The Importance of CD11b$^+$ Dendritic Cells in CD4$^+$ T Cell Activation In Vivo: With Help from Interleukin 1

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Control of Leishmania Major Infection in a Mouse Model of Cutaneous Leishmaniasis A murine model of cutaneous leishmaniasis, using needle inoculation of *Leishmania major,* represents perhaps the most widely used system to study the contrasting effects of Th1 versus Th2 immunity in disease control. One of the intriguing aspects of this disease model is that certain strains of mice, such as BALB/c and SWR/J, fail to control infection leading to progressive lesion development and systemic infection, whereas the majority of other mouse strains control infection at the local site of inoculation. Most humans infected with *L. major* manifest with self-healing lesions limited to the skin. Thus, the susceptible strains of mice represent an abnormal situation seen in rare patients who succumb to systemic infection with the cutaneous *Leishmania* species. The susceptibility of BALB/c mice to *L. major* is a multigenic phenomenon (1) in which both the T cell and the non-T cell compartments are impaired (2). It is generally accepted that a predominant IL-4 response is associated with disease progression whereas an IL-12/IFN-γ–mediated Th1 response is associated with lesion resolution and control of parasite spread. However, recent evidence suggests that a transient Th2 response occurs in both resistant and susceptible strains of mice (3), but a sustained Th2 response is required for the development and progression of disease (for a review, see reference 4). Of importance in this regard are the abilities of the APCs in redirecting the early Th2 response toward a protective Th1 response. Two papers published in this issue by Filippi et al. (5) and by von Stebut and colleagues (6) address whether DCs and the factors secreted by these cells contribute to the susceptible versus resistant phenotypes after intradermal (i.d.) *L. major* challenge, and point to a selective role for the CD11b^{hi} subset of DCs in this process (5).

Identification of a Subset of Cutaneous DCs that Present Leishmania Antigens In Vivo. At the site of parasite inoculation, a number of distinct potential APCs are present including epidermal Langerhans cells, dermal DCs, and dermal macrophages. Within the epidermal layer are the Langerhans cells that are characterized by their unique intracellular organelles known as the Birbeck granules in which the Langerin molecule is expressed (7, 8). In response to stimulation, Langerhans cells emigrate in increased numbers from the epidermis to the draining LNs. This process is accompanied by the increase in their expression of CD40 and MHC class II molecules (9–13). Within the LNs, these Langerhans cells can be distinguished from other DC types as CD11b^{mod}/CD8α^{lo}/CD11c$^+$/CD205^{hi}/CD40^{hi}/MHC class II^{hi}/Langerin$^+$ cells. Beneath the basement membrane, a relatively understudied population of DCs known as dermal DCs is present. These cells express CD11c, CD11b (9–12, 14), and a variety of lectins including MMGL (15) and in humans, DC-SIGN (16–19). After migration into LNs, they increase in cell size and become CD11b^{hi}/CD8α$^-$/CD11c$^+$/CD205^{mod}/CD40^{hi}/MHC class II^{hi} (9–13). This population is likely distinct from the CD14$^+$ dermal Langerhans cell precursors that has been identified (20) as the dermal DCs are negative for CD14 and lack Birbeck granules (14). Upon invasion by microbial pathogens, however, the composition of DCs at the site of infection may change dramatically from that of the steady state. Assault by a pathogen triggers an inflammatory response, resulting in the release of various cytokines and chemokines. These inflammatory chemokines can mediate the recruitment of leukocytes including DCs to the site of infection, whereby enhancing the chances for DCs to capture pathogens. Blood-derived DCs that migrate into peripheral and lymphoid tissues express CD11b and low levels of MHC class II (9, 11, 13, 21, 22). Thus, upon infection with *L. major*, Langerhans cells, dermal DCs, dermal macrophages, and blood-derived DCs may all contribute to the presentation of parasite antigens to CD4$^+$ T cells in the draining LNs.

