

Dendritic cells in *Leishmania major* infections: mechanisms of parasite uptake, cell activation and evidence for physiological relevance

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Abstract Leishmaniasis is one of the most important infectious diseases worldwide; a vaccine is still not available. Infected dendritic cells (DC) are critical for the initiation of protective Th1 immunity against *Leishmania major*. Phagocytosis of *L. major* by DC leads to cell activation, IL-12 release and (cross-) presentation of *Leishmania* antigens by DC. Here, we review the role of Fcγ receptor- and B cell-mediated processes for parasite internalization by DC. In addition, the early events after parasite inoculation that consist of mast cell activation, parasite uptake by skin-resident macrophages (MΦ), followed by neutrophil and monocyte immigration and DC activation are described. All these events contribute significantly to antigen processing in infected DC and influence resulting T cell priming in vivo. A detailed understanding of the role of DC for the development of efficient anti-*Leishmania* immunity will aid the development of potent anti-parasite drugs and/or vaccines.

Keywords *Leishmania* · Skin immune system · Dendritic cells · Adaptive immunity

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Introduction

Cutaneous leishmaniasis as model disease

Infections with *Leishmania* spp. represent a serious health problem in large parts of the world. Leishmaniasis is endemic in 88 countries of southern Europe, Central and South America, Africa, the Middle East and the Indian subcontinent [1, 2]. Currently, 12 million people are suffering from leishmaniasis, and an estimated 1–1.5 million new cases every year and a death toll of around 70,000 annually are observed [3]. Due to these numbers, the development of new drugs or vaccines against leishmaniasis has recently received more attention. In addition to being a disabling and socioeconomically important disease, leishmaniasis has emerged as a model system of parasitic skin infections and studies in leishmaniasis have greatly improved our understanding of skin/parasite interactions.

Leishmaniasis is caused by protozoan parasites of the genus *Leishmania*. The disease is transmitted to the host via sand flies. Dermatotropic species of *Leishmania*—the main focus of this review—induce granulomatous skin reactions, while viscerotropic species induce hepatosplenomegaly.

The early events that take place in skin and lymph node (LN) after infection have been studied extensively. After inoculation of infectious stage metacyclic promastigotes into the upper dermis by the bite of a sand fly, *Leishmania* primarily locate to the phagolysosomes of skin-resident macrophages (MΦ) (Fig. 1) [2]. Here, the parasite transforms to obligate intracellular amastigotes and replicates. Release of free amastigotes into the tissue leads to infection of other phagocytes. Finally, the parasite's life cycle is complete upon re-infection of a sand fly during another blood meal.

