The immunobiology of *Leishmania braziliensis* infection

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**Infection with New World Leishmania Species:**

CLINICAL ASPECTS

Rebelo et al. (2010) proposed the term tegumentary (skin or mucosal) leishmaniasis (TL) to the French Society of Dermatology in 1925, the first contribution of Brazilian researchers to the study of this disease. TL is a major health problem in Brazil, where the main Leishmania species associated with this disease are *Leishmania (Viannia) braziliensis*, *L. (V) naiffi*, *L. (V) shawi*, *L. (V) lamarquii*, and *L. (Leishmania) amazonensis*. Of these, *L. braziliensis* is the predominant species in the regions of Brazil where TL is endemic, and it occurs in areas of both ancient and recent colonization with a low prevalence in the Amazonas state (Sanches et al., 1987). *L. braziliensis* transmission is associated with the presence of domestic animals, which are implicated as potential reservoirs. It is transmitted by several different sand fly species, including *Luattemia intermedia*, *Lu. whitmani*, and *Lu. welcomi* (Miranda et al., 2002; Rebelo et al., 2010). *L. amazonensis* has been identified in different areas of Brazil and induces cutaneous ulcers, including diffuse cutaneous leishmaniasis. The main reservoirs for *L. amazonensis* are rodents and marsupials, and the main vector species associated with its transmission are *Lu. flaviscutellata* and *Lu. olmeca*. Host–parasite interactions can lead to a series of events culminating in clinical manifestations; the clinical forms of TL vary due to this complexity. In Brazil, TL can present as a single lesion (LCL, localized cutaneous leishmaniasis) that can be unapparent or discrete and can spontaneously heal. Multiple ulcerations may be present, compromising the mucosal areas (ML, mucosal leishmaniasis). ML is particularly important in South America and is caused primarily by *L. braziliensis*, although *L. amazonensis* has also been implicated (Costa et al., 1986). ML is characterized by latency and chronicity. Parasitological diagnosis is difficult, and a significant number of cases do not respond to treatment. Generally, 2–5% of TL cases in which the primary infection heals subsequently develop ML (Maiden, 1990). Leishmaniasis persistence following clinical treatment may be responsible for recurrence of the disease (Schubach et al., 1998). LCL and ML represent responsive facets of the disease, with immune responses that are readily detected.

Diffuse cutaneous leishmaniasis, caused by *L. amazonensis*, is a rare but severe manifestation of the disease that develops in anergic patients and is characterized by a defective cellular immune response to *Leishmania* antigens (Convy et al., 1962). In this case, a single ulcer slowly evolves, developing plaques or multiple non-ulcerated nodules. Diffuse cutaneous leishmaniasis responds poorly to treatment, and *Leishmania* skin tests are negative.

In this review, we will focus on recent advances in understanding the complexity of TL caused by *L. braziliensis*, focusing on both experimental models of infection and pathogenesis in the human host. We will also discuss certain aspects of infection with *L. amazonensis*, given its sole association with diffuse cutaneous leishmaniasis and the singular characteristics of this disease. We aim to provide a current picture of the complex host–parasite interactions involved in leishmaniasis while also taking into account the role of the vector’s saliva, an area of intense research in the past several years.

It is important to understand recent findings regarding intrinsic differences within the *Leishmania* species when addressing *L. braziliensis* pathogenesis. These differences came to light following vaccination attempts using the *L. braziliensis* homologs of the receptor for activated C kinase (LACK), thiol-specific antioxidant (TSA), Leishmania elongation and initiation factor (LeFI1), and *L. major* stress-inducible protein 1 (LmSTI1; Salay et al., 2007). All four open reading frames have a high degree of homology at the
that, in RNAi-competent L. braziliensis, infection with LRV increased parasite survival and pathogen-dow-modulate RNAi activity. L. braziliensis caused by mobile elements (Shi et al., 2004). Double-strand (ds) RNA viruses (LRVs) can infect L. braziliensis (Tarr et al., 1988; Patterson, 1993), and the RNA response may help to pro-tect against such infection (Anderson et al., 2007). However, infection with LRV increased parasite survival and pathogen-esis in L. guyanensis (Ives et al., 2011), raising the possibility that, in RNAi-competent Leishmania, LRV infection may actually down-modulate RNAi activity.

