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LEISHMANIASIS

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Abstract:

Leishmaniasis is a vector-borne disease that is transmitted by sandflies and caused by obligate intracellular protozoa of the genus Leishmania. Human infection is caused by about 21 of 30 species that infect mammals. These include the L. donovani complex with 3 species (L. donovani, L. infantum, and L. chagasi); the L. mexicana complex with 3 main species (L. mexicana, L. amazonensis, and L. venezuelensis); L. tropica; L. major; L. aethiopica; and the subgenus Viannia with 4 main species (L. (V.) braziliensis, L. (V.) guyanensis, L. (V.) panamensis, and L. (V.) peruviana). The different species are morphologically indistinguishable, but they can be differentiated by isoenzyme analysis, molecular methods, or monoclonal antibodies.

Keywords

Introduction

Leishmaniasis is caused by a protozoa parasite from over 20 Leishmania species. Over 90 sandfly species are known to transmit Leishmania parasites. There are 3 main forms of the disease:

Visceral leishmaniasis (VL), also known as kala-azar, is fatal if left untreated in over 95% of cases. It is characterized by irregular bouts of fever, weight loss, enlargement of the spleen and liver, and anaemia. Most cases occur in Brazil, east Africa and India. An estimated 50 000 to 90 000 new cases of VL occur worldwide annually, with only 25–45% reported to WHO. It has outbreak and mortality potential.

Cutaneous leishmaniasis (CL) is the most common form and causes skin lesions, mainly ulcers, on exposed parts of the body. These can leave life-long scars and cause serious disability or stigma. About 95% of CL cases occur in the Americas, the Mediterranean basin, the Middle East and central Asia. It is estimated that 600 000 to 1 million new cases occur worldwide annually but only around 200 000 are reported to WHO.

Mucocutaneous leishmaniasis leads to partial or total destruction of mucous membranes of the nose, mouth and throat. Over 90% of mucocutaneous leishmaniasis cases occur in Bolivia (the Plurinational State of), Brazil, Ethiopia and Peru (1).