One of the key questions in this field has been, "which cell type initiates protective immunity to *L. major*"? Although dermal macrophages are the primary cells infected with *L. major*, these cells are unlikely to participate in the generation of CD4$^+$ T cell immunity for the following reasons: (a) dermal macrophages do not become activated as a result of infection with promastigotes or amastigotes (23–26), (b) their ability to secrete Th1-promoting cytokines is suppressed (23–26), and (c) infected macrophages do not migrate to the draining LNs where encounter with naive

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$CD4^+$ T cells can be initiated. In contrast, a role for Langerhans cells in the immune induction to *L. major* has been described. 4 d after intradermal injection of *L. major* promastigotes, $CD205^+$ DCs containing the parasites were found to appear within the draining LNs and present antigens to $CD4^+$ T cells (27). Although Langerhans cells express the highest levels of CD205 in the LNs, one cannot rule out the possibility that the $CD205^+$ cells in this study included the dermal DCs, as they can express moderate levels of CD205 in the LNs (9). Another support for the role of Langerhans cells in $CD4^+$ T cell priming comes from a study of CCR2 deficient mice (28). These mice have a defect in Langerhans cell migration in response to FITC painting, and also have a defective anti-parasitic response after i.d. injection of *L. major* promastigotes. These two events may or may not be related, however, as distribution and migration of other types of DCs are also abnormal in these mice (28, 29).

The role of dermal DCs in *L. major* antigen presentation has been unclear until now. In the study by Filippi et al. (5), by using a $CD4^+$ T cell hybridoma specific for the LACK peptide 158–163 bound to $I\text{-}A^d$, the unique capacity of the $CD11b^+$, but not $CD11b^-$, DCs in presenting the LACK antigen to $CD4^+$ T cells was demonstrated. Moreover, using naive $CD4^+$ LACK-specific TCR transgenic T cells, the $CD11b^+$ DCs isolated from 2d *L. major*–infected susceptible strains of mice were shown to induce Th2 differentiation while those from resistant strains induced Th1 differentiation ex vivo (5). These results revealed that there is an intrinsic difference in the ability of the $CD11b^+$ DCs to differentiate $CD4^+$ T cells depending on the strains of mice from which they were isolated, and that this difference correlates with the T cell phenotypes that develop following *L. major* infection in the respective mouse strains. Although the tissue origin of the antigen-presenting $CD11b^+$ DCs was not examined, these $CD11b^+$ DCs likely represent the dermal DCs, as they are $CD11b^+/CD8\alpha^-/CD11c^+/CD40^{hi}$/MHC class II^{hi} (5). To better identify these cells as dermal DCs, and rule out Langerhans cells, however, the expression levels for CD205, Langerin, and epithelial cell adhesion molecule (Ep-CAM)-1 need to be examined as Langerhans cells express high levels of these three markers (7–13, 30). Alternatively, these $CD11b^+$ DCs may represent blood monocyte-derived DCs that have captured *L. major* antigens in the dermis and migrated to the LNs. However, monocyte-derived DCs are $CD11c^{dim}$ (31) and would have been excluded from the $CD11c^+$ cells used by Filippi et al. (5). The importance of the $CD11b^+$ DCs in $CD4^+$ T cell priming was also demonstrated in a recent study by Itano and colleagues (32). By generating a recombinant protein consisting of a fusion between the peptide 45–75 from the I-E molecule with a red fluorescence protein, they were able to follow both the antigen itself (red fluorescence) and the presentation of the I-Ep45–75 peptide bound to $I\text{-}A^b$ (using the Y-Ae antibody that recognizes this complex). Using this clever system, the antigen presentation in the draining LNs was found to occur in two distinct waves. The first wave occurred within the first 4–8 h, which resulted in the proliferation of antigen-specific T cells in the draining LN. The second wave of antigen presentation occurred around 24 h, which induced T cell activation in the draining LNs. Interestingly, the second wave of antigen presentation was required for the generation of functional T cell immunity (as measured by DTH), and this response was mediated by the $CD11b^+$ DCs migrating from the dermis into the draining LNs. Thus, in the case of *L. major* infection, the time course of the $CD11b^+$ DCs found to present the LACK antigen to T cells in the draining LNs (48 h; reference 5) likely reflects the migration of antigen-loaded dermal DCs to the draining LN corresponding to the "second wave" of antigen presentation described by Itano et al. (32).