**INNATE IMMUNITY IN LEISHMANIASIS TRIGGERED BY L. BRAZILIENSIS**

BALB/c mice infected in the ear dermis with L. braziliensis develop cutaneous lesions at the site of inoculation (de Moura et al., 2005), and histological analysis of ear sections demonstrated constant recruitment of neutrophils to the inoculation site. Interestingly, neutrophil depletion during L. braziliensis infection increased parasite load, where BALB/c mice co-inculated with both parasites and live neutrophils displayed lower parasite loads at the site of infection and in the draining lymph nodes (Novais et al., 2009). In vitro, co-cultures of live neutrophils and L. braziliensis-infected macrophages led to a decrease in parasite load, and elimination of L. braziliensis elimination was associated with TNF-α and superoxide production (Novais et al., 2009). In experiments using L. amazonensis, phagocytosis of apoptotic human neutrophils by L. amazonensis-infected macrophages led to an increase in para-site burden in a TGF-β and PGE2-dependent manner. Conversely, uptake of necrotic neutrophils by infected macrophages led to killing of L. amazonensis. Leishmanicidal activity was depend-ent on TNF-α and neutrophil elastase and was also associated with superoxide production (Afonso et al., 2008). Another func-tion attributed to the neutrophils is the production of neutrophil extracellular traps (NETs) which are composed of filamentous genomic DNA containing antimicrobial peptides. L. amazonensis parasites are susceptible to killing by humans NETs, and LPG iso-lated from these parasites triggered NET release (Guimaraes-Costa et al., 2009).

L. braziliensis induced the production of CXCL-10 and IL-10 by human peripheral blood mononuclear cells (PBMCs) and macrophages, but the enhanced expression of CXCL10 and its receptor, CXCR3, was predominately detected in CD14+ monocytes. Interestingly, sera from TL patients, and espe-cially those from ML patients, have significantly higher levels of CXCL10, CCL4, and soluble TNF receptor II (sTNFRII) than sera from control individuals. These multiple inflammatory media-tors produced by the host may contribute to disease severity by increasing cellular recruitment (Vargas-Inchaustegui et al., 2010). However, IL-10 production is important in controlling the exagger-ated inflammatory response characteristic of TL. Antonelli et al. (2004) showed a strong positive correlation between IL-10 and TNF-α-producing monocytes in PBMC cultures from LCL patients stimulated with soluble Leishmania antigens (SLA), sug-gestning that an intrinsic macrophage auto-regulation mechanism appears to be active in LCL patients.

Interferon-β increases the parasite load in infected human macrophages following infection with New World parasites (L. braziliensis and L. amazonensis) in a manner that is indepen-dent of endogenous and exogenous NO (Khouri et al., 2009). In parallel, IFN-β significantly reduces superoxide release by both Leishmania-infected and uninfected human macrophages. This reduction was accompanied by a significant increase in superoxide dismutase (SOD1) protein levels. Biosynthes from New World cuta-neous leishmaniasis patients show pronounced SOD-1 expression in vivo (Khouri et al., 2009). Importantly, these results suggest that IFN-β production in human Leishmaniasis may be deleteri-ous, particularly in DCL cases where the parasite load is elevated. Similarly, TGF-β also plays a major role in macrophage deactiva-tion, leading to increased parasite load in an experimental model of L. amazonensis infection (Barra et al., 1992). Subsequently, para-site killing following L. amazonensis infection has been observed in vitro in experiments using the superoxide-dismutase inhibitor diethyldithiocarbamate (DETC; Khouri et al., 2009). Moreover, in vivo treatment with DETC significantly decreased lesion size and parasite load in an experimental model of L. braziliensis infection.

Systemic administration of Leishmania parasites, including L. braziliensis, induces in vivo DC maturation characterized by DC migration to T cell areas and costimulatory molecule upregu-lation (Antonelli et al., 2004). DCs co-cultured with L. braziliensis up-regulate DC activation markers and produce IL-12 and TNF-α. However, up-regulation of activation markers and IL-12 pro-duction was primarily confined to bystander (uninfected) DCs (Carvalho et al., 2006). The authors of this study proposed that bystander DCs in L. braziliensis infection lead to T cell activa-tion, while infected DCs contribute to parasite control through enhanced TNF-α production. Indeed, experimentally infected TNF-α−/− mice developed non-healing skin lesions (Rocha et al., 2007). Likewise, axenic L. braziliensis amastigotes successfully stimulated DCs to produce IL-12p40, inducing an activated phenotype (Vargas-Inchaustegui et al., 2008). DCs infected with L. braziliensis show increased phosphorylation of STAT molecules and ISG15 expression (IFN-stimulated gene 15). Accordingly, in vivo infection with L. braziliensis led to a self-healing phenotype characterized by increased numbers of IFN-γ- and IL-17-secreting CD4+ T cells. DCs from MyD88−/− mice exhibited less acti-vation and decreased production of IL-12 during experimental L. braziliensis infection, suggesting a role for TLR involvement (Vargas-Inchaustegui et al., 2009). Furthermore, MyD88−/− mice developed larger lesions than control mice. However, a lack of...
TLR2 resulted in enhanced DC activation, increased IL-12 production and successful priming of naïve CD4+ T cells. Fully understanding the role of TLRs in *L. braziliensis* infection will require further research.