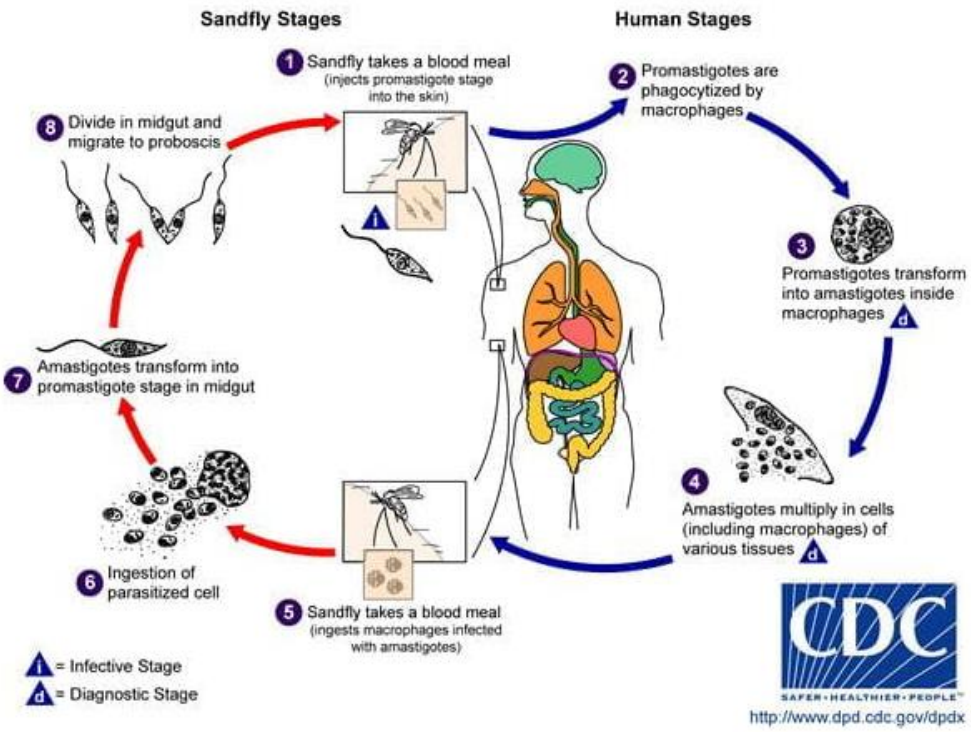
Classification and Morphology

Leishmania are eukaryotic protozoan parasites of vertebrates in the family Trypanomastidae (order Kinetoplastida). Characteristic of members of the order Kinetoplastida is the presence of a conspicuous Feulgen stain-positive (i.e., DNA-containing) kinetoplast (see 'Other membrane-bound organelles'). All members of the family Trypanomastidae are parasitic for vertebrates or invertebrates and undergo morphological changes during transition between stages of their life cycle. Of the many genera in this family, only species of Leishmania and Trypanosoma are human pathogens. Two subgenera of Leishmania, L. (Leishmania) and L. (Viannia) are recognized. Conservatively, at least 14 Leishmania species are pathogenic for mammals, of which nine are recognized parasites of humans (2).

Leishmania exist as morphologically distinct forms. In mammals, amastigotes are an obligate intracellular parasite of mononuclear phagocytes. The elongated motile promastigote form is found in female sandflies (genus Phlebotomus in the Old World and Lutzomyia and Psychodopygus in the New World), which are the only known vector for Leishmania. Promastigotes possess a single nucleus and are variable in length (15–25 μm) and shape (ellipsoid to slender). The most prominent features of stained promastigotes are the nucleus, the kinetoplast, and the flagellum, the kinetoplast and the origin of the flagellum define the anterior region of the parasite. Amastigotes are round to oval in shape with a 2–10 μm diameter. This stage is aflagellar (i.e., the flagellum does not extend past the cell boundary), but the kinetoplast and nucleus remain visible in stained amastigotes (3).

Life Cycle of *Leishmania*

1. People are infected when they are bitten by an infected female sand fly. Sand flies inject a form of the protozoa (called promastigotes) that can cause infection.
2. Promastigotes are ingested by certain immune cells called macrophages. (The process of a cell ingesting a microorganism, another cell, or cell fragments is called phagocytosis, and cells that ingest are called phagocytes.)
3. In these cells, promastigotes develop into another form (called amastigotes).
4. Amastigotes multiply inside macrophages in various tissues.
5–6. When a sand fly bites an infected person or animal, it becomes infected by ingesting blood containing macrophages with amastigotes inside.
7. In the middle part of the fly's gut (midgut), amastigotes develop into promastigotes.
8. In the fly's midgut, promastigotes multiply, develop, and migrate to the fly's mouth parts. They are injected when the fly bites another person, completing the cycle (4).



P-glycoprotein

Leishmania contains in its genome at least one P-glycoprotein homologue. The Leishmania gene product is highly homologous to the mammalian MDR1 protein and it was characterized in several Leishmania species(5). The Leishmania MDR1 gene was amplified in Leishmania mutants selected for vinblastine or daunomycin resistance and transfection experiments indeed indicated that this MDR1 gene can cause multidrug resistance (6). The interactions between flavenoids and the ABC domain of the Leishmania MDR1 were characterized and some derivatives with high affinity for the nucleotide-binding domain reversed the multidrug resistance phenotype of resistant cells (7,8).