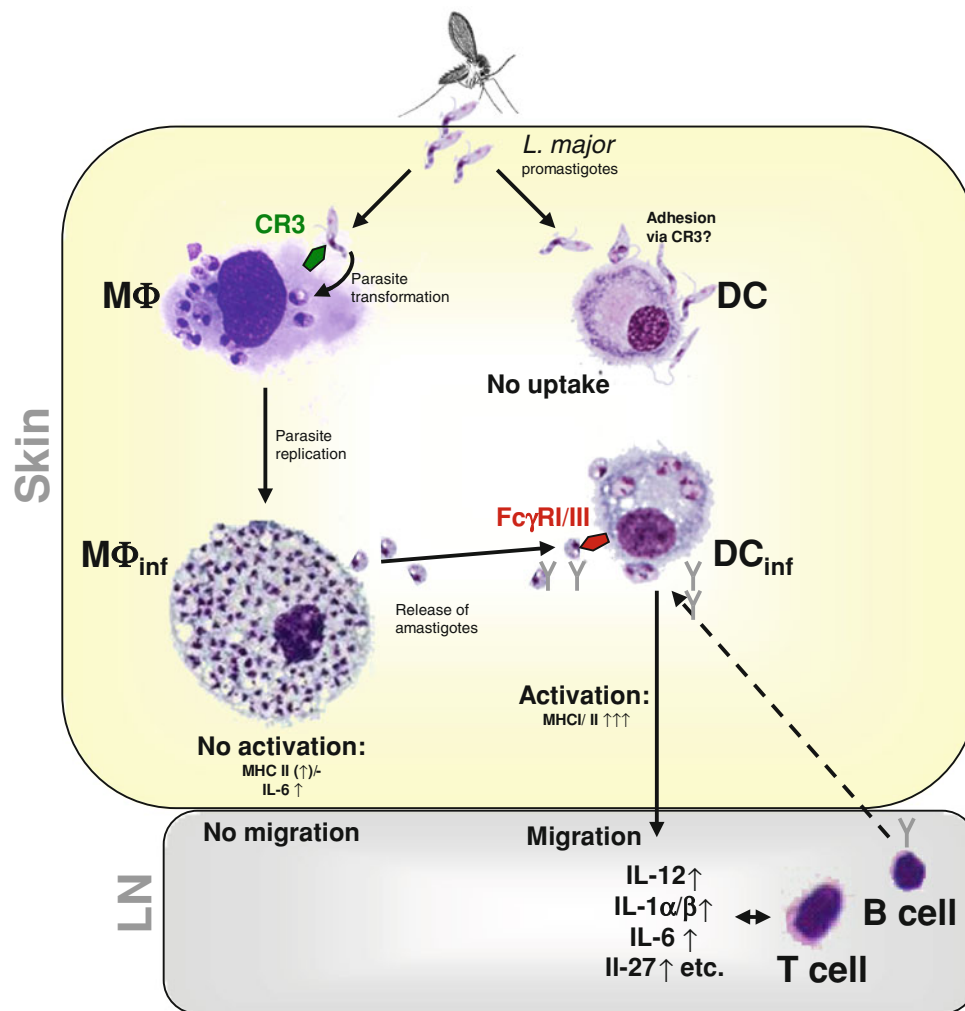


Fig. 1 Role of MΦ and DC in cutaneous leishmaniasis. The parasite *L. major* is inoculated into skin by the bite of a sand fly. The infectious stage metacyclic promastigotes adhere to MΦ via complement receptor (CR) 3 which mediates phagocytosis. Within MΦ, parasites transform into amastigotes and replicate, the infection remains 'silent'. Promastigote parasites adhere to surfaces of DC without entering the cell. Later, when free amastigotes are released from lysed MΦ, DC internalize this parasite life form via Fc γ RI or

Fc γ RIII requiring parasite opsonization with IgG produced by B cells. In contrast to MΦ, infection of DC with *L. major* leads to cell activation, upregulation of MHC class I and II as well as co-stimulatory molecules. Infected DC migrate to draining LN, where they present parasite antigens to both CD4⁺ and CD8⁺ T cells. In parallel, cytokine production by DC contributes to T helper cell (Th) differentiation towards protective Th1/Tc1-dependent immunity

Defence mechanisms against *Leishmania major*

After several weeks post-infection, an influx of inflammatory neutrophils (PMN), monocyte-derived MΦ and (later) also dendritic cells (DC) into skin lesions is observed, after which adaptive immune responses are elicited with priming and activation of antigen-specific T cells (Fig. 1) [4–6]. At this stage, clinically apparent skin lesions are observed. Finally, IFN γ -producing antigen-specific CD4⁺ Th1 and CD8⁺ Tc1 cells induce healing of the lesions by activating infected MΦ to eliminate the parasite via nitric oxide (NO).

Prior studies showed that infected DC are the critical APC responsible for T cell priming in *Leishmania* infections [7–10]. Uptake of *L. major* by DC results in activation and interleukin (IL)-12 release. Infected DC efficiently stimulate CD4⁺ and CD8⁺ T cells and vaccinate against leishmaniasis. In contrast, complement receptor (CR) 3-dependent phagocytosis of *L. major* by MΦ leads exclusively to MHC class II-restricted antigen presentation to primed, but not naive, T cells, and no IL-12 production [4, 11].

Due to the fact that an effective *Leishmania* vaccine does not exist and (skin) DC are critical regulators of the anti-*Leishmania* immune response, DC are attractive targets for

immuno-therapeutic approaches. Thus, it is essential to understand the precise role of DC in leishmaniasis.

Phagocytosis of *Leishmania* parasites by antigen-presenting cells

As described above, both M Φ and DC play important roles in the defence mechanisms against *Leishmania* infections. Even though both cell types are antigen-presenting cells (APC) and of myeloid lineage, *L. major* uptake by these cells leads to major differences in the functional consequences. Additionally, we documented strong differences in parasite internalization [12]: (1) Skin DC preferentially take up *L. major* amastigotes, the obligate intracellular life form of the parasite, rather than promastigotes transmitted by sand flies, whereas M Φ efficiently phagocytose both life forms [7, 13, 14], (2) the phagocytotic capacity of DCs is limited as compared with that of M Φ [7], (3) *L. major*-infected DC, unlike infected M Φ , release IL-12 and efficiently induce Th1/Tc1 differentiation of naive cells [7, 8, 15, 16], and (4) although both cell types present *Leishmania* antigen via the MHC class II pathway, only DC prime and restimulate *L. major*-specific CD8⁺ T cells [17]. Prior studies showed that M Φ internalize *L. major* parasites via CR3 [18] and that CR3 signalling in M Φ is responsible for their inability to release IL-12 after infection with the parasites [18].