**ADAPTIVE IMMUNITY IN HUMAN LEISHMANIASIS**

In general, patients with LCL and ML have a strong type 1 immune response to *Leishmania* antigen, with high production of IFN-γ and TNF-α and decreased efficacy of IL-10 in down-modulating IFN-γ production (Follador et al., 2002). With disease progression, ML patients tend to develop stronger intradermal skin test reactions, and their lymphocytes exhibit stronger proliferative responses and IFN-γ production than cells from LCL patients. However, antigen-stimulated PBMCs from 50% of subjects who developed the disease within the previous 60 days exhibited low or absent IFN-γ levels. This response can be restored by either IL-12 or anti-IL-10 monoclonal antibodies (Ribeiro-de-Jesus et al., 1998). Later in the disease course, both LCL and MCL patients exhibited high levels of IFN-γ and TNF-α, but TNF-α levels decreased following treatment. IFN-γ and TNF-α seem to be involved in both controlling parasite multiplication during the early phases of *Leishmania* infection and mediating the tissue damage observed in TL (Ribeiro-de-Jesus et al., 1998). In a recent study, Oliveira et al. (2011) observed a positive correlation between ulcer size at the time of the first evaluation, time to recovery, and TNF-α levels, supporting the use of TNF-α inhibitors combined with standard therapy to improve recovery time in LCL patients with severe lesions. In fact, pentoxifylline has been successfully used to decrease recovery time in ML patients, even in those that were refractory to conventional treatment (Lessa et al., 2001; Baffica et al., 2003).

Infection with *L. braziliensis* has been associated with lymphadenopathy in the absence of tegumentary lesions (skin or mucosal) (Barral et al., 1992, 1993a). Lymphadenopathy can precede the appearance of skin ulcers and must be differentiated from the satellite lymph node enlargement associated with lesion establishment. Cells obtained from lymph nodes from LCL patients including CD4+ and CD8+ T cells, and numerous IL-17+ cells. In contrast, CD19+ B cells and plasma cells were more frequently observed in patients showing lymphadenopathy with ulcerations (late phase; Botrón et al., 2007). IL-10 transcription was significantly higher in patients than in LCL patients, suggesting a possible protective role of this cytokine at both the protein and mRNA levels were very similar in both groups. IL-27 is a cytokine that both initiates a Th1 response and regulates inflammation (Trinchieri, 2005; Yoshimura et al., 2006). IL-27 mRNA levels were higher in cells from LCL patients than in those from SC patients following stimulation with *L. braziliensis*. The mechanisms by which SC individuals control parasite growth are unknown. Because the adaptive immune responses in these individuals is less prominent, we can speculate that parasite control may be dependent on innate immune responses, with the participation of neutrophils (Novoa et al., 2011) and macrophages and NK cells of particular importance. Interestingly, PBMC IL-17 production is slightly higher in SC patients than in LCL patients, suggesting a possible protective role for this cytokine. However, IL-17 could be exerting different functions based on the phase of disease. Our group recently illustrated the involvement of IL-17 and IL-17-inducing cytokines in biopsy specimens from ML patients. IL-17 was expressed by CD4+, CD8+, and CD14+ cells, and numerous IL-17+ cells co-expressed CCR6. We also observed the presence of neutrophils in necrotic and perinecrotic areas; these neutrophils stained positive for neutrophil elastase, myeloperoxidase, and MMP-9, indicating that IL-17 could be involved in ML pathogenesis (Boaventura et al., 2010). In fact, in vitro infection of human macrophages with *L. braziliensis* increased the secretion and activation of MMP-9, and macrophages from cured individuals with previous histories of ML exhibited positive correlation was observed between IFN-γ and TNF-α and IL-10 production from lymphocytes. Higher frequencies of specific T cells identified by their TCR Vβ expression. They observed an increase in CD4 Vβ 5.2- and Vβ 24-positive T cells in LCL patients compared to controls, a profile suggesting previous activation of the CD4 Vβ 5.2-, 11-, and 24-positive T cells characterized by increased expression of CD45RO, HLA-DR, IFN-γ, TNF-α, and IL-10 compared to the other Vβ-expressing subpopulations and a positive correlation between higher frequencies of CD4 Vβ 5.2 T cells and lesion size. The identification of active subpopulations in this form of the disease could allow for the identification of the immunodominant *Leishmania* antigens responsible for triggering an efficient host response against the parasite and could also allow for identification of the cell populations involved in disease pathology.

