interleukin 17 production among patients with American cutaneous leishmaniasis. *J Infect Dis* 2009;**200**:75–8.

Barbosa W, Moreira MC, Souza M *et al.* Note on the classification of the *Leishmania sp.* responsible for cutaneous leishmaniasis in the East Central Region of Brazil. *Ann Trop Med Parasitol* 1976;**70**:389–99.

Berman JD, Badaro R, Thakur CP *et al.* Efficacy and safety of liposomal amphotericin B (AmBisome) for visceral leishmaniasis in endemic developing countries. *Bull World Health Organ* 1998;**76**:25–32.

Bourreau E, Ginouves M, Prévot G *et al.* Presence of *Leishmania* RNA virus 1 in *Leishmania guyanensis* increases the risk of first-line treatment failure and symptomatic relapse. *J Infect Dis* 2016;**213**:105–11.

Bourreau E, Prévot G, Gardon J *et al.* High intralesional interleukin-10 messenger RNA expression in localized cutaneous leishmaniasis is associated with unresponsiveness to treatment. *J Infect Dis* 2001;**184**:1628–30.

Bourreau E, Prévot G, Pradinaud R *et al.* Interleukin (IL)-13 is the predominant Th2 cytokine in localized cutaneous leishmaniasis lesions and renders specific $CD4^+$ T cells unresponsive to IL-12. *J Infect Dis* 2001;**183**:953–9.

Bourreau E, Prévot G, Pradinaud R *et al.* Unresponsiveness of specific T cells to IL-12 is associated with active cutaneous leishmaniasis owing to *Leishmania guyanensis*. *Scand J Immunol* 2001;**54**:335–9.

Bourreau E, Ronet C, Couppié P *et al.* IL-10 producing $CD8^+$ T cells in human infection with *Leishmania guyanensis*. *Microbes Infect* 2007;**9**:1034–41.

Bourreau E, Ronet C, Darcissac E *et al.* Intralesional regulatory T-cell suppressive function during human acute and chronic cutaneous leishmaniasis due to *Leishmania guyanensis*. *Infect Immun* 2009;**77**:1465–74.

Brasil, Brasil. Ministério da Saúde Manual de vigilância da leishmaniose tegumentar. 2017, http://bvsms.saude.gov.br/bvs/publicacoes/manual_vigilancia_leishmaniose_tegumentar.pdf.

Brettmann EA, Shaik JS, Zangger H *et al.* Tilting the balance between RNA interference and replication eradicates *Leishmania* RNA virus 1 and mitigates the inflammatory response. *Proc Natl Acad Sci U S A* 2016;**113**:11998–2005.

Calvopina M, Aguirre C, Cevallos W *et al.* Coinfection of *Leishmania guyanensis* and human immunodeficiency virus—Acquired immune deficiency syndrome: report of a case of disseminated cutaneous leishmaniasis in Ecuador. *Am J Trop Med Hyg* 2017;**96**:16–0431.

Cantanhêde LM, da Silva Júnior CF, Ito MM *et al.* Further evidence of an association between the presence of *Leishmania RNA virus 1* and the mucosal manifestations in tegumentary leishmaniasis patients. *PLoS Negl Trop Dis* 2015;**9**:e0004079.

Carvalho EM, Barral A, Costa JM *et al.* Clinical and immunopathological aspects of disseminated cutaneous leishmaniasis. *Acta Trop* 1994;**56**:315–25.

Castiglioni P, Hartley M-A, Rossi M *et al.* Exacerbated leishmaniasis caused by a viral endosymbiont can be prevented by immunization with its viral capsid. *PLoS Negl Trop Dis* 2017;**11**:e0005240.

Couppié P, Clyti E, Sainte-Marie D *et al.* Disseminated cutaneous leishmaniasis due to *Leishmania guyanensis*: case of a patient with 425 lesions. *Am J Trop Med Hyg* 2004;**71**:558–60.