Parasite recognition by DC

Receptors involved in DC phagocytosis

Because of the differences in cellular behaviour of M Φ and DC after infection, we speculated that differences in the receptors utilized for parasite internalization exist. Confirmingly, we showed that parasite uptake by DC was independent of CR3. To this aim, DC deficient in CD18—not expressing CR3 or CR4—were used for in vitro infections. Here, no difference in the percentage of infected DC and in the number of parasite per cell was observed [12]. However, a previous report suggested that uptake of *L. major* amastigotes by cell suspensions containing 30 % primary Langerhans cells (LC) was decreased in the presence of anti-CR3 [14]. Interestingly, even though we were unable to demonstrate an effect of CR3/4 involvement in parasite uptake by DC, we consistently observed tight adherence of even serum-opsonized promastigote life forms to the surface of DC after 18 h co-cultures (Fig. 1). Thus, it is possible that the initial steps leading to parasite/DC contact are mediated by complement-associated mechanisms. In line, in other inflammatory processes, co-engagement of CR3 with other receptors such as Fc γ R has been described [19].

We also assessed the involvement of other candidate phagocytosis receptors, for example, the mannose receptor or CD205 [12]. None of the tested inhibitors affected the uptake of *L. major* by DC. Thus, similar to CR3 and CR4, C-type lectins appeared to be dispensable for phagocytosis of the parasites by DCs.

As described above, DC preferentially internalize *L. major* amastigotes as compared to promastigotes. Experimentally, amastigotes need to be prepared from lesional tissue, whereas infectious stage metacyclic promastigotes can be isolated and enriched from parasite cultures. While assessing potential differences between these life forms, we studied the isotypes of parasite-specific antibodies that are bound to parasite surfaces. Using flow cytometry, we determined that complement-opsonized parasite preparations displayed membrane-bound natural IgM (found on both promastigotes and amastigotes) [20]. Thus, IgM was not involved in parasite uptake by DC. However, only amastigotes additionally displayed IgG (IgG1 as well as IgG2a/b) on their surface. This suggested that immunoglobulins are involved in *L. major* internalization by DC. To confirm this, we next used amastigotes isolated from B cell-deficient JHT or μ MT mice. These parasites are devoid of IgG on their surface and were not phagocytosed by DC in vitro, and, in line, in subsequent experiments, promastigotes opsonized with antibodies derived from serum of immunized mice were taken up rapidly [12]. Finally, we determined that parasite uptake was dramatically decreased in vitro and in vivo only if DC from Fc γ R^{-/-} or Fc γ RI \times Fc γ RIII^{-/-} were used. In summary, we identified Fc γ RI and Fc γ RIII as important mediators for DC infection in leishmaniasis. Interestingly, both receptors were capable of efficiently compensating for each other.

Disease outcome in B cell- or Fc γ receptor-deficient mice

In vivo, DC infiltration of *L. major*-infected skin lesions coincides with the appearance of lesional B cells and parasite-specific antibodies in sera [12, 17]. Consequently, skin of infected B cell-deficient μ MT and JHT mice and Fc γ R^{-/-} mice contained fewer parasite-infected DC in vivo [12]. Infected B cell-deficient mice as well as Fc γ R^{-/-} or Fc γ RI^{-/-} \times Fc γ RIII^{-/-} mice (all on C57BL/6 background) showed similarly increased disease susceptibility as assessed by lesion volumes and parasite burdens. The B cell-deficient mice also displayed impaired T cell priming and dramatically reduced IFN γ production, and all these deficits were normalized by infection with IgG-opsonized parasites. As expected, since Fc γ RI and Fc γ RIII compensated for each other, Fc γ RI^{-/-}, Fc γ RII^{-/-}, as well as Fc γ RIII^{-/-} mice all exhibited no phenotype.

Genetical differences matter

All data described above were relevant especially in physiological low dose infections with 1,000 infectious stage metacyclic promastigotes inoculated into dermal ear skin [17]. Interestingly, our findings in C57BL/6 mice were not in line with those of prior reports using BALB/c mice. It is well accepted that genetically determined disease outcome in leishmaniasis is determined on the level of various immune cells [2]: Whereas C57BL/6 mice—similar to infected humans with no immune deficits—develop skin lesions that ultimately heal due to parasite-eradicating IFN γ release from antigen-specific Th1/Tc1 cells, BALB/c mice succumb to infection with *L. major* because of enhanced Th2/Th17 development [2, 21]. In BALB/c mice, the strong pathological Th2-dominated immune response is associated with excessive immunoglobulin levels of all classes. In addition, a strongly improved disease outcome of BALB/c Fc γ R^{-/-} and B cell-deficient BALB/c JHT mice was explained by decreased IL-10 release from M Φ [22].

Summary and open questions

Altogether, these data demonstrated that DC and M Φ use different receptors to recognize and ingest *L. major* with different outcomes, and indicate that B cell-derived, parasite-reactive IgG and DC Fc γ RI and Fc γ RIII are essential for optimal development of protective immunity. It appears that at later stages of infection, the balance between (1) Fc γ R-mediated induction of IL-12 from DC and (2) Fc γ R-associated IL-10 release from infected M Φ is ultimately responsible for disease outcome.