In *L. braziliensis* endemic areas, approximately 10% of the individuals have a positive delayed-type hypersensitivity (DTH) skin test to *Leishmania* antigen but have neither a previous history of LCL nor a typical LCL scar. These individuals are categorized as having a subclinical (SC) *L. braziliensis* infection (Follador et al., 2002). Individuals with SC *L. braziliensis* infection produce significantly lower levels of IFN-γ and TNF-α than patients with active LCL. However, IL-10 levels are higher in these individuals than in LCL patients (Bittar et al., 2007). Recently, Novoa et al. (2011) reported stronger Th1 responses in LCL patients than in SC individuals. This finding seems be unaffected by IL-10, as levels of this cytokine in both the protein and mRNA levels were similar in both groups. IL-27 is a cytokine that both initiates a Th1 response and regulates inflammation (Trinchieri, 2005; Yoshimura et al., 2006). IL-27 mRNA levels were higher in cells from LCL patients than in those from SC patients following stimulation with *L. braziliensis*. The mechanisms by which SC individuals control parasite growth are unknown. Because the adaptive immune responses in these individuals is less prominent, we can speculate that parasite control may be dependent on innate immune responses, with the participation of neutrophils (Novoa et al., 2011) and macrophages and NK cells of particular importance. Interestingly, PBMC IL-17 production is slightly higher in SC patients than in LCL patients, suggesting a possible protective role for this cytokine. However, IL-17 could be exerting different functions based on the phase of disease. Our group recently illustrated the involvement of IL-17 and IL-17-inducing cytokines in biopsy specimens from ML patients. IL-17 was expressed by CD4+, CD8+, and CD14+ cells, and numerous IL-17+ cells co-expressed CCR6. We also observed the presence of neutrophils in necrotic and perinecrotic areas; these neutrophils stained positive for neutrophil elastase, myeloperoxidase, and MMP-9, indicating that IL-17 could be involved in ML pathogenesis (Boaventura et al., 2010). In fact, *in vitro* infection of human macrophages with *L. braziliensis* increased the secretion and activation of MMP-9, and macrophages from cured individuals with previous histories of ML exhibited
increased MMP-9 activity than those from cured LCL patients (Marotti-Mura et al., 2011).

Macrophage leishmaniasis patients display an exacerbated and unregulated immune response. They have a higher frequency of activated T cells than patients with LCL, as measured by different activation markers. While LCL patients displayed a positive correlation between IL-10 and TNF-α-producing monocytes, ML patients did not. This lack of correlation between IL-10-producing and TNF-α-producing monocytes in ML patients could lead to a poorly controlled inflammatory response in vivo, and cytokine networks may be involved in the development of immunopathology in ML patients (Gaze et al., 2006). Additionally, IL-10 receptor expression was lower in ML lesions than in LCL lesions (Faria et al., 2005).

