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Modulation of Dendritic Cell Function by Leishmania Parasites

Lynn Soong

The interactions between Leishmania parasites and dendritic cells (DCs) are complex and involve paradoxical functions that can stimulate or halt T cell responses, leading to the control of infection or progression of disease. The magnitude and profile of DC activation vary greatly, depending upon the Leishmania species/stains, developmental stages, DC subsets, serum opsonization, and exogenous DC stimuli involved in the study. In general, the uptake of Leishmania parasites alone can trigger relatively weak and transient DC activation; however, the intracellular parasites (amastigotes) are capable of down-modulating LPS/IFN-γ-stimulated DC activation via multiple mechanisms. This review will highlight current data regarding the initial interaction of DC subsets with invading parasites, the alterations of DC signaling pathways and function by amastigotes, and the impact of DC functions on protective immunity and disease pathogenesis. Available information provides insight into the mechanisms by which DCs discriminate between the types of pathogens and regulate appropriate immune responses. The Journal of Immunology, 2008, 180: 4355–4360.

Leishmaniasis is a vector-transmitted disease distributed throughout the world’s tropical and subtropical regions. At least 20 Leishmania species can give rise to a wide spectrum of clinical manifestations, ranging from self-healing skin ulcers to disfiguring mucosal lesions and fatal visceral infections. Although the determinants of parasite tissue tropism remain unclear, the diverse clinical forms are believed to be attributable to the host immune status and the species of parasite involved. Leishmania parasites have a dimorphic life cycle. Initially, the infectious promastigote form of the parasite is transmitted to the mammalian host by the bite of sand flies. Once phagocytosed by cells in the macrophage (Mφ) lineage, the promastigotes differentiate into a nonmotile amastigote form that replicates within the acidified parasitophorous vacuoles. The promastigotes interact with mammalian hosts very briefly without causing clinical manifestations, whereas amastigotes persist in their hosts for years or for a lifetime and are hence responsible for clinical diseases. Those working in this field have focused on gaining a better understanding of the processes by which the promastigotes establish the infection and the amastigotes modulate or take advantage of their host’s immune system. However, investigation into the molecular details of the means by which the host cell interacts with amastigotes has been partially hampered by the limited availability of amastigotes for some Leishmania species. Although axenically cultured amastigotes are readily available for some species (e.g., Leishmania amazonensis and Leishmania mexicana), other amastigote species need to be freshly prepared from mouse/hamster lesions or generated from cultured promastigotes (via reduction of culture pH and increase of temperature).

Another challenge in defining the molecular details of the host-Leishmania interaction is the complex regulation of species- and stage-specific genes. The Leishmania genome is spread over 34–36 chromosome pairs containing ~8,160 genes. Global analyses of gene expression indicate that the vast majority (>95%) of genes are constitutively expressed in all life stages and that for the few dozen of the amastigote-specific genes identified, they are relatively specific for a given Leishmania species. Therefore, information learned from one Leishmania species may not be applicable to other species. For example, there is a great need for comparative studies of Leishmania major and Leishmania donovani (etiologic agents of the Old World cutaneous and visceral leishmaniasis, respectively), as well as of L. amazonensis and Leishmania braziliensis (etiologic agents of diffuse cutaneous and mucosal leishmaniasis in South America, respectively). In this regard, animal studies have clearly indicated that L. major can cause a Th2-mediated, lethal infection in BALB/c mice but a Th1-mediated, self-healing infection in C57BL/6 and C3H mice, whereas L. amazonensis often causes progressive, nonhealing lesions in all of these mouse strains.

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due to an IL-4/IL-10-independent impairment in innate and acquired immunity (4, 5). This review will highlight available evidence at the DC level that may explain the diverse outcomes of *Leishmania* infection.