This pivotal role for antibodies to parasites in the priming of T cell immunity by DC raises the question of how the initial B cell response to the parasite itself develops [23]. Does it evolve in the absence of infected DC? Alternatively, early on after parasite inoculation, natural IgG may substitute for the antigen-specific IgG that are found at later stages post-infection. Former work has suggested that parasites can enter host cells via a process called ‘apoptotic mimicry’ and it is also known that *L. major* parasites are covered with phospholipids on their surface [24, 25]. Thus, natural IgG recognizing, for example, antiphospholipid antibodies may play a role in early parasite uptake by DC.

Proper DC activation requires skin mast cells

Role of mast cells for disease outcome in cutaneous leishmaniasis

Mast cells (MC) are key effector cells in type I hypersensitivity reactions or in response to parasites and play an

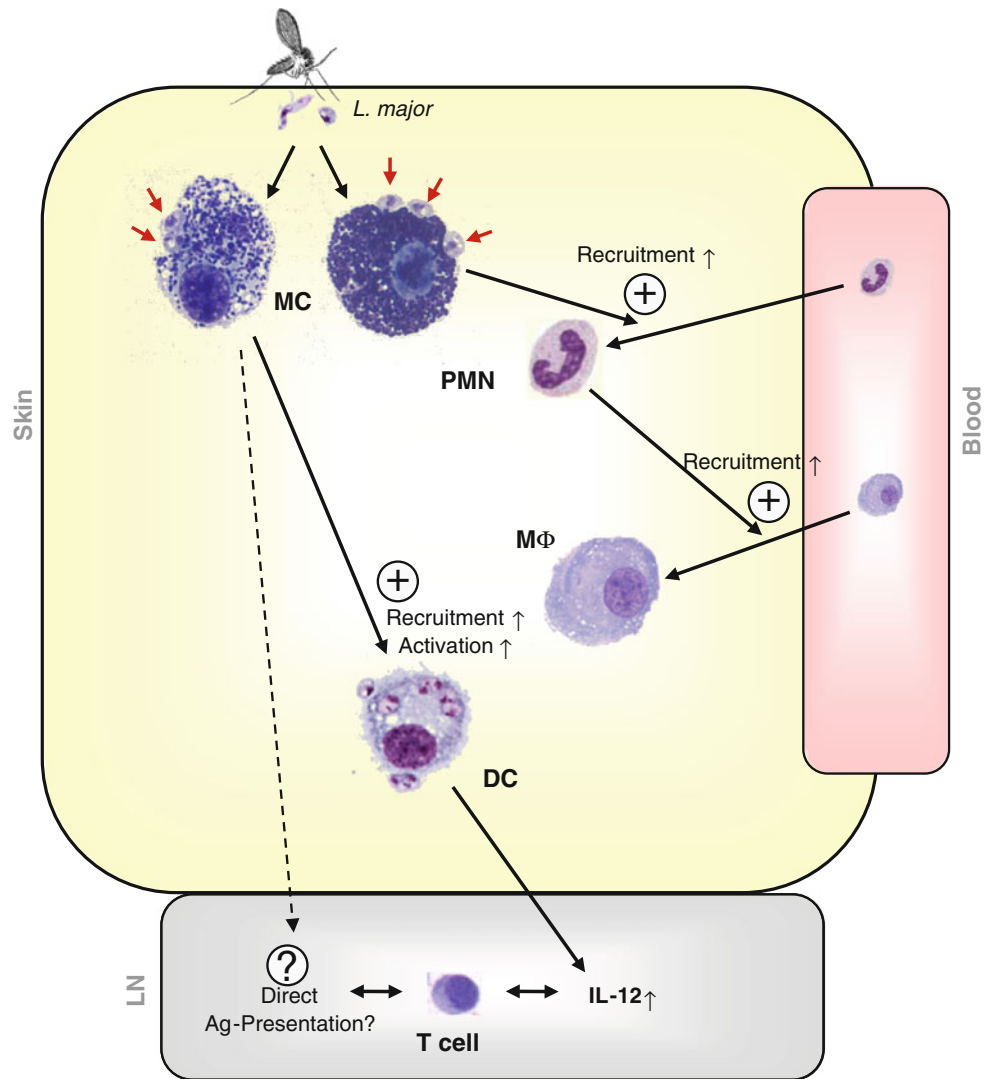
important role in the regulation of protective adaptive immune responses against pathogens. Strategically located in the skin, they are known to contribute to the control of parasitic skin infections by *L. major* [26, 27]. First, MC degranulation and activation were demonstrated after interaction with *L. major* both in vitro and in vivo (Fig. 2) [26–29]. Next, *L. major*-infected MC-deficient *Kit^W/Kit^{W-v}* mice (on a *Leishmania*-resistant C57BL/6 background) developed markedly larger skin lesions than normal *Kit^{+/+}* mice did, and cutaneous reconstitution with MC resulted in normalization of lesion development. *Kit^W/Kit^{W-v}* lesions contained significantly more parasites, and infections resulted in enhanced spreading of parasites to the spleens as compared to controls. Antigen-specific T cell priming was delayed in *Kit^W/Kit^{W-v}* mice, and cytokine responses were skewed towards Th2. Notably, local skin MC reconstitution at sites of infection was sufficient for the induction of systemic protection.