**ROLE OF CD8+ T CELLS**

The role of cytotoxicity in host defense and tissue damage during human LCL is not yet well understood. Machado et al. (2002) observed the presence of NK cells, CD8+ and CD45RO+ T cells, and strong expression of TIA-1, a molecule associated with cytotoxicity, in the dermal cell infiltrates of lesions from LCL patients. The presence of these cytolytic cells in LCL lesions suggests active participation of NK and CD8+ T cells in the pathogenesis of this disease. These cells may play a role in both parasite killing and ulcer development (Machado et al., 2002). More recently, Faria et al. (2009) characterized the immunological kinetics associated with LCL progression, comparing the cellular composition and cytokine and granzyme expression in lesions of patients with early-stage (E-LCL) and late-stage LCL (L-LCL). Histopathological analysis showed that lesions from L-LCL patients displayed more exuberant inflammatory infiltration than those from E-LCL patients. Although E-LCL and L-LCL lesions were predominantly mononuclear, lesions from E-LCL patients presented higher neutrophil and eosinophil counts than those from L-LCL patients. Although E-LCL and L-LCL lesions were predominantly mononuclear, lesions from E-LCL patients presented higher neutrophil and eosinophil counts than those from L-LCL patients. Although E-LCL and L-LCL lesions were predominantly mononuclear, lesions from E-LCL patients presented higher neutrophil and eosinophil counts than those from L-LCL patients. These results suggest that the recruitment of CD8+ and granzyme A+ T cells is involved in lesion progression in human LCL. Our group found similar results, showing that lesions from CL patients presented higher frequencies of CD8+ T cells displaying CLA (cutaneous lymphocytes antigen); these cells are mainly cytotoxic, with strong expression of CD107 and granzyme B. They also produce IFN-γ and IL-10, but in lower frequencies than CD4+ T cells (Silva et al., submitted manuscript). Mendes-Aguiar Cde et al. (2009) also observed that the CLA receptor could direct Leishmania-specific CD8+ T lymphocytes toward inflammatory lesions, affecting the cell composition of the inflammatory infiltrate in Leishmaniasis. CD8+ T cells seem to play distinct roles in different phases of the disease. Using an in vitro priming assay (IVP), we observed that CD8+ T cells are the first cells to be activated by Leishmania promastigotes and to produce IFN-γ, which is partially responsible for directing the differentiation of Th1 cells (Pompeu et al., 2001). However, CD8+ T cells and NK cells also contribute to the tissue destruction observed in ML patients in late stages of disease (Brodkyn et al., 1997).

**IMMUNOSUPPRESSION IN LEISHMANIASIS**

In spite of a robust immune response, a small number of parasites persist following the resolution of leishmaniasis (Mendezona et al., 2004; Figueroa et al., 2009; Martins et al., 2010). In mice infected with L. braziliensis in the ear dermis, parasites also persist in draining lymph nodes, despite lesion resolution and parasite clearance from the infection site (de Moura et al., 2005). In mice, IL-10 blockade following infection with low doses leads to a sterile cure of the disease (Belkaid et al., 2001). In Leishmaniasis, this cytokine can be produced by several different cell sources, including Treg cells (Belkaid et al., 2002), Th1 cells (Stager et al., 2006; Anderson et al., 2007; Nilen et al., 2007), CD8+ T cells (Belkaid et al., 2002), B cells (Ronet et al., 2010), NK cells (Maroof et al., 2008), regulatory DCs (Svensson et al., 2004), macrophages (Miles et al., 2005), and neutrophils (McFarlane et al., 2008). In patients, functional Treg cells could be found in the skin lesions of patients with LCL. These cells expressed phenotypic markers of Treg cells, including CD25, CTLA-4, Foxp3, and GITR (glucocorticoid-induced tumor necrosis factor receptor), and were able to produce large amounts of IL-10 and TGF-β. CD4+CD25+ T cells derived from the lesions of patients with LCL suppressed the PHA-induced proliferative T cell responses of allogeneic PBMCs from healthy controls (Campanelli et al., 2006). These findings suggest that functional Treg cells accumulate at sites of Leishmania infection in humans and possibly contribute to the local control of effector T cell functions.

Another important immunosuppressive cytokine involved is TGF-β. This cytokine has different effects on cells in the immune system, including down-regulation of certain macrophage functions. As commented earlier, this cytokine is produced by macrophages present in the lesions of mice infected with L. amazonensis (Barral-Neto et al., 1992). Human macrophages produce active TGF-β after infection with L. amazonensis, L. chagasi, and L. braziliensis. The addition of this cytokine to cultures of L. braziliensis-infected macrophages led to an increase in parasite numbers compared to untreated cultures. Fibroblasts in the dermis could be immunostained for TGF-β, as could inflammatory cells and monocytes in the dermis. The mechanism behind the effects of TGF-β on Leishmania infection is not fully understood, but it is likely that TGF-β can inhibit the accumulation of T cells and NK cells at the site of infection, leading to a reduced immune response and persistence of the parasite. However, analysis of the immune response has yet to be fully elucidated, and more research is needed to understand the role of TGF-β in the pathogenesis of Leishmaniasis.