The immunological relevance of the dermal $CD11b^+$ DCs during infection with pathogenic microbes needs to be further examined. In this regard, the submucosal DCs, which are the mucosal equivalent of the dermal DCs that express similar markers, have been shown to play a dominant role in Th1 induction after genital infection with herpes simplex virus type 2 (HSV-2; reference 33). Within the vaginal mucosa, the replication of the virus occurred exclusively in the vaginal epithelium, beneath which the submucosal DCs were found to accumulate within the first 24 h. Subsequently, by 48 h, the $CD11b^+$ DCs that have captured viral antigens have migrated to the draining LNs and began presenting viral antigens to $CD4^+$ T cells (33). Surprisingly, Langerhans cells in the LNs did not present viral antigens to $CD4^+$ T cells after vaginal HSV-2 infection. At other portals of pathogen entry, the subepithelial $CD11b^+$ DCs may possess a unique capacity to mediate tissue-appropriate immune responses. For instance, in the small intestine, $CD11b^+$ DCs are found beneath the follicle-associated epithelial cells in the Peyer's patches (34, 35). In the absence of pathogens, these $CD11b^+$ DCs remain in the subepithelial dome regions and secrete high levels of IL-10 to promote Th2 differentiation ex vivo (35–37). Upon microbial stimulation in vivo, the $CD11b^+$ DCs up-regulate CCR7 and migrate toward the T cell regions (35), presumably to activate naive T cells. Taken together, data from Filippi et al. (5) and others (32, 33, 35) all point toward the importance of the $CD11b^+$ DCs in capturing antigen in the dermis or submucosa, migrating to the draining LNs and activating $CD4^+$ T cells in a productive fashion (Fig. 1). One obvious question that arises from these studies is "what do Langerhans cells do"? Recent evidence suggests that Langerhans cells continually migrate from the skin to the LNs in the absence of pathogen-induced maturation (38, 39). Whether these cells contribute to the immune initiation process for *Leishmania* and other pathogens needs to be further explored. Moreover, as these dermal/submucosal DCs express distinct set of pathogen-recognition molecules (15–19) compared with the Langerhans cells (7, 8), their role in the capture and presentation of bacterial, parasitic, and viral pathogens must be carefully examined in vivo.

What Really Happens during Natural Infection with L. major? Although the study by Filippi et al. provided the long awaited answer to the question, "what cells present *L. major* antigens to $CD4^+$ T cells in vivo"?, there are important questions that still remain with regards to what happens after transmission of *L. major* in humans. *Leishmania* spp. are transmitted through the bite of an infected sandfly vector, *Phlebotomus paratasi*, which inoculates a small number (100–1,000) of metacyclic promastigotes into the skin (40). The natural route of infection by the sandfly is accompanied by the inoculation of salivary secretion, which has been shown to exacerbate the lesion development at the site of infection (24). Further, compared with the widely-used subcutaneous inoculation of 10^5–10^7 promastigotes, the inoculation of the physiological number of promastigotes (100–1,000) into dermal sites induces a very different disease course in mice, which consists of a "silent" phase in which no T cell responses are generated for the first 4–5 wk while parasites replicate in the skin macrophages (41). This silent phase is followed by the development of a lesion associated with acute inflammation and reduction in the parasite number peaking around 6 wk after infection. Therefore, in keeping with the results from studies using 100–1,000 promastigotes (41), the immune induction in humans infected by sandflies may only begin after an incubation time, at the end of which amastigotes are release from rupturing dermal macrophages. Either the dying macrophages containing amastigotes or the amastigotes themselves can be ingested by the dermal DCs. This results in the infection, activation and migration of dermal DCs to the draining LNs where they can present the parasite antigens to $CD4^+$ T cells (Fig. 1). In the study by Filippi et al., presentation of the LACK antigen by the $CD11b^+$ DCs at 48 h after injection presumably occurred in the absence of productive infection of these DCs as *L. major* promastigotes have been shown not to infect skin resident DCs in vitro (23) and in vivo (41). Thus, it is likely that in their study (5), the dermal $CD11b^+$ DCs phagocytosed *L. major* promastigotes without becoming infected, migrated, and presented the LACK antigen to $CD4^+$ T cells in the draining LNs. Therefore, during a natural course of infection with a sandfly vector, the question of whether dermal DCs at the site of inoculation take up metacyclic promastigotes (early) and/or amastigotes released from the macrophages (late) and migrate to the draining LNs and activate $CD4^+$ T cell responses must be revisited using smaller inocula, with or without salivary secretion from the sandfly.