The c-kit mutation in *Kit^W/Kit^{W-v}* mice impairs melanogenesis and results in anaemia, sterility, and markedly reduced levels of tissue MC. In contrast, *Kit^{W-sh}/Kit^{W-sh}* mice, bearing the *W-sash* (*W^{sh}*) inversion mutation, also show MC deficiency, but normal levels of major classes of other differentiated hematopoietic and lymphoid cells [30]. As a result, intradermal inoculation of high doses and physiologically relevant low doses of *L. major* resulted in significantly worsened disease with larger lesions in MC-deficient *Kit^{W-sh}/Kit^{W-sh}* compared to infected C57BL/6 control mice ([31], and unpublished data). This was correlated to enhanced parasite burdens in ears and spleens in *Kit^{W-sh}/Kit^{W-sh}* mice. Additionally, skin draining LN cells from infected *Kit^{W-sh}/Kit^{W-sh}* and C57BL/6 mice were isolated and pulsed with soluble *Leishmania* antigen to analyse antigen-specific cytokine production. Here, the IL-10 production was significantly increased in MC-deficient mice, which strongly supports a shift towards a Th2 response. Thus, using two independent mouse models for MC deficiency, we showed that MC are critically involved in regulating disease outcome after *L. major* infection into skin.

Involvement of MC in inflammatory cell recruitment

The early inflammatory response after inoculation of *L. major* is dominated by an influx of polymorphonuclear neutrophils (PMN) and M Φ [4, 6]. M Φ are the major constituents of skin granulomas, and parasite containment is the result of efficient granuloma formation in cutaneous leishmaniasis. In the end, M Φ are the main effector cells for parasite elimination, since IFN γ release from T cells induces NO-mediated parasite killing by M Φ [2]. PMN, in contrast, are recruited to the inoculation site within minutes post-infection [32]. Interestingly, *Leishmania* possess some

Fig. 2 Skin MC regulate the development of protective anti-parasite immunity. Parasite inoculation into skin leads to activation of MC as assessed by degranulation and $TNF\alpha$ release. MC-derived $TNF\alpha$ leads to recruitment of neutrophils (PMN) into the tissue, which in turn release $MIP-1\alpha/\beta$ that is chemotactic for immigrating inflammatory $M\Phi$. In parallel, improper activation of DC was observed in the absence of MC, which leads to decreased levels of $IL-12$ and defects in the development of efficient anti-parasite immunity. Finally, it is unclear to date whether MC directly contribute to antigen presentation and T cell priming in this T cell-dependent disease



means to survive their early encounter with recruited PMN at the bite site [6]. In addition, PMN serve as Trojan horse that mediates parasite survival and entry into tissue $M\Phi$ [33, 34] and DC [35].

In some models, MC are the main cells initiating cell recruitment to effector organs. Thus, using a model of biogel-induced skin granuloma formation, we assessed the role of MC for PMN followed by $M\Phi$ recruitment [36]. Kit^W/Kit^{W-v} mice as well as mice deficient in the MC product $TNF\alpha$ exhibited markedly reduced $M\Phi$ numbers in cutaneous granulomas. $M\Phi$ recruitment was restored in Kit^W/Kit^{W-v} mice reconstituted with MC from $Kit^{+/+}$ or $TNF\alpha^{+/+}$, but not from $TNF\alpha^{-/-}$ mice. MC- $TNF\alpha$ -dependent $M\Phi$ influx required prior recruitment of $MIP-1\alpha/\beta$ -producing PMN, as PMN depletion before biogel injection completely inhibited $M\Phi$ influx, which was restored after reconstitution with PMN supernatants. These findings

showed that $M\Phi$ recruitment to skin granulomas is the result of a sequence of inflammatory processes initiated by MC-derived $TNF\alpha$ followed by PMN influx and $MIP-1\alpha/\beta$ release.

As a conclusion, we hypothesized that MC could be crucial for recruiting $M\Phi$ to sites of infection as well. And indeed, in infections with *L. major*, we and others detected significantly fewer $7/4^+$ PMN, $F4/80^+$ $M\Phi$, and—interestingly— $CD11c^+/MHC$ class II $^+$ DC in leishmaniasis skin lesions in Kit^W/Kit^{W-v} mice (Fig. 2) ([26] and Nathan Peters/Bethesda, personal communication). The latter was associated with decreased levels of $IL-12$ production found in draining LN. Thus, MC-dependent recruitment of the most important APC to skin, $M\Phi$, PMN and DC, contributes to control of disease in leishmaniasis and facilitates proper T cell priming against *L. major* infection.