**EXPERIMENTAL INFECTION WITH L. BRAZILIENSIS: INSIGHTS INTO HUMAN PATHOGENESIS?**

Initial studies conducted with inbred mice infected with L. braziliensis revealed a broad range of responses. AKR/J and CBA/J mice showed only a mild and transient swelling of the nose. SWR/J, C57L/J, A/J, A/HeJ, and DBA/1J mice showed initial nodules, which eventually healed. In contrast, BALB/cJ mice were considered susceptible based on progressive dermal lesions (Childs et al., 1984). However, analysis of the immune response has yet to be fully elucidated, and more research is needed to understand the role of TGF-β in the pathogenesis of Leishmaniasis.
Importantly, in the same study, the authors demonstrated that parasite multiplication in the draining lymph nodes (Maioli et al., 2004). This phenotype was associated with increased levels of parasite multiplication in the draining lymph nodes (Maioli et al., 2004). In a recent study, mice inoculated with an antigen-resistant L. braziliensis strain displayed an increased IL-4 response and elevated Arginase I expression (Costa et al., 2011). Treatment with an anti-IL-4 monoclonal antibody resulted in decreased lesion thickness and parasite load. Therefore, the capacity of L. braziliensis isolates to induce a Th2-type response, characterized by presence of IL-4, contributes to the virulence and severity of disease.

Using the footpad model of infection, we showed that mice infected with a L. braziliensis strain from Ceará (H3227) developed detectable lesions, whereas mice infected with a different strain (BA788), isolated in Bahia, did not (Indiani de Oliveira et al., 2004). Early after parasite inoculation, lymph node cells from BA788-parasitized mice produced higher levels of IFN-γ and had higher numbers of NK cells than H3227-infected mice. Importantly, the L. braziliensis strain from Ceará (H3227) is genetically different from the L. braziliensis strain from Bahia (BA788). Therefore, variation in the pathogenicity of these different L. braziliensis strains correlated with their genetic diversity. Interestingly, in Ceará state, where the H3227 L. braziliensis isolate was obtained, prominent lymphadenopathy can precede skin lesion appearance (Souza Ade et al., 1995), suggesting that important correlates can be drawn from the mouse model. In a subsequent study using the same two strains, the H3227 L. braziliensis strain induced significantly stronger cellular recruitment than the BA788 strain, which correlated with higher expression of CCL2, CCL5, and CXCCL1 (Teixeira et al., 2005). In contrast, L. braziliensis BA788 significantly up-regulated CXCCL10 expression, which correlated with earlier IFN-γ production and with a higher number of NK cells present at the infection site (Indiani de Oliveira et al., 2004).

BALB/c mice infected with a Leishmania strain isolated from a ML patient developed a rapidly progressing and widely metastatic disease resembling diffuse cutaneous leishmaniasis (Barral et al., 1983). Although C57BL/6 mice initially contained parasite multiplication and appeared clinically cured, subsequent disease development that was characterized by distinctive ulcerative metasizes and destruction of the nasal region, similar to what is observed in ML. Disease development in these mouse strains was associated with a decrease in cell-mediated immunity, as monitored by delayed type hypersensitivity and lymphoproliferative responses. Footpad injection of metacyclic L. braziliensis into C57BL/6 mice confirmed the initial findings regarding disease outcome in this mouse strain, as the mice control infection and parasite multiplication in the draining lymph nodes (Mazoli et al., 2004). This phenotype was associated with increased levels of IFN-γ and TNF-α and a superior lymphoproliferative response. Importantly, in the same study, the authors demonstrated that L. amazonensis infection leads to chronic lesion development with elevated parasite numbers and a decreased cellular response, highlighting the differences in immune regulation induced by the distinct New World Leishmania species.