**Mφs** are the main host cells for *Leishmania* replication and the effector cell for parasite killing. *Leishmania* infection does not trigger inflammatory Mφs to produce IL-12 (6), and parasite-carrying Mφs are incompetent in priming naïve CD4+ T cells or stimulating Ag-specific CD4+ T cells (7). The molecular details have been reviewed elsewhere with respect to how promastigotes and amastigotes bind to Mφ surface receptors and interfere with their effector functions (8, 9). The recent advancements in the biology of DC subsets, as well as in the reagents used, have greatly enhanced our understanding of DC-*Leishmania* interactions, and several reviews have summarized the critical role of myeloid DCs (mDCs) in generating protective immunity against *L. major* infection (10–12). New data also call for re-evaluation of the accepted paradigm in *L. major* infection (13, 14). This review will focus on recent data related to the initial interactions of DC subsets with promastigote infection in vivo, alterations of DC signaling pathways by amastigotes, the impact of these alterations on disease outcomes, and the potential of DC-based vaccines in animal models of cutaneous leishmaniasis. Emphasis will be placed on DC responses to the species of amastigotes in the *L. mexicana* complex, especially to *L. amazonensis* because of its association with profound T cell suppression in patients with diffuse cutaneous leishmaniasis (15, 16) and its ability to cause nonhealing skin lesions in several inbred mouse strains that are genetically resistant to *L. major* parasites.

**The interaction of DC subsets with *Leishmania* promastigotes in vivo**

Because leishmaniasis is initiated by promastigote infection in the skin, extensive studies have been conducted in mouse models of *L. major* infection to define the early source of IL-12 and the role of different DC subsets in infection. Most of the reported studies, however, have used supraphysiologic doses of promastigotes, which is known to modulate DC maturation/activation (17) and local immune responses (18). Following skin injection of *L. major* promastigotes, polymorphonuclear neutrophils (PMNs) are recruited within hours to the site of infection and constitute the majority of the cellular infiltrates in the first day of infection. Parasite infection in PMNs may trigger the expression of TLR2, 7, and 9, as well as the production of some IL-12. Although pDCs do not endocytose promastigotes, they can be activated via released *Leishmania* genomic DNA (gDNA) to produce appreciable amounts of IFN-α and some IL-12 in a TLR9-dependent manner. mDCs and monocyte-derived DCs can efficiently engulf promastigotes, serving as a critical source of early IL-12 production. Activated DCs can migrate to draining LNs, activate resting NK cells via cell contact-dependent manner, and trigger NK cells to produce IFN-γ. The cognate interactions among multiple innate cell types and cytokines at the site of infection and in draining LNs are required for appropriate activation of protective Th1 responses against the invading pathogens. However, the magnitude of DC and Th1 cell activation in vivo varies greatly, depending upon the species of parasites involved.

**For some time the nature and specific functions of migratory DCs in cutaneous leishmaniasis have been matters of controversy.** Although both dermal DC immigrants (MHC IIhigh, CD11c+, CD11b+, CD86+, and CD205low) and epidermal Langerhans cells (LCs) (MHC IIhigh, CD11c+, CD11b+, CD8α+, and CD205high, and Langerin+) home to the draining lymph nodes (LNs), they may have different capacities in engulfing promastigotes and distinct functions in experimental leishmaniasis (13, 26). In *L. major* infection, parasite Ags are believed to be transported from the site of infection to draining LNs by CD11c+CD8α–Langerin– dermal DCs rather than by LCs (27) because LCs are less efficient in the uptake of promastigotes. These recent data challenge our previous view on LCs (28) and support the hypothesis that dermal DCs carry parasite Ag to activate Th1 effector cells, whereas epidermal LCs take up free Ag released from parasites or damaged host cells to activate other T cell subsets (13). More recent data from *L. major*-infected tissues and adoptive transfer of purified monocytes have provided new evidence, indicating the importance of the kinetics of cellular recruitment during infection and the de novo differentiation of monocytes to DCs, as well as the role of this DC subset in inducing parasite-specific Th1 responses in the draining LNs (14). Collectively, these in vivo studies suggest the dynamics of the DC network during infection-induced inflammatory reactions and the importance of fully activated mDCs (CD86high, CD40high, CCR7+, and IL-12+) in TLR-9-mediated activation of NK cells and in the generation of protective Th1 responses against *Leishmania* parasites (Fig. 1). At present, there is still limited information on initial DC responses to other species of *Leishmania* at the site of infection and on the priming of pathogenic T cells.
transient expression of the IL-12p40 gene in DCs following infection with L. amazonensis promastigotes. Bone marrow-derived C57BL/6-DCs were infected with promastigotes (red lines) and amastigotes (blue lines) for 8 and 24 h, respectively. To examine the effect of parasitic infection on DC responsiveness to other stimuli, cells were infected with parasites for 6 h and then treated with IFN-γ (100 ng/ml) plus LPS (100 ng/ml) for an additional 2 or 18 h, respectively. LPS/IFN-γ-treated DCs are denoted by a dashed line. The levels of IL-12p40 mRNA were quantified by real-time RT-PCR (normalized to the β-actin gene) and are presented as relative expression fold in comparison to those of untreated DCs.