DC activation and T cell priming is regulated by MC

Prior work suggested that MC can promote T cell activation indirectly through the stimulation of APC in vivo; for example, MC induced the migration of DC [37] and LC [38, 39] to draining LN, where antigen presentation occurs. To further identify the underlying mechanism of the immunoregulatory capacity of MC, we recently investigated the impact of MC on DC maturation and function in more detail [31]. Peritoneal cultured MC directly bound to immature DC and this cell-to-cell crosstalk resulted in an increased expression of the DC maturation markers CD86, CD80 and CD40 [31]. The supernatants of MC/DC co-cultures contained several T cell modulating cytokines, for example, IL-6 and TGF β . Interestingly, in these in vitro experiments, the presence of MC further increased the amount of IL12p70 release of lipopolysaccharide (LPS)-matured DC (Fig. 2). Our results also demonstrated that the crosstalk of DC with MC strongly impacts the subsequent capacity of DC to activate CD4⁺ T cells and to polarize the T cells towards a Th1 or Th17 response. In line, using *Kit^{W-sh}/Kit^{W-sh}* mice, we showed that after *L. major* infection, decreased production of Th1 and Th17 cytokines is observed also in vivo.

MC are a potential source of many mediators that can influence the development, recruitment, phenotype, proliferation and activation of T cells in vitro and in vivo. Thus, during the initial phase of infection MC could also directly influence T cell priming and subsequent immune responses. Here, MC may act as APC themselves [40]. MC are most often associated with Th2-type inflammatory responses, but, more recently, MC were shown to be an important source of IL-12 during peritonitis, which promoted PMN and survival of infected mice [41].

Antigen processing in DC

Role of T cells for disease outcome

It is well accepted that protection against *Leishmania* infections is T-cell-dependent. For example, T cell-deficient nude or severe combined immunodeficient (SCID) mice failed to control fatal dissemination of *L. major* mice [42, 43]. An inability to control disease was also observed in MHC class II^{-/-} and CD4-deficient mice. Interestingly, despite the absence of CD4⁺ T cells, a population of functional helper T cells (CD8⁻ $\alpha\beta$ TCR⁺ T cells) developed after infection of CD4-deficient C57BL/6 mice with *L. major* [44]. These cells were MHC II-restricted and produced IFN γ after challenge with parasite antigens. These data indicated that T cell lineage commitment and peripheral function may not stringently depend on the

function of the CD4 molecule [44]. Disease susceptibility also resulted from sustained depletion of CD4⁺ cells by monoclonal anti-CD4 antibodies, although transient depletion at the time of infection allows the side-effect of protective Th1 cells in otherwise susceptible BALB/c mice [45–47]. The latter effect is the result of transient depletion of so-called pre-primed, *Leishmania* homolog of receptors for activated c kinase (LACK)-reactive V β 4 V α 8⁺ CD4⁺ T cells, with most LACK-specific T cells producing IL-4, but not IFN γ [48]. LACK-induced preferential expansion of parasite-specific Th2 cells and a low level of IFN γ production resulted in progressive infection of BALB/c mice and fatal outcome [49]. Interestingly, similar LACK-reactive T cells with preferential IL-4 release also exist in resistant C57BL/6 cells, but in smaller numbers [2].

Several studies have indicated that IFN γ release by CD8⁺ *L. major*-specific T cells (Tc1) also promotes the development of protective immunity [17, 50–52]. Thus, CD8 as well as MHC class I knockout mice were unable to control infection [17]. In line, antigen-specific IFN γ release from total LN cells results from equal production on this cytokine by CD4⁺ and CD8⁺ T cells in vivo, [21]. In addition, using (DC-based) vaccinations with fusion proteins consisting of HIV-1 TAT and the *Leishmania*-specific protein LACK leading to preferential induction of LACK-specific CD8 responses resulted in significantly smaller lesion volumes compared to mice immunized with LACK alone [53].

Evidence for T cell priming by DC

In experimental cutaneous leishmaniasis, infected DC appear to be the most important inducers of protective immunity. Once activated by parasite uptake, they process the antigen both in the MHC class I and in the MHC class II pathway, thereby efficiently stimulating both CD4⁺ and CD8⁺ T cells [12, 17]. In line, other groups have previously shown that murine skin-derived DC (e.g. epidermal LC) internalize *L. major* and transport the parasites from the skin via the lymphatics into the T cell areas of draining LN to stimulate parasite-specific T cell responses [54–56]. In addition, using low dose infections mimicking physiological sand fly bite transmission, DC immigration into the infection site and local IL-12 production is observed between week 5 and 6 post-infection shortly before the first T cells are recruited [5], whereas infected M Φ can already be found within days after parasite inoculation. Others have previously shown that impaired DC activation/migration (e.g. in CCR2^{-/-} mice) results in worsened disease outcome due to defects in T cell priming [57]. Finally, Baldwin et al. [10] systematically examined the parasite load in different APC subsets isolated from draining LN of *Leishmania*-susceptible BALB/c and resistant C57BL/6

mice. Although parasites were already detectable in LN a few hours after infection with 10^3 metacyclic promastigotes, parasites were not found in DC until week 3, indicating that at this point of time the main infected cell type may be M Φ . Interestingly, when using this physiologically relevant low dose model, T cell priming does not occur before 4–5 weeks post-infection [10].