Croft SL, Coombs GH. Leishmaniasis—current chemotherapy and recent advances in the search for novel drugs. *Trends Parasitol* 2003;**19**:502–8.

DaMata JP, Mendes BP, Maciel-Lima K *et al.* Distinct macrophage fates after *in vitro* infection with different species of *Leishmania*: induction of apoptosis by *Leishmania (Leishmania) amazonensis*, but Not by *Leishmania (Viannia) guyanensis*. *PLoS One* 2015;**10**:e0141196.

da Silva IA, Morato CI, Quixabeira VBL *et al.* *In Vitro* metacyclogenesis of *Leishmania (Viannia) braziliensis* and *Leishmania (Leishmania) amazonensis* clinical field isolates, as evaluated by morphology, complement resistance, and infectivity to human macrophages. *BioMed Res Int* 2015;**2015**:1–15.

de Araujo FJ, da Silva LDO, Mesquita TG *et al.* Polymorphisms in the *TOLLIP* gene influence susceptibility to cutaneous leishmaniasis caused by *Leishmania guyanensis* in the Amazonas State of Brazil. *PLoS Negl Trop Dis* 2015;**9**:e0003875.

de Benicio EA, Gadelha EPN, Talhari A *et al.* Combining diagnostic procedures for the management of leishmaniasis in areas with high prevalence of *Leishmania guyanensis*. *An Bras Dermatol* 2011;**86**:1141–4.

de Souza Freitas MT, Ríos-Velasquez CM, Costa CRL *et al.* Phenotypic and genotypic variations among three allopatric populations of *Lutzomyia umbratilis*, main vector of *Leishmania guyanensis*. *Parasit Vectors* 2015;**8**:448.

Dietze R, Talhari S, Chrusciak Talhari C *et al.* Randomized controlled clinical trial to access efficacy and safety of miltefosine in the treatment of cutaneous leishmaniasis caused by *Leishmania (Viannia) guyanensis* in Manaus, Brazil. *Am J Trop Med Hyg* 2011;**84**:255–60.

Dorta ML, Oliveira MAP, Fleuri AKA *et al.* Improvements in obtaining New World *Leishmania* sp from mucosal lesions: Notes on isolating and stocking parasites. *Exp Parasitol* 2012;**132**:300–3.

Eren RO, Reverte M, Rossi M *et al.* Mammalian innate immune response to a *Leishmania*-resident RNA virus increases macrophage survival to promote parasite persistence. *Cell Host Microbe* 2016;**20**:318–28.

Espir TT, de Figueira LP, Naiff MF *et al.* The role of inflammatory, anti-inflammatory, and regulatory cytokines in patients infected with cutaneous leishmaniasis in Amazonas State, Brazil. *J Immunol Res* 2014;**2014**:1–10.

Espir TT, Guerreiro TS, Naiff MF *et al.* Evaluation of different diagnostic methods of American Cutaneous Leishmaniasis in the Brazilian Amazon. *Exp Parasitol* 2016;**167**:1–6.

Fonseca MS, Comini MA, Resende BV. Ornithine decarboxylase or gamma-glutamylcysteine synthetase overexpression protects *Leishmania (Vianna) guyanensis* against antimony. *Exp Parasitol* 2017;**175**:36–43.

Gadelha EPN, Talhari S, de Oliveira Guerra JA *et al.* Efficacy and safety of a single dose pentamidine (7mg/kg) for patients with cutaneous leishmaniasis caused by *L. guyanensis*: a pilot study. *An Bras Dermatol* 2015;**90**:807–13.

Ginouvès M, Simon S, Bourreau E *et al.* Prevalence and Distribution of Leishmania RNA Virus 1 in Leishmania Parasites from French Guiana. *Am J Trop Med Hyg* 2016;**94**:102–6.