The high degree of homology between Leishmania and human MDR1 suggests that the former could confer resistance by active extrusion of the drug. The efflux of rhodamine 123 in Leishmania amazonensis-resistant cells, the absence of accumulation of puromycin in vinblastine-resistant Leishmania donovani (9) and the reduction of daunomycin accumulation in resistant L. tropica (10) were all consistent with this hypothesis. This putative transport defect due to an efflux pump does not fit, however, with subcellular localization studies done in the laboratory of D. Wirth at Harvard. Their studies suggest that the majority of Leishmania MDR1 protein is not located in the plasma membrane but in an organelle close to the mitochondria of Leishmania enriettii (11). Further work is required to understand how MDR1 confers drug resistance in Leishmania and to determine its exact cellular location. Recently, it was suggested that MDR1 could confer resistance to

miltefosine (F. Gamarro, personal communication), a promising alkyl-lysophospholipid that can be taken orally and is highly active against Leishmania (12). Thus MDR1 has the potential for conferring resistance against useful anti-leishmanial compounds.

Leishmania and immunity

Infection of humans with *Leishmania* surmounts 1.3 million new cases each year. Parasites infect and survive within phagolysosomal vesicles in host macrophages. Following infection with *Leishmania*, macrophages produce reactive oxygen species (ROS), cytokines, and chemokines and recruit an early inflammatory reaction (13, 14). Interactions with inflammatory neutrophils either increase or decrease *L. major* replication in macrophages depending on host genotype and through mechanisms involving TGF-*β* or neutrophil elastase (15,16). Recent studies suggest that additional phagocytes such as monocytes and dendritic cells (DCs) play important roles in infection, both as host and as effector cells. Here we discuss recent experimental findings regarding regulation of *L. major* infection by these major phagocyte populations. In addition, the role of IL-4 on DC and monocyte responses to infection is discussed.

The Innate Immune Response in Leishmaniasis

2.1.1. Neutrophils

In leishmaniasis, the innate immune response is mainly mediated by macrophages, dendritic cells, natural killer cells, and neutrophils. These cells interact to determine susceptibility/resistance to the infection by modulating the ensuing adaptive immune response (19,20,21). There are divergent views on the role of neutrophils in the host immune response to leishmaniasis. At the early stages of infection, neutrophils migrate through the vasculature and are recruited to the site of infection, where they phagocytose parasites and eliminate them through a variety of mechanisms (22,23). One such mechanism involves the use of neutrophil extracellular traps (NETs) (22,24). The antileishmanial potency of NETs varies depending on the species of *Leishmania* involved. For instance, although NETs are effective against *L. amazonensis*, they are ineffective against *L. infantum*, *L. donovani*, and *L. mexicana* (22). In another study, Novais et al. showed that depletion of neutrophils in vivo after *L. braziliensis* infection resulted in a remarkable increase in parasite load in infected BALB/c mice, suggesting that they contribute to resistance (25). Nitric oxide (NO) is regarded as the major anti-*Leishmania* effector molecule in infected cells(26,27,28,29)A study conducted by Charmoy et al. showed that neutrophils isolated from *Leishmania*-resistant C57BL/6 mice harbored significantly fewer parasites than those isolated from *Leishmania*-susceptible BALB/c mice. Furthermore, neutrophils from C57BL/6 were shown to significantly secrete more NO than those from the susceptible BALB/c mice in response to IFN-γ stimulation *in vitro*. This observation from Charmoy et al. suggests that neutrophils may contribute to anti-*Leishmania* immunity by utilizing NO-dependent mechanisms to