DC responsiveness to Leishmania promastigote infection in vitro

To define the means by which DCs respond to Leishmania parasites and Ag-specific CD4+ T cells are activated, extensive studies have been performed with bone marrow-derived mouse mDCs, skin-derived mouse LCs, or monocyte-derived human DCs. In vitro infection studies have collectively indicated that mDCs (but not LCs) can efficiently engulf promastigotes, and that infection with parasites in the absence of other stimuli can activate DCs to produce little IL-12p70 but a range of IL-12p40 and IL-10 (29–31). Nevertheless, IL-12p40 and IL-12p70 production by infected DCs can be markedly enhanced by the addition of exogenous stimuli (e.g., IL-1, IFN-γ, IFN-γ/LPS, CD40L, and anti-CD40) at the time of infection (29, 31, 32). Promastigote-infected, IL-12p40-producing mDCs have a CD11chighCD45RB+CD83−CD40+ phenotype (31). Although infection with L. donovani and L. major promastigotes can trigger the release of preformed IL-12p70 and the production of IL-12p40 (6, 33), the up-regulation of the IL-12p40 gene in L. amazonensis-infected mDCs appears to be relatively weak and transient (31) (L. Xin, K. Li, and L. Soong, submitted for publication). Although there was an ~16- and 1300-fold induction in IL-12p40 gene expression at 8 h of infection in the absence or presence of LPS/IFN-γ, respectively, the expression levels dropped dramatically by 24 h postinfection (Fig. 2). Depending on the species of the parasites involved, promastigote infection was able to marginally or partially reduce DC responsiveness to exogenous stimuli (29, 31) and to impair DC differentiation in vitro (34).

The responsiveness of mDCs to different Leishmania promastigotes can be roughly ranked in the order of L. major and L. braziliensis > L. donovani and Leishmania infantum > L. mexicana and L. amazonensis as judged by the expression levels of surface markers and cytokines and APC potential in priming naive CD4+T cells (31, 32, 35) (D. A. Vargas-Inchaustegui, L. Xin, and L. Soong, submitted for publication). It is well known that promastigote surface molecules, such as lipophosphoglycan (LPG), can suppress MΦ functions (36); however, LPG, but not the lipid-free LPG, can also activate murine mDCs and NK cells through TLR2 (37). Given the marked multiplicity in LPG glycan side chains (composition and position on the LPG core structure) (38), it will be important to examine whether diverse DC responses to promastigotes are solely due to LPG (32) or to other undefined pattern-recognition molecules. In comparison to other pathogens that have chemically defined ligands for multiple TLRs (39), TLR ligand involvement is less clear for Leishmania parasites. Although circumstantial evidence suggests the involvement of LPG as well as TLR2, 3, 4, 7, and 9, in Leishmania infection (20, 40–42), the lack of strong TLR ligands in Leishmania infection may partially explain the relatively weak DC activation induced by promastigote infection alone.