Gomes CM, Ávila LR, Pinto SA *et al.* *Leishmania braziliensis* amastigotes stimulate production of IL-1β, IL-6, IL-10 and TGF-β by peripheral blood mononuclear cells from nonendemic area healthy residents. *Parasite Immunol* 2014;**36**:225–31.

Gontijo B, de Carvalho M. Leishmaniose tegumentar americana. *Rev Soc Bras Med Trop* 2003;**36**:71–80.

Goto H, Lauletta Lindoso JA. Cutaneous and mucocutaneous leishmaniasis. *Infect Dis Clin North Am* 2012;**26**:293–307.

Grimaldi G, McMahan-Pratt D. Leishmaniasis and its etiologic agents in the New World: an overview. *Prog Clin Parasitol* 1991;**2**:73–118.

Guerra JA, Prestes SR, Silveira H *et al.* Mucosal leishmaniasis caused by *Leishmania (Viannia) braziliensis* and *Leishmania (Viannia) guyanensis* in the Brazilian Amazon. *PLoS Negl Trop Dis* 2011;**5**:e980.

Hartley M-A, Bourreau E, Rossi M *et al.* *Leishmaniavirus*-dependent metastatic leishmaniasis is prevented by blocking IL-17A. *PLoS Pathog* 2016;**12**:e1005852.

Hartley M-A, Drexler S, Ronet C *et al.* The immunological, environmental, and phylogenetic perpetrators of metastatic leishmaniasis. *Trends Parasitol* 2014;**30**:412–22.

Hartley M-A, Ronet C, Zangger H *et al.* *Leishmania* RNA virus: when the host pays the toll. *Front Cell Infect Microbiol* 2012;**2**:99.

Hashiguchi Y, Gomez EL, Kato H *et al.* Diffuse and disseminated cutaneous leishmaniasis: clinical cases experienced in Ecuador and a brief review. *Trop Med Health* 2016;**44**:2.

Ito MM, Catanhêde LM, Katsuragawa TH *et al.* Correlation between presence of Leishmania RNA virus 1 and clinical characteristics of nasal mucosal leishmaniosis. *Braz J Otorhinolaryngol* 2015;**81**:533–40.

Ives A, Masina S, Castiglioni P *et al.* MyD88 and TLR9 dependent immune responses mediate resistance to *Leishmania guyanensis* infections, irrespective of *Leishmania* RNA virus burden. *PLoS One* 2014;**9**:e96766.

Ives A, Ronet C, Prevel F *et al.* *Leishmania* RNA virus controls the severity of mucocutaneous leishmaniasis. *Science* 2011;**331**:775–8.

Kariminia A, Bourreau E, Pascalis H *et al.* Transforming growth factor β1 production by CD4$^+$ CD25$^+$ regulatory T cells in peripheral blood mononuclear cells from healthy subjects stimulated with *Leishmania guyanensis*. *Infect Immun* 2005;**73**:5908–14.

Killick-Kendrick R. Phlebotomine vectors of the leishmaniases: a review. *Med Vet Entomol* 1990;**4**:1–24.

Kuhlmann FM, Robinson JI, Bluemling GR *et al.* Antiviral screening identifies adenosine analogs targeting the endogenous dsRNA *Leishmania* RNA virus 1 (LRV1) pathogenicity factor. *Proc Natl Acad Sci U S A* 2017;**114**:E811–9.

Lainson R, Shaw JJ, Ready PD *et al.* Leishmaniasis in Brazil: XVI. Isolation and identification of, *Leishmania* species from sandflies, wild mammals and man in north Pará State, with particular reference to *L. braziliensis guyanensis* causative agent of "pian-bois". *Trans R Soc Trop Med Hyg* 1981;**75**:530–6.

Mans DRA, Kent AD, Hu R V *et al.* Monitoring the response of patients with cutaneous leishmaniasis to treatment with pentamidine isethionate by quantitative real-time PCR, and identification of *Leishmania* parasites not responding to therapy. *Clin Exp Dermatol* 2016;**41**:610–5.