The contribution of M Φ to T cell activation in *L. major* infections is less clear. Comparative migration assays with infected epidermal LC and M Φ from BALB/c and athymic BALB/c nude mice as well as mixed labelling immunohistology of draining LN early in infection provided evidence that infected M Φ were, in contrast to LC, not able to migrate to dLN and therefore may not induce antigen-specific T cell responses [58]. In the mammalian body, IL-12 is the main and most important cytokine inducing anti-*Leishmania* immunity, which requires IFN γ release by primed T cells. Host M Φ from susceptible and resistant mice were, in contrast to DC, not efficiently activated by uptake of parasites via CR3-dependent phagocytosis, and IL-12 synthesis is selectively inhibited. This leads to the production of a broad range of cytokines (e.g. IL-2, IL-10) from CD4 $^+$ T cells, which may favour survival of the host organism by MHC class II-restricted antigen presentation by M Φ to primed, but not naïve, T cells [59, 60]. Overall, infected M Φ may thus be less important for T cell priming against *L. major* antigens. However, they may contribute to restimulation of antigen-specific CD4 $^+$ T cells that have immigrated to the site of infection at later stages post-infection.

PMN and DC co-localize at sites of acute inflammation in the skin after parasite inoculation. Interestingly, a recent study indicated that infected PMN in skin expressed elevated apoptotic markers, resulting in enhanced capturing by skin DC [35]. Thus, after 1 day, the majority of infected DC recovered from skin stained positive for neutrophil markers, indicating that they acquired their parasites via uptake of infected PMN. When infected, these DC were actively downmodulated. Consequently, PMN depletion led to enhanced activation marker expression on DC and improved DC-mediated *Leishmania* antigen presentation of CD4 $^+$ T cells. The findings suggest that during the acute PMN response early after infection, the parasites actively promote immunosuppression by entering DC via PMN shuttles, whereas at later stages, ‘direct’ DC uptake of *L. major* supports the development of protective T cell responses.

Cross-presentation of exogenous parasite antigens by DC

It is long believed that exogenous antigens, like parasite proteins, are mainly presented via the MHC class II pathway (a vesicular system) for priming of CD4 $^+$ T cells.

Interestingly, however, only infected DC, but not M Φ , are also capable of priming and restimulation of CD8 $^+$ T cells [17, 61, 62]. The presentation of exogenous antigen as endogenous antigen such as self proteins was named ‘cross-presentation’ [63, 64]. In general, APC present endogenous cytosolic proteins via MHC class I molecules towards CD8 $^+$ T cells. Degradation of the proteins is performed by the proteasome [65] and resulting peptides are then transported to the endoplasmic reticulum by the transporter associated with antigen presentation (TAP), where they bind to MHC class I molecules which are then presented on the surface to CD8 $^+$ T cells [65].

How the parasite antigens enter the cytoplasm is unknown so far. Interestingly, prior work using immune complexes and tumour models has already demonstrated that the development of efficient CD8 T-cell-dependent anti-tumour immunity was dependent on Fc γ signalling [64, 66]. Thus, the utilization of Fc γ R by DC for parasite internalization (as compared to CR3 by M Φ) may explain their ability to prime CD8 $^+$ T cells for parasite antigens. To further understand CD8 $^+$ T cell priming by infected DC, *L. major* promastigotes were transfected with a plasmid to express ovalbumin (OVA) fragments [62]. Here, it was shown that parasite-secreted OVA was more immunogenic compared to nonsecreted OVA, suggesting that secreted parasite proteins are more efficiently processed as endogenous antigens than others [62]. Interestingly, studies by Bertholet et al. and our group have furthermore revealed that DC-mediated antigen presentation of *L. major* peptides to CD8 $^+$ T cells is (immuno-)proteasome/TAP-independent [67, 68], thus independent of these two major nonlysosomal protein degradation machineries. The involvement of alternative cytosolic pathways of cross-presentation encompassing protease candidates such as tripeptidyl peptidase II and nardilysin will have to be investigated.

Summary and outlook

In summary, these data indicate that infected DC are crucial for T cell priming in cutaneous leishmaniasis, whereas other infected APC such as M Φ mainly contribute to MHC class II-restricted CD4 $^+$ T cell restimulation. However, until now, at least five different DC subsets have been described in skin. As described below, all of these may have different functions for disease outcome, whereby epidermal LC may be the most important cells best equipped to prime CD8 $^+$ T cells against *L. major* [69]. However, using novel tools such as cell-specific ablation of, for example, MHC class I or II on CD11c $^+$ or LysM $^+$ cells will allow for a detailed analysis of the respective role of these APC for T cell activation.