The Role of IL-1α and IL-1β in the Generation of Protective Immunity to Leishmania major. The two papers published in this issue both examined the possibility that the nature of the DCs contribute to the susceptible versus resistant phenotype. Remarkably, upon examination of the cytokines released from DCs either in vitro (6) or ex vivo (5), both groups ended up identifying the members of the IL-1 cytokine to be synthesized at higher levels in DCs from the resistant strains compared with those from the susceptible strains. The IL-1 system consists of two distinct ligands, IL-1α and IL-1β, both of which bind to the same receptor, IL-1R type I (IL-1RI). Both types of IL-1 are made as precursor cytokines, and require processing by the proteases calpain (for IL-1α) or caspase-1 (for IL-1β) to be cleaved

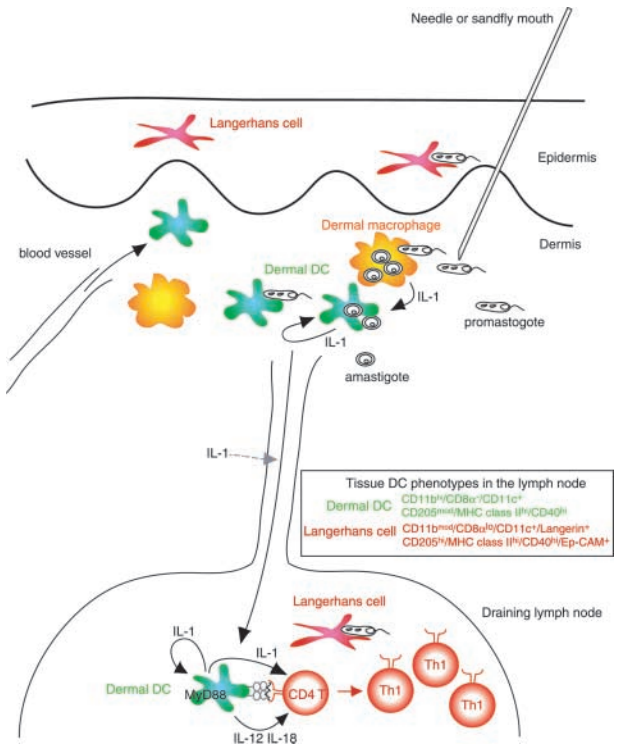

Figure 1. A proposed mechanism of $CD4^+$ T cell priming after *Leishmania major* infection. Infectious metacyclic promastigotes are introduced into the dermis of the host by the bite of a sandfly or through needle inoculation. The promastigotes are taken up by skin dermal macrophages, within which the parasite can replicate in the form of amastigotes. Amastigotes released from ruptured macrophages can infect nearby dermal DCs, which results in the activation of dermal DCs and release of factors such as IL-1 and IL-12. In the case of needle injection of a large number of promastigotes (10^5–10^7), the promastigotes can be taken up by the dermal DCs and processed for MHC class II presentation. Either the amastigote-infected dermal DCs (following sandfly bite) or promastigote-loaded dermal DCs (after needle injection of $>10^5$ parasite) migrate to the draining LNs and enter the T cell area to present parasite antigens to $CD4^+$ T cells in the context of MHC class II (reference 5). Langerhans cells infected with the parasite may also migrate to the draining LNs but whether they present parasite antigens and induce $CD4^+$ T cell activation needs to be further elucidated. IL-1 secreted from the DCs promotes Th1 differentiation of the antigen-specific T cells in several mutually nonexclusive ways: (a) IL-1 may facilitate the migration of dermal DCs from the site of infection to the draining LNs (reference 44), (b) IL-1 may induce activation of DCs through signaling of their IL-1RI/IL-1RAcP complex via the MyD88 pathway, and (c) IL-1 may directly activate $CD4^+$ T cells leading to their Th1 differentiation. Signaling of IL-1RI on DCs may lead to increase in the expression levels of costimulatory molecules and release of cytokines such as IL-12 and IL-18. Alternatively, a separate microbial stimulus present on Leishmania parasite may trigger the secretion of IL-12, which is enhanced by the concomitant IL-1 signaling. In the resistant strains, this IL-1 production from DCs is optimal, leading to the activation of $CD4^+$ T cells to secrete IFN-γ (references 5 and 6). In contrast, IL-1 secretion from DCs is reduced in the susceptible strains, leading to suboptimal DC activation and Th2 differentiation (references 5 and 6). Factors other than IL-1 likely also contribute to the intrinsic differences between the $CD11b^+$ DCs of the susceptible and the resistant strains, such as differences in the expression of costimulatory molecules (reference 5), other cytokines, chemokine/chemokine receptors, and perhaps TLRs.