Martinez JE, Travi BL, Valencia AZ *et al.* Metastatic capability of *Leishmania (Viannia) panamensis* and *Leishmania (Viannia) guyanensis* in golden hamsters. *J Parasitol* 1991;**77**:762–8.

Martínez JE, Valderrama L, Gama V *et al.* Clonal diversity in the expression and stability of the metastatic capability of *Leishmania guyanensis* in the golden hamster. *J Parasitol* 2000;**86**:792.

Matta NE, Nogueira RS, Franco AMR *et al.* *Leishmania (Viannia) guyanensis* induces low immunologic responsiveness in leishmaniasis patients from an endemic area of the Brazilian Amazon Highland. *Am J Trop Med Hyg* 2009;**80**:339–44.

Mellors JW, Muñoz A, Giorgi JV *et al.* Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection. *Ann Intern Med* 1997;**126**:946–54.

Mendez S, Reckling SK, Piccirillo CA *et al.* Role for CD4$^+$ CD25$^+$ regulatory T cells in reactivation of persistent leishmaniasis and control of concomitant immunity. *J Exp Med* 2004;**200**:201–10.

Molyneux DH. Virus-like particles in *Leishmania* parasites. *Nature* 1974;**249**:588–9.

Montalvo Alvarez AM, Nodarse JF, Goodridge IM *et al.* Differentiation of *Leishmania (Viannia) panamensis* and *Leishmania (V.) guyanensis* using *Bcc*I for *hsp70* PCR-RFLP. *Trans R Soc Trop Med Hyg* 2010;**104**:364–7.

Monte-Neto R, Laffitte M-CN, Leprohon P *et al.* Intrachromosomal amplification, locus deletion and point mutation in the aquaglyceroporin AQP1 gene in antimony resistant *Leishmania (Viannia) guyanensis*. *PLoS Negl Trop Dis* 2015;**9**:e0003476.

Muskus C, Segura I, Oddone R *et al.* Carbohydrate and LPG expression in *Leishmania Viannia* subgenus. *J Parasitol* 1997;**83**:671–8.

Naiff RD, Talhari S, Barrett TV. Isolation of *Leishmania guyanensis* from lesions of the nasal mucosa. *Mem Inst Oswaldo Cruz* 1988;**83**:529–30.

Neves LO, Talhari AC, Gadelha EPN *et al.* Estudo clínico randomizado comparando antimoniato de meglumina, pentamidina e anfotericina B para o tratamento da leishmaniose cutânea ocasionada por *Leishmania guyanensis*. *An Bras Dermatol* 2011;**86**:1092–101.

Nunes VL, Dorval ME, Oshiro ET *et al.* Estudo epidemiológico sobre Leishmaniose tegumentar (LT) no município de corguinho, mato grosso do Sul: estudos na população humana. *Rev Soc Bras Med Trop* 1995;**28**:185–93.

Ogg MM, Carrion R, Botelho AC *et al.* Short report: quantification of leishmaniavirus RNA in clinical samples and its possible role in pathogenesis. *Am J Trop Med Hyg* 2003;**69**:309–13.

Pacheco RS, Fernandes O, Salinas G *et al.* Intraspecific heterogeneity in the mini-exon gene localization of *Leishmania (Viannia) panamensis* and *Leishmania (Viannia) guyanensis* from Colombia. *J Parasitol* 2000;**86**:1250–3.

Pereira L, Maretti-Mira AC, Rodrigues KM *et al.* Severity of tegumentary leishmaniasis is not exclusively associated with *Leishmania* RNA virus 1 infection in Brazil. *Mem Inst Oswaldo Cruz* 2013;**108**:665–7.

Pires A, Borges AF, Cappellazzo Coelho A *et al.* Identification and biological characterization of *Leishmania (Viannia) guyanensis* isolated from a patient with tegumentary leishmaniasis in Goiás, a nonendemic area for this species in Brazil. *Biomed Res Int* 2015;**2015**:350764.