How important is DC infection for disease outcome?

DC as major source of Th1/Tc1-promoting IL-12

C57BL/6 mice lacking IL-12 showed progressive disease. Similarly, continuous treatment of BALB/c mice with recombinant IL-12 redirected the early Th2 response to Th1 and promoted resistance [70–72]. Interestingly, a strong, more sustained Th2 response in anti-IL-12-treated resistant mice led to disease exacerbation. Of note, the anti-IL-12 antibody treatment has the greatest effect when delayed until seven days after infection [73]. This indicated that production of IL-12 is delayed normally even in resistant mice. Indirectly, this coincides with the delayed appearance of infected DC both at the site of infection and in the draining LN [17]. As described above, DC appear to be the major and only cellular source for bioactive IL-12 after infection [7, 8].

DC subsets in skin: it's not that easy...

Overall, however, only indirect evidence is present so far that points to an important role of DC for disease outcome in leishmaniasis. Only recently, new experimental approaches such as constitutive or conditional lineage ablation allow for an investigation into specific cell functions in vivo. The situation is further complicated by the fact that DC in skin can be subdivided into at least 6 subtypes. In addition, all of these subsets may have different functions for resulting immune responses [74]. Evidence exists that suggest that the different DC subsets express different activation markers, such as Toll-like receptors (TLR) [74], supporting the hypothesis that they are triggered by different signals in vivo resulting in divergent immune responses.

DC are divided in type-1 interferon-producing plasmacytoid DC (pDC) [75] and conventional DCs (cDC) [76] in nonlymphoid tissues, in circulation, and in lymphoid tissues. The cDC can be further subdivided into lymphoid tissue-resident DC that are present in the thymus, spleen and LN, and into migratory DC that act as sentinels in the periphery. These latter migratory DC are, according to their tissue localization, LC in the epidermis or dermal DC (dDC) in the dermis. The migratory DC have a mature phenotype upon reaching the LN, whereas the lymphoid-tissue-resident DC have an immature phenotype and are active in antigen uptake and processing [77]. Finally, DC that are not found in the steady state, but develop after inflammation or infection, include the monocyte-derived DCs (mo-DC) [78]. These mo-DC are found in particularly large numbers in lesions after *L. major* infection. Even though it is well accepted that APC are crucial for T cell priming during infection and that skin-derived LC and dDC

are the main APC to activate T cells during infections with *L. major*, it is still unclear which of the mentioned DC subtypes is the key player [69].

Langerin (CD207) is a C-type lectin predominantly expressed by LC [79, 80], but Langerin expression was also found on some murine CD8 α^+ LN DC [81, 82]. Recently, a new subset of Langerin $^+$ dDC that is independent from epidermal LC was identified [83–85]. In addition to LC in transit from the epidermis, the dermis appears to contain two more subsets of Langerin $^+$ dDC (distinguished by differential CD103 expression) and two subsets of Langerin $^{\text{neg}}$ dDC that differ in CD11b expression [86].

Experimental approaches such as inducible cell lineage ablation enable the investigation into specific cell functions in vivo. Langerin-DTR knock-in mice express the human DTR under control of the *langerin* promoter and allow for the inducible ablation of Langerin $^+$ cells [87]. We recently demonstrated that after physiological low dose infection with metacyclic promastigotes of *L. major*, mice lacking Langerin $^+$ DC developed significantly smaller ear lesions, reduced parasite burdens, and an increased Th1 response as compared to their control littermates. Depending on the timing of the DT treatment protocol used, it was possible to completely deplete LC, while Langerin $^+$ dDC are largely restored [88]. Alternatively, bone marrow chimera with Langerin-DTR and wild-type mice allow for depletion of LC or dDC only. Interestingly, selective depletion of LC alone led to significantly reduced lesion sizes, enhanced IFN γ /IL-4 and IFN γ /IL-10 ratios, and reduced numbers of regulatory T cells (Treg), indicating that LC and not Langerin $^+$ dDC were responsible for the suppressive effect (Fig. 3) [89]. Interestingly, in contrast, bone marrow chimeras, in which only Langerin $^+$ dDC can be depleted, showed no phenotype.

Summary and conclusion

To induce protective adaptive immunity against *Leishmania spp.*, IL-12 release by DC (and that of other cytokines such as IL-1 α [90, 91], IL-27 [92], IL-23 [21]) is a crucial step and known to be an important promoter of Th1 differentiation. Fc γ R-mediated phagocytosis appears to be one important trigger of this type of response by DC after parasite internalization. In addition, MHC class I- and II-restricted antigen presentation towards both CD4 $^+$ and CD8 $^+$ T cells by DC is essential for appropriate T cell priming against parasite antigens. Some of the underlying mechanisms, for example, such as how cross-presentation of exogenous parasite epitopes occurs, need to be studied in detail in the future. Overall, it is still unclear which skin DC subtype is the definitive key population to induce protective immunity and there is only indirect evidence