into their active forms. The mature forms of IL-1α and IL-1β have multiple effects in vivo as revealed by the analysis of mice deficient for various components of the IL-1 system (for a review, see reference 42). Upon binding of either IL-1α or IL-1β to IL-1RI, the IL-1RI forms a heterodimeric receptor complex with the IL-1R accessory protein (IL-1RAcP) resulting in signal transduction. The cytoplasmic domains of the IL-1RI and IL-IRAcP contain the Toll-like receptor (TLR)/IL-1 (TIR) domain, which upon activation recruits the MyD88 adaptor protein, leading to further activation of a signaling cascade involving IRAK and TRAF-6.

It is intriguing that the two reports demonstrate preferentially enhanced production of IL-1α (6) or IL-1β (5) by the DCs from the resistant strains of mice. Each study found exclusive role for either IL-1α but not IL-1β (6) or vice versa (5). This discrepancy may relate to the differences in their experimental approaches. Specifically, Filippi et al. used ex vivo $CD11b^+$ DCs isolated from the draining LNs of 2 d *L. major* infected B10.D2 mice and found that IL-1β, but not IL-1α mRNA, was expressed at 10- to 15-fold higher levels compared with the $CD11b^+$ DCs of the infected BALB/c mice (5). On the other hand, von Stebut et al. used C57BL/6 fetal skin-derived DCs stimulated in vitro with amastigotes and demonstrated that they expressed 1.7-fold higher IL-1α mRNA and 3–4-fold higher IL-1α protein compared with those from BALB/c mice (6). The results from these two studies suggest that in the susceptible BALB/c mice, the ability of the DCs to secrete IL-1 is reduced (5, 6), resulting in the failure to generate Th1 responses to *L. major*. On the other hand, in resistant C57BL/6 and B10.D2 strains, the DCs secrete higher levels of IL-1, allowing efficient induction of Th1 responses in vivo. To provide support for this hypothesis, both groups tested the ability of IL-1 injection in the control of *L. major* infection in the susceptible host, and convincingly demonstrated that the recombinant IL-1α (6) or IL-1β (5) inoculated around the time of *L. major* challenge enhanced protective immunity. However, a definitive proof that the levels of IL-1 secreted from the DCs dictate the susceptibility of mouse strains is yet to be provided. Future studies must examine the definitive importance of IL-1 secreted by DCs in the generation of the protective immunity to *L. major* challenge by using conditional knockout mice that specifically lack the IL-1 genes in DCs, or by the adoptive transfer of $IL\text{-}1^{+/+}$ DCs into $IL\text{-}1^{-/-}$ host.