Down-modulation of DC functions by Leishmania amastigotes

The molecular details of how DCs deal with the disease-forming amastigotes are still convoluted. In contrast to promastigotes, Leishmania amastigotes are largely deficient in LPG and have several unique biological features (9). First, the avidity of Leishmania for the surface C-type lectin called DC-specific ICAM-3-grabbing nonintegrin (DC-SIGN or CD209) and DC-SIGN-related molecules varies greatly with the parasite species and maturation stage in question. For example, amastigotes of L. amazonensis, L. mexicana, L. donovani, but not L. major, can bind to DC-SIGN (43, 44). Because this binding is independent of LPG and does not induce DC maturation (44), the benefits of engaging to DC-SIGN in these DCs remain enigmatic (45). Second, lesion-derived amastigotes are commonly coated with host Abs. Whereas Ab opsonization promotes the uptake of L. major amastigotes by murine mDCs and skin-derived LCs (6, 46), amastigotes of L. amazonensis (47), L. mexicana (48), and L. braziliensis (D. A. Vargas-Inchaustegui, L. Xin, and L. Soong, submitted for publication) are highly efficient in infecting mDCs, even in the absence of any Abs. Nevertheless, Ab opsonization of amastigotes (and promastigotes) significantly enhances DC activation and the production of IL-10 (and IL-12p40, to some extent) (6, 47) via the FcyR-mediated pathway (49). Consequently, such DCs preferentially prime IL-10-producing CD4+ T cells and promote lesion progression in mice (47, 48). In contrast, other studies have shown that the uptake of Ab-opsonized L. major amastigotes by murine mDCs or lesion-derived L. donovani amastigotes by human mDCs activates DCs to produce IL-12 and to induce protective immunity (46, 50). In further contrast, uptake of opsonized L. major amastigotes by BALB/c mDCs preferentially stimulates the production of IL-12p40 and the formation of inhibitory IL-12p40 homodimers (51). The differences in DC cytokine profiles may partially explain the complex roles of Ab in leishmaniasis (12, 47, 52).

Although L. amazonensis amastigotes can infect >80% of CD11c+ mDCs in <30 min, they are especially poor at activating these cells (35, 48). Live L. amazonensis amastigotes also markedly alter DC responsiveness to exogenous stimuli (IFN-γ, LPS, and LPS/IFN-γ), and amastigote-infected DCs are poor APCs in priming naive CD4+ T cells in vitro and in vivo (35) (L. Xin, J. Li, and L. Soong, submitted for publication). Although IL-10 can be readily detected in amastigote-infected mDCs (30), current evidence suggests that the down-modulation of DC activation following amastigote infection is not due to endogenous IL-10 but rather to reduced phosphorylation and/or accelerated degradation of key molecules in the JAK/STAT, NF-κB, and IFN regulatory factor (IRF) pathways via parasite-derived proteinases and oligopeptidases (Fig. 3). This hypothesis is supported by several lines of evidence. First, heat inactivation (56°C for 30 min) or pretreatment of amastigotes with protease inhibitors averts host protein degradation,
permitting DC activation to some extent (30)(L. Xin, K. Li, and L. Soong, submitted for publication). Second, Leishmania parasites are known to contain a vast repertoire of proteolytic enzymes encoding at least 154 peptidases (aspartic, cysteine, metallo, serine, and threonine peptidases) (53), and amastigotes of L. amazonensis and L. mexicana are notorious for their high cysteine protease/oligopeptidase B activities in specialized structures called megasomes (54, 55). The cathepsin B-like cysteine proteinase is central to the parasites’ ability to modulate NF-κB signaling and to consequently inhibit IL-12 production by murine Mφs (56), and targeted depletion of its encoding gene greatly reduces parasite virulence (57). At present, it is unclear how parasite components travel through the parasitophorous vacuole membrane to interfere with host signaling pathways and whether such down-modulation is specific to activation-related pathways. Nevertheless, alterations in DC activation/migration may be a general phenomenon in Leishmania infection, especially during the chronic stages (58, 59).