As has been thoroughly described in the literature, mice from BALB strains are highly susceptible to L. major infection, and this susceptibility is linked to a predominant Th2 response characterized by the presence of IL-4 (reviewed in Bellaud et al., 2002). In a comparative study, BALB/c mice infected subcutaneously with L. braziliensis developed small, nodular lesions that self-healed, in contrast to L. major-infected BALB/c mice, which displayed progressive ulcers (Rocha et al., 2007). This phenotype was confirmed by intradermal infection with L. braziliensis, after which mice develop ulcerated lesions (similar to the lesions that develop upon natural infection) that heal spontaneously (de Moura et al., 2005). In this model, a mixed Th1/Th2 immune response was observed that was characterized by the presence of IFN-γ, IL-4, and IL-10-secreting cells, which is distinct from the Th2-polarized response observed following L. major infection. Interestingly, parasites are cleared from the infection site following lesion healing but persist within draining lymph nodes, suggesting that immunoregulatory mechanisms allow for parasite survival. Indeed, the presence of CD4+CD25+ T cells expressing regulatory markers such as Foxp3, GITR, and CD103 has been described following L. braziliensis infection (Costa et al., 2011; Falcão et al., 2012).

A similar phenotype was also observed in C57BL/6 mice. The “resistance” observed in L. braziliensis-infected mice of this strain was associated with significantly lower IL-4 and IL-13 production in parallel with increased presence of IFN-γ and TNF. Previous work has shown that IFN-γ−/− mice infected with L. braziliensis develop uncontrolled lesions (DeKrey et al., 1998), while IL-12p40−/− mice, which lack both IL-12 and IL-23, develop chronic lesions (de Souza-Neto et al., 2004). As lymphocytes from the latter produce decreased levels of IFN-γ, this further implicates IFN-γ in the control of L. braziliensis infection. These results were confirmed by infecting IL-12p35−/− mice, which lack IL-12 only. These mice display uncontrolled lesions, as do IL-12p40−/− mice (Rocha et al., 2007). A similar phenotype was observed following infection of STAT4−/− mice, implicating IL-12 in the immune response to L. braziliensis. Lastly, IL-10−/− mice develop progressive non-healing lesions and have an increased parasite load within the draining lymph nodes (Rocha et al., 2007). The lesions self-heal in mice lacking pp19iphox, indicating that only IL-10 is essential for controlling L. braziliensis infection.

### NEW WORLD LEISHMANIASIS AND THE ROLE OF SAND FLY SALIVA

Several attempts have been made to reproduce the biology of natural transmission, taking into account parasite load (sand flies inoculate low numbers of parasites), the presence of saliva (parasites are injected into the host’s skin in conjunction with sand fly saliva) and the site of inoculation (parasites are injected by the sand fly into the dermal compartments of the skin). BALB/c mice inoculated in the ear dermis with L. braziliensis develop ulcerated lesions (de Moura et al., 2005), but co-inoculation of parasites plus sand fly saliva exacerbates infection...
to modify the inflammatory environment and, in doing so, favors *L. braziliensis* establishment.

Several groups have examined the possibility of vaccination using salivary antigens. Immunization with a DNA plasmid coding for the SP15 *P. falciparum* paptatia protein was effective in providing protection against infection by *L. major* (Valenza et al., 2001). In parallel, we have shown that hamsters immunized with a DNA plasmid coding for LJM19, a protein present in *L. longipalpis* saliva, were protected against the development of visceral leishmaniasis (Gomes et al., 2008). LJM19-immunized hamsters maintained a low parasite load that correlated with high IFN-γ/TGF-β ratio and iNOS production. Importantly, a delayed-type hypersensitivity (DTH) response with high expression of IFN-γ was also detectable in the skin of LJM19-immunized animals. These studies suggested that a DTH response generated against salivary antigens such as SP15 or LJM19 might be the mechanism underlying protective anti-Leishmania immunity. Based on this hypothesis, we have shown that immunization with a DNA plasmid coding for LJM19, a protein present in *L. longipalpis* saliva, conferred protection against *L. braziliensis* (Tavares et al., 2011). These results suggest the possibility of using salivary antigens to generate protection against different species of *Leishmania*.

**CONCLUDING REMARKS**

Although *Leishmania* infection has been widely used to elucidate many aspects of the immune response to intracellular pathogens, we still do not fully understand the immunopathogenesis of the human disease. It is clear that LCL and ML are caused by *L. braziliensis* are diseases in which immunoregulation, rather than parasite multiplication per se, plays a major role. Moreover, DCL caused by *L. amazonensis* is a disease that completely lacks a cellular immune response. Collectively, these major differences may offer unique opportunities for more studies focused on human immunology. The experimental mouse intradermal infection model using *L. braziliensis* recapitulates many aspects of the human infection; as such, it will prove a useful tool for dissecting aspects of both the innate and the adaptive immune response to this important representative of New World leishmaniasis.

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