eliminate *Leishmania* parasites (30). In contrast, the pro-*Leishmania* activity of neutrophils was demonstrated by Peters et al., who showed that depletion of neutrophils in *L. major*-infected C57BL/6 mice resulted in decreased parasite load and diminished the progression of disease (31). Neutrophils have also been shown to enhance the infection of macrophages by upregulating a macrophage chemokine, MIP-1β (CCL4) (32). Neutrophils are rapidly mobilized to the site of *Leishmania* infection via *Leishmania* chemotactic factor (LCF), a selective neutrophil chemokine (33). These neutrophils are able to phagocytose *Leishmania* and secrete Interleukin 8 (IL-8), which, in turn, increases the number of neutrophils migrating to the site of infection (34). The infected neutrophils become apoptotic and secrete macrophage chemokines such as MIP-1β (CCL4), which leads to the chemotaxis of macrophages to the site of infection. At the site of infection, these macrophages phagocytose the infected apoptotic neutrophils. The phagocytosed *Leishmania* parasites (from the apoptotic neutrophils) are able to survive and multiply within macrophages, further establishing the disease (34). This observation leads to neutrophils being referred to as "Trojan horses." The participation of neutrophils in leishmaniasis has been shown to be modulated to some extent by the mode of *Leishmania* infection. Peter et al. observed massive, sustained, and localized neutrophil recruitment to the site of a sand fly bite compared to a needle injection in the dermis (35). Taken together, these studies indicate that the role of neutrophils in host immune response to *Leishmania* infection may depend on the parasite species, mouse model used, and the mode of infection.

2.1.2. Macrophages

Macrophages are also present at the site of *Leishmania* infection. Although neutrophils and macrophages are both infected by *Leishmania* parasites, macrophages serve as the major infected cells that perpetuate the infection in the host because they live longer than neutrophils (26,36,37).

As well, *Leishmania* parasites are able to promote their survival in macrophages. One mechanism by which they do so is by suppressing Interleukin 12 (IL-12) production in infected macrophages (38,39). IL-12 is a key cytokine that induces the T-helper 1 (Th1) response which is essential for parasite elimination in infected macrophages (40,41,42). It has been shown that the outcome of *Leishmania* infection in macrophages depends on their activation status **(**38**,**43**,**44**,**45**).** Classical activation of macrophages is mediated by Interferon gamma (IFN-γ), an effector cytokine secreted by $CD4^+$ T helper type 1 (Th1) cells, $CD8^+$ T cells, and natural killer (NK) cells (46)**.** Upon IFN-γ stimulation, macrophages produce inducible nitric oxide synthase (iNOS), which converts L-arginine to nitric oxide (NO), a critical effector molecule for killing of intracellular amastigotes (46). iNOS production is dependent on NF-κB transcription, and is important in facilitating the clearance of parasites. Gregory et al. reported that *Leishmania* parasites inhibit the production of NO by suppressing the production and/or activation of iNOS

via *Leishmania* protease (gp63)-mediated cleavage of p65 subunit of NF- κB (47). Also, *Leishmania* parasites inhibit NO production by increasing the expression of arginase which competitively cleaves L-arginine into ornithine (48). Ornithine favors the proliferation of *Leishmania* parasites in macrophages (49). Indeed, Wei et al. showed that the normally resistant (C57BL/6) mice that lost the capacity to synthesize iNOS became susceptible to *L. major* infection, although they maintained a strong IFN-γ type response (50). An iNOS inhibitor, N(G)-monomethyl-L-arginine, suppressed IFN-γ mediated parasite clearance in both mice and human macrophages in vitro (51). Furthermore, the administration of another iNOS inhibitor, L-N6-iminoethyl-lysine, in C57BL/6 mice, which healed their primary *L. major* infection, resulted in disease recrudescence and a 10-fold increase in parasite burden at both the draining lymph node and cutaneous site of infection. This observation suggests that continuous iNOS expression is important for primary resistance to *L. major* infection, as well as for the maintenance of infection-induced immunity in healed mice (52).