Prestes SR, Guerra JA, Romero GAS *et al.* Polymerase chain reaction-based method for the identification of *Leishmania (Viannia) braziliensis* and *Leishmania (Viannia) guyanensis* in mucosal tissues conserved in paraffin. *Rev Soc Bras Med Trop* 2015;**48**:555–9.

Puentes SM, Sacks DL, da Silva RP *et al.* Complement binding by two developmental stages of *Leishmania major* promastigotes varying in expression of a surface lipophosphoglycan. *J Exp Med* 1988;**167**:887–902.

Ribeiro-de-Jesus A, Almeida RP, Lessa H *et al.* Cytokine profile and pathology in human leishmaniasis. *Braz J Med Biol Res* 1998;**31**:143–8.

Romero GAS, de Farias Guerra MV, Paes MG *et al.*Comparison of cutaneous leishmaniasis due to *Leishmania (Viannia) braziliensis* and *L. (V.) guyanensis* in Brazil: Clinical findings and diagnostic approach. *Clin Infect Dis* 2001;**32**:1304–12.

Romero GAS, de la Gloria Orge M, de Farias Guerra MV *et al.* Antibody response in patients with cutaneous leishmaniasis infected by *Leishmania (Viannia) braziliensis* or *Leishmania (Viannia) guyanensis* in Brazil. *Acta Trop* 2005;**93**:49–56.

Ross R. Further notes on Leishman's bodies. *BMJ* 1903;**2**:1401.

Rossi M, Castiglioni P, Hartley M-A *et al.* Type I interferons induced by endogenous or exogenous viral infections promote metastasis and relapse of leishmaniasis. *Proc Natl Acad Sci U S A* 2017;**114**:4987–92.

Rotureau B, Ravel C, Nacher M *et al.* Molecular epidemiology of *Leishmania (Viannia) guyanensis* in French Guiana. *J Clin Microbiol* 2006;**44**:468–73.

Rougeron V, Bañuls A-L, Carme B *et al.* Reproductive strategies and population structure in *Leishmania*: substantial amount of sex in *Leishmania Viannia guyanensis*. *Mol Ecol* 2011;**20**:3116–27.

Sacks DL, Perkins PV. Identification of an infective stage of *Leishmania* promastigotes. *Science* 1984;**223**:1417–9.

Saiz M, Llanos-Cuentas A, Echevarria J *et al.* Short report: detection of Leishmaniavirus in human biopsy samples of leishmaniasis from Peru. *Am J Trop Med Hyg* 1998;**58**:192–4.

Salinas G, Zamora M, Stuart K *et al.* *Leishmania* RNA viruses in *Leishmania* of the *Viannia* subgenus. *Am J Trop Med Hyg* 1996;**54**:425–9.

Santrich C, Segura I, Arias AL *et al.* Mucosal disease caused by *Leishmania braziliensis guyanensis*. *Am J Trop Med Hyg* 1990;**42**:51–55.

Scarpassa VM, Alencar RB. *Lutzomyia umbratilis*, the main vector of *Leishmania guyanensis*, represents a novel species complex? *PLoS One* 2012;**7**:e37341.

Scheffter SM, Ro YT, Chung IK *et al.* The complete sequence of *Leishmania* RNA virus LRV2-1, a virus of an Old World parasite strain. *Virology* 1995;**212**:84–90.

Schröder NW, Schumann RR. Single nucleotide polymorphisms of Toll-like receptors and susceptibility to infectious disease. *Lancet Infect Dis* 2005;**5**:156–64.

Shah JA, Vary JC, Chau TTH *et al.* Human TOLLIP regulates TLR2 and TLR4 signaling and its polymorphisms are associated with susceptibility to tuberculosis. *J Immunol* 2012;**189**:1737–46.