DC-based immunotherapy in murine models of leishmaniasis

Examinations of the utility of DC vaccination as cellular immunotherapy for cancers have yielded some encouraging results and prompted similar studies in animal models of leishmaniasis. Although DC-based vaccination may lack a conspicuous application for parasitic diseases endemic in developing countries, it serves as a valuable tool for defining DC-targeted control methods. Using L. major infection as a model, Moll and colleagues have shown that a single i.v. injection of ex vivo Ag-pulsed LCs (60), mDCs (61), or pDCs (24) (5 × 10^4 ~ 5 × 10^5 cells per mouse) is sufficient to induce protective immunity against single or repeated parasite challenges in susceptible BALB/c mice. Interestingly, Ag-pulsed LCs and pDCs can mediate protection independent of additional DC stimuli (CpG, TNF-α, LPS, or CD40 ligation); however, overnight exposure to CpG motifs is required for Ag-loaded mDCs to induce specific Th1 responses (61), a phenomenon also observed for mDCs pulsed with L. infantum Ag (62). In addition, while a polarized Th1 cytokine profile is linked to protection in LC- and CpG/mDC-immunized mice, no strong correlation (or specific roles for IL-12 and IFN-α) is observed in the case of pDC-immunized mice (24). These findings indicate the functional diversity of DC subsets in the control of L. major infection and the importance of mounting adequate local immune responses to control the infection (63). However, direct lesional injection of L. amazonensis Ag-pulsed mDCs (10^6 cells per mouse) together with rIL-12 failed to promote healing in mice chronically infected with L. amazonensis (35). Therefore, simply providing in vitro-activated DCs or primed Th1 cells is not sufficient to ensure healing in L. amazonensis-infected hosts (35, 64). Additional treatments aimed at reducing intracellular parasite load and/or blocking a parasite’s enzymatic activities are needed to outwit the parasites and thereby stem their persistence.

The development of DC-based immunotherapy for the control of cutaneous leishmaniasis and other infectious diseases requires a better understanding of the following: 1) the differences in the homing of transferred DCs to draining LNs and into a particular T cell activation compartment (13); 2) the requirement for additional DC stimuli in vaccination protocols (63); and 3) the contribution of microenvironmental factors in DC functions during the early and late phases of infection (65). Given the unique features of DC-amastigote interaction, DC-based vaccination studies should be extended to those hosts having an established infection. Targeted delivery of leishmanial Ag to endogenous DCs (66) and the use of genetically engineered parasites that express the DC activator (67) are other attractive approaches.

Conclusions

Leishmania parasites, especially their intracellular forms, have evolved complex strategies to evade DC functions. While infection with promastigotes alone can induce dermal DCs and mDCs to mature, these activated DCs only transiently produce relatively low amounts of proinflammatory cytokines. In vitro studies have suggested that promastigote-infected DCs retain their responsiveness to exogenous DC stimuli, producing appreciable levels of IL-1, TNF-α, IL-12p70, and IL-10, depending upon the nature and combination of stimuli. Infection with Leishmania amastigotes, however, often fails to induce DC activation, presumably due to the general lack of LPG and the direct effect of amastigote proteinases/oligopeptidases in host cell signaling pathways. There is an increasing awareness of species- and strain-dependent differences in DC responsiveness to Leishmania parasites. Likewise, marked differences are evident in the biological roles of different DC subsets. The molecular basis of parasite-derived and DC-derived differences remains enigmatic, although DC-based studies have provided valuable information. The details are largely unexplored with regard to how different DC subsets interact with promastigotes during natural infection in vivo and with amastigotes, especially at the chronic stages of infection. The availability of molecule- engineered, traceable, and suicidal parasites (68, 69) would provide additional tools to advance our understanding of host-parasite interactions.

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