2.1.3. Monocytes

Monocytes are also recruited to the site of *Leishmania* infection via chemokines (such as MIP-1β and CCL3), cytokines (such as Interleukin 8 and Tumor necrosis factor- alpha), and complement proteins, notably C5a, the breakdown product of C5 (53). Romano et al. reported that monocytes serve as the main cell types where parasite replication occurs in vivo (54). They further reported that early on during primary *L. major* infection, Major histocompatibility complex (MHC) II maturation of inflammatory monocytes is disrupted, unlike in secondary sites of infection. They showed that monocytes from secondary sites of infection expressed significantly higher CD86 than those from primary sites of infection. This suggests that infected monocytes at secondary sites of infection undergo extensive maturation compared to those at the primary sites of infection. This finding suggests that the in vitro infection of monocytes (as opposed to the conventional macrophage models) may be a more suitable model to study the role of *Leishmania* parasites as modulators of phagocyte maturation. This is because, in conventional macrophage models, the ability of parasites to suppress the maturation of phagocytes are assessed in already mature cells (macrophages) (54). Furthermore, Romano et al. showed that following secondary *L. major* challenge, $CCR2^+$ monocytes, which respond to CXCL10 (a chemokine produced by inflammatory cells like neutrophils in response to IFN-γ), are the major source of $iNOS^+$ cells and are essential to the clearance of intracellular *L. major* parasites. The authors also suggested that neutrophils rely on $CCR2^+$ monocytes for parasite clearance, as they observed that neutrophils from CCR2 depleted mice (mice which lacked monocytes) as opposed to those from CCR2 competent mice showed no evidence of leishmanicidal activity, but instead served as a safe haven for the parasites in *L. major*-infected CCR2-depleted mice.

2.1.4. Dendritic Cells

Dendritic cells (DCs) are also infected by *Leishmania* and are known to be critical for antigen presentation and induction and differentiation of naïve $CD4^+$ T cells into effector Th1 cells (55,56,57,58,59). These effector Th1 cells are necessary for resistance to *Leishmania* infection (60,61,62,63). As *Leishmania* infection progresses, DCs serve as major producers of IL-12, which is needed to prime naïve $CD4^+$ T cells to differentiate into protective Th1 cells (55,57,58,59,60,64). DCs also express costimulatory molecules such as CD40, CD80, and CD86 (65).. These costimulatory molecules have been reported to contribute to host immunity to leishmaniasis. Okwor et al. showed that antibody-mediated blockade of CD40-CD40L interaction in *L. major*-infected mice resulted in decreased production of IL-12 and IFN-γ and increased parasite burden (66). Also, Kamanaka et al. reported that CD4- deficient C57BL/6 mice were more susceptible to *L. major* infection compared to their WT counterparts. They observed that CD40 deficiency in *L. major*-infected C57BL/6 mice tilted the Th1/Th2 balance in the favor of pro-*Leishmania* Th2 as opposed to anti-*Leishmania* Th1. This was accompanied by decreased IL-12p40 and IFN-γ response following infection (67). In the same study, it was shown that deficiency of CD40 ligand (CD40L, normally expressed on T cell) in *L. major*-infected C57BL/6 mice resulted in a decreased Th1 immune response against *L. major* infection, leading to the development of ulcerating lesions. This increased disease severity in CD40L deficient mice was associated with inability of their Th1 cells to induce IL-12 production in macrophages. The administration of recombinant CD40L to infected CD40L deficient mice resulted in decreased lesion size and parasite burden (67). Hathcock et al. reported that Th2 response in BALB/c mice is dependent on CD86 expression (68). Interestingly, they also showed that the early Th1 immune response that confers protection to *L. major* in C57BL/6 relies on CD86 signaling, suggesting that the role of CD86 in leishmaniasis may be dependent on the genetic background of the mice (68).

2.1.5. Natural Killer (NK) Cells

Another group of innate immune cells that play a role in immunity to leishmaniasis are NK cells (69,70,71). Mouse infection studies showed that early (seven days post infection) depletion of NK cells decreased IFN-γ production and led to significant increase in parasite burden (72). It was also reported that HeN mice were resistant to *L. major* infection, and this was attributed to NK cell-mediated early IFN-γ production (73). In contrast, the enhanced susceptibility to leishmaniasis observed in BALB/c mice was associated with diminished NK cell activity (69). Collectively, these studies suggest that NK cells contribute to host protection during *Leishmania* infection.