Smith DF, Peacock CS, Cruz AK. Comparative genomics: from genotype to disease phenotype in the leishmaniases. *Int J Parasitol* 2007;**37**:1173–86.

Soto J, Berman J. Treatment of New World cutaneous leishmaniasis with miltefosine. *Trans R Soc Trop Med Hyg* 2006;**100**:S34–40.

Soto J, Toledo J, Valda L *et al.* Treatment of Bolivian mucosal leishmaniasis with miltefosine. *Clin Infect Dis* 2007;**44**:350–6.

Sousa-Franco J, Araújo-Mendes E, Silva-Jardim I *et al.* Infection-induced respiratory burst in BALB/c macrophages kills *Leishmania guyanensis* amastigotes through apoptosis: possible involvement in resistance to cutaneous leishmaniasis. *Microbes Infect* 2006;**8**:390–400.

Stuart KD, Weeks R, Guilbride L *et al.* Molecular organization of Leishmania RNA virus 1. *Proc Natl Acad Sci U S A* 1992;**89**:8596–600.

Sundar S, Jha TK, Thakur CP *et al.* Oral miltefosine for the treatment of Indian visceral leishmaniasis. *Trans R Soc Trop Med Hyg* 2006;**100**:S26–33.

Tarr PI, Aline RF, Smiley BL *et al.* LR1: a candidate RNA virus of Leishmania. *Proc Natl Acad Sci U S A* 1988;**85**:9572–5.

Thakur CP, Singh RK, Hassan SM *et al.* Amphotericin B deoxycholate treatment of visceral leishmaniasis with newer modes of administration and precautions: a study of 938 cases. *Trans R Soc Trop Med Hyg* 1999;**93**:319–23.

Torres DC, Adaui V, Ribeiro-Alves M *et al.* Targeted gene expression profiling in *Leishmania braziliensis* and *Leishmania guyanensis* parasites isolated from Brazilian patients with different antimonial treatment outcomes. *Infect Genet Evol* 2010;**10**:727–33.

Trinchieri G. Interleukin-10 production by effector T cells: Th1 cells show self control. *J Exp Med* 2007;**204**:239–43.

Trujillo C, Ramírez R, Vélez ID *et al.* The humoral immune response to the kinetoplastid membrane protein-11 in patients with American leishmaniasis and Chagas disease: prevalence of IgG subclasses and mapping of epitopes. *Immunol Lett* 2000;**70**:203–9.

Ueno N, Wilson ME. Receptor-mediated phagocytosis of *Leishmania*: Implications for intracellular survival. *Trends Parasitol* 2012;**28**:335–44.

van der Meide WF, Sabajo LOA, Jensema AJ *et al.* Evaluation of treatment with pentamidine for cutaneous leishmaniasis in Suriname. *Int J Dermatol* 2009;**48**:52–58.

Walker J, Acestor N, Gongora R *et al.* Comparative protein profiling identifies elongation factor-1β and tryparedoxin peroxidase as factors associated with metastasis in *Leishmania guyanensis*. *Mol Biochem Parasitol* 2006;**145**:254–64.

Widmer G, Comeau AM, Furlong DB *et al.* Characterization of a RNA virus from the parasite Leishmania. *Proc Natl Acad Sci U S A* 1989;**86**:5979–82.

WHO, World Health Organization. Leishmaniasis in high-burden countries: an epidemiological update based on data reported in 2014. 2016, http://www.who.int/leishmaniasis/resources/who_wer9122/en/.

Zangger H, Hailu A, Desponds C *et al.* *Leishmania aethiopica* field isolates bearing an endosymbiontic dsRNA virus induce pro-inflammatory cytokine response. *PLoS Negl Trop Dis* 2014;**8**:e2836.

Zangger H, Ronet C, Desponds C *et al.* Detection of *Leishmania* RNA virus in *Leishmania* parasites. *PLoS Negl Trop Dis* 2013;**7**:e2006.