The in vivo importance of IL-1 in anti-*Leishmania* immunity has been demonstrated in a previous study in which IL-1RI–deficient mice infected with *L. major* promastigotes were found to harbor higher parasite burden and increased Th2 cytokine secretion in the draining LNs (43). The IL-1 secreted during *L. major* infection can have an effect on both the T cells and the APCs. The importance of IL-1 in DC migration (44), activation, and acquisition of Th1-inducing ability has been demonstrated (45). On the other hand, IL-1α has been shown to have a direct effect on T cells, serving as a cofactor for IL-12–induced Th1 differentiation for BALB/c but not C57BL/6 T cells (46). In the study by von Stebut et al., the timing at which the injection of recombinant IL-1α optimally enhanced protective immunity to *L. major* was determined to coincide with that of $CD4^+$ T cell priming in vivo (6). Taken together, these results lead to the hypothesis that IL-1α and IL-1β secreted by the DCs at the time of $CD4^+$ T cell priming in the draining LNs promote the generation of a protective Th1 immunity through activating DCs and/or T cells via the IL-1RI/MyD88-pathway (Fig. 1). Of interest in this regard is the recent demonstration that MyD88-deficient C57BL/6 mice failed to develop Th1 responses and control lesion after i.d. *L. major* inoculation (47). The immune responses generated following *L. major* infection in $MyD88^{-/-}$ and $IL\text{-}1RI^{-/-}$ mice share the heightened Th2 responses and parasite burden, but differ in that only the latter was able to control lesion size (43, 47). Thus, a MyD88-dependent pathway distinct from the IL-1RI must be involved in the full activation of protective immunity in the resistant mouse strains.

Future Vaccine Design and Implications. The current studies highlight the importance of the DCs in the establishment of protective Th1 immunity in the draining LNs (5, 6). Whether IL-1 secretion from the relevant DCs accounts for the resistance to *L. major* infection or not, the benefit of IL-1 injection at the time of parasite challenge was clearly demonstrated in both studies (5, 6). A number of vaccine strategies have been designed and tested in an effort to prevent disease in susceptible mice to high dose challenge with live *L. major*. Now that we understand the DC population responsible for presenting the parasite antigens and inducing protective Th1 immunity in the resistant strains, namely the $CD11b^+$ DCs, we can begin to design vaccine approaches that target these DCs in the dermis. As IL-12 was necessary for the injected IL-1α to mediate its protective effects in vivo (6), an optimal vaccine to *L. major* must ultimately result in DC activation and IL-12 secretion. The best-known agents that accomplish these ends are the TLR ligands. Further, a sustained IL-12 secreting environment with persistent antigen presence has been shown to be important in maintaining an effective immunity against *L. major* (48). Given these constraints, it is not surprising that plasmid DNA vaccines encoding *L. major* proteins are effective in providing long lived protection in susceptible mice (48–52), as bacterial DNA can both trigger TLR9 (DC activation) and provide a source of immunogen through its prolonged expression in vivo. Future vaccine strategies may include the use of bicistronic plasmid DNA vaccines in which both the *Leishmania* antigen and the mature forms of the IL-1α or IL-1β are expressed, or the injection of *L. major* antigens with recombinant IL-1 in conjunction with an adjuvant that activates the TLR pathway in the susceptible hosts.

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