2.2. The Adaptive Immune Response in Leishmaniasis

The adaptive immune system is composed of T and B cells that mediate cell-mediated and humoral immunity, respectively. Unlike innate immunity, adaptive immunity is specific, highly specialized, and possesses immunologic memory. T cells are the major components of the host immune system that are responsible for cell-mediated immunity. Cell-mediated

immunity has been shown to play a critical protective role against *Leishmania* parasites. The protective role of T cells in leishmaniasis is well established, and this was evidenced by the observation that mice that are ordinarily resistant to *Leishmania* infection became highly susceptible when their T cell response was disrupted. Furthermore, the adoptive transfer of functional T cells to these susceptible mice (with a disrupted T cell response) restored resistance to *Leishmania* infection (74).

2.2.1. $CD4^+$ T Cells

Various subsets of $CD4^+$ T cells play different roles in leishmaniasis. IFN-γ-producing $CD4^+$ Th1 cells are the major cells involved in parasite control in resistant mice. IFN-γ, which is generally regarded as the 'signature' Th1 cytokine, activates macrophages into an M1 phenotype, resulting in nitric oxide production and enhanced elimination of parasites resident in these macrophages (60,61,62,63). The C57BL/6 mice are resistant to *L. major* because they mount a strong Th1 immune response against *Leishmania* parasites. In contrast, BALB/c mice are highly susceptible due to their relatively weaker Th1 but stronger Th2 responses (75). Th2 cells contribute to susceptibility of BALB/c mice to leishmaniasis by producing cytokines (such as IL-4 and IL-10) that deactivate infected macrophages and suppress the iNOS pathway, which is important for eliminating intracellular *Leishmania* parasites (75). Interestingly, the immunosuppressive effect of Th2 cells in BALB/c mice was reversed following depletion of neutrophils, and this was shown to be related to the downregulation of primary IL-4 response, thereby conferring more protection to the mice (76).

The role T-helper 17 (Th17) cells, play in immunity to *Leishmania* infection depends on the parasite species. This is because some studies have shown that Th17 cells promote susceptibility to *L. major* infection (77), but contribute to resistance to *L. infantum* (78), and *L. donovani* (79), infections. Gonzalez-Lombana et al. reported that Th17 cells enhanced host susceptibility to *L. major* infection by promoting excessive immune response (77). On the other hand, Nascimento et al. identified IL-17A as a critical host molecule in immunity against *L. infantum*. In this study, IL-17R deficient C57BL/6 mice displayed increased susceptibility to *L. infantum* infection, as evidenced by higher parasite burden when compared to their WT counterpart mice. The increased parasite burden in IL-17R deficient C57BL/6 mice was attributed to the significant increase in IL-10-secreting regulatory T cells, coupled with a reduction in the number of $CD4^+$ Th1 cells (78). In another study, Th17 cells were also reported to be associated with resistance to *L. donovani* infection in mice due to their ability to induce chemokine secretion, which attracts neutrophils and Th1 cells to the infected sites (79).

Naturally occurring regulatory T cells (Tregs) are also found at the infected sites in cutaneous leishmaniasis, where they limit the function of effector $CD4^+$ T cells through both IL-10 independent or dependent mechanisms, thus promoting long-term parasite persistence (80). This parasite persistence is beneficial to the host because it enhances the establishment of durable immunity against the parasite (81). This model also illustrates the

importance of a host-parasite equilibrium that appears to benefit both the host and the parasite (82). Persistence ensures the maintenance of effector memory cells that mediate infection-induced immunity in the host, as well a stable source or reservoir of parasites for transmission from one host to the other by the sand fly. In line with this, the development of lesions in mice infected with *L. major* has been associated with a high number of IL-10-secreting Tregs present at the infected site (83).As well, the adoptive transfer of Tregs isolated from acutely infected mice to chronically infected mice was shown to lead to disease recurrence and the disruption of effector memory T cell response (84).

2.2.2. $CD8^+$ T Cells

Although $CD4^+$ T cells are the key cells responsible for anti-*Leishmania* immunity, $CD8^+$ T cells that are specific to *Leishmania* antigens have also been identified (85). The role of $CD8^+$ T cells in regulating host immune response in leishmaniasis is controversial. In one study, mice deficient in $CD8^+$ T cells or class I MHC (which is necessary for $CD8^+$ T cell development and activation) were able to effectively control primary *L. major* infection (86). However, another study demonstrated that $CD8^+$ T cells are crucial for resistance during low-dose parasite infection and for mounting an effective secondary immune response (87). Uzonna et al. studied the mechanism of $CD8^+$ T cell-mediated protection during low-dose infection (88). Their study demonstrated that a transient early Th2 response was induced in C57BL/6 mice following low-dose *L. major* infection. However, this transient Th2 response was suppressed by IFN-γ produced by $CD8^+$ T cells, leading to sustained Th1 response and the development of protective immunity (88). In line with this, infection of $CD8^+$ T cell-deficient C57BL/6 mice with low parasite dose resulted in persistent Th2 response and susceptibility, evidenced by increased cutaneous lesion and high parasite burden (88). Okwor et al. also showed that although $CD8^+$ T cells are selectively activated and contribute to optimal primary immunity after low-dose infection, they are dispensable during secondary immunity (89).

Whereas the role of $CD8^+$ T cells in primary immunity is equivocal, their role in vaccine-induced immunity is unequivocal. Mendez et al. showed that $CD8^+$ T cells are important for vaccine-induced immunity in *L. major* infection (90). In this study, they reported that depletion of $CD8^+$ T cells in vivo before vaccination with DNA expressing LACK (*Leishmania* homologue of receptors for activated C kinase), TSA (*L. major* recombinant protein homologue to eukaryotic thiol-specific-antioxidant protein), and LmSTI1 (*L. major* recombinant protein homologue to eukaryotic stress-inducible protein) compromised the ability of the vaccine to confer full protection following *L. major* challenge (90). Although there was an observed delay in lesion development in vaccinated C57BL/6 mice treated with anti-CD8 antibody, the lesions progressed to a size comparable to unvaccinated controls (90). In addition, $CD8^+$ T cells were also shown to be important in vaccine studies with BALB/c mice. Gurunathan et al. reported that LACK DNA vaccination in conjunction with $CD8^+$ T cell depletion in BALB/c mice failed to confer protection to the vaccinated BALB/c mice following *L. major* challenge (91). When

LACK DNA vaccinated mice were treated with anti-CD8, the frequency of IFN-γ producing $CD4^+$ T cells were diminished both at 2 and 12 weeks post vaccination, suggesting that $CD8^+$ T cells are critical for maintaining Th1 immune response in vaccinated mice (91). Collectively, these observations indicate that $CD8^+$ T cells play a critical role in vaccine-induced immunity in leishmaniasis.

2.2.3. B Cells

Generally, B cells are not regarded as major contributors to protective immunity against leishmaniasis. This is because *Leishmania* are intracellular parasites and reside in the vacuole of macrophages, and as such, may not be easily accessed by antibodies (92). To demonstrate that B cells are not protective in leishmaniasis, BALB/c mice that were sub lethally irradiated became resistant to leishmaniasis when the donor cells were $CD4^+$ T cells but not when B cells were transferred (93). Furthermore, the transfer of serum from healed mice did not confer protection (94). However, there is some evidence to suggest that B cells may play a role in protective immune response to *Leishmania* infection. Woelbing et al. reported that IgG antibodies secreted by activated B cells in *L. major*-infected C57BL/6 mice increased antigen uptake capacity by dendritic cells via Fc gamma 3 receptor (FcγR III) (95). This increased antigen uptake was associated with increased antigen presentation by dendritic cells, which resulted in an increased Th1 response (95). In line with this, *L. major*-infected B cell deficient C57BL/6 mice displayed significant reduction in IFN-γ secretion and increased lesion and parasite burden, and this was reversed upon infection with IgG-opsonized parasites (95).

Immunity to Pathogens and Tumors

Cesar Terrazas, ... Bradford S. McGwire, in Encyclopedia of Immunobiology, 2016

Intracellular Survival and Growth of Leishmania in Macrophages

Leishmania parasites primarily reside within macrophages. Although macrophages are an important niche for parasite growth and replication, they are also critical for parasite elimination. Leishmania parasites infect macrophages in two ways: either directly based on interactions between receptors on the surfaces of the parasite and the macrophage or indirectly when macrophages engulf apoptotic infected neutrophils. Inside the macrophage, Leishmania is shuttled into the endosome which then develops into a phagolysosome which harbors an acidic environment. While the promastigote form is typically destroyed by the acidic environment, the amastigote form survives. Leishmania molecules such as LPG delay the maturation of the phagolysosome providing the parasites a longer time frame to successfully differentiate from promastigotes into amastigotes (88). In order to survive, Leishmania parasites also subvert the microbicidal activation of the macrophage. The parasites secrete proteins which target the in.

Leishmaniasis and drug resistance

Leishmaniasis, one of the most dreaded parasitic diseases, continues to rely on chemotherapy in the absence of effective vaccines and efficient vector control measures. Conversely, the lack of effective and non-toxic drugs; variation in efficacy as result of intrinsic variation in drug sensitivity; and the emergence of drug resistance limits the arsenal of anti-leishmanial drugs (96). Miltefosine, the first oral treatment of human visceral leishmaniasis, has shown remarkable activity and offers great promise for the treatment of parasitic infections including those caused by *Leishmania sp.* non-responsive to antimony (97). However, limited reports are available on the sensitivity of miltefosine towards drug-resistant *Leishmania* (98).

Differential sensitivity of a drug results from the differences in net drug accumulation regulated by influx and/or efflux pathways. P-glycoproteins (Pgps) and multiple drug resistance (MDR) have been implicated in miltefosine resistance among different mammalian cell lines (7). Conversely, Pgp overexpressing cell lines displaying MDR have also been reported to be sensitive to miltefosine or other lysophospholipid analogues (5,12). In *Leishmania*, daunomycin-resistant *L. tropica* cells overexpressing a Pgp-like transporter have been found to be cross-resistant to miltefosine (99). However, an *in vitro* generated miltefosine-resistant strain of *L. donovani* was observed to have defective inward translocation of drug involving a P-type lipid translocase without any participation of Pgp-like proteins (96,99). Thus, there is ambiguity in the role played by Pgp-like proteins in miltefosine resistance and the sensitivity of miltefosine among MDR/Pgp overexpressing *Leishmania* cell lines. An arsenite-resistant *L. donovani* (*Ld-As20*) strain generated in our laboratory has been reported to overexpress Pgp-like protein and display MDR-like phenotypes (100). In an attempt to address these issues, we have used this strain.

Apoptosis involves series of morphological and biochemical changes in which mitochondria serves as a major regulator of apoptosis (13). Change in lipid content on oxidative damage leads to change in the permeabilization of mitochondrial membrane and release of proapoptotic proteins into the cytosol including cytochrome *C* (13). Relocation of cytochrome *C* serves as the irreversible commitment to death by activating cellular proteases. Different signaling pathways converge at this stage, leading to cleavage of multiple downstream substrates. Miltefosine has been found to induce apoptosis-like death in wild type *L. donovani* (101), although its mode of action on drug-resistant strains of *Leishmania* and pathways and the proteins implicated in apoptosis-like death process is not known. In the present study, we investigated the sensitivity of the arsenite-resistant *L. donovani* (*Ld-As20*) to miltefosine, and its cytotoxic effects, followed by death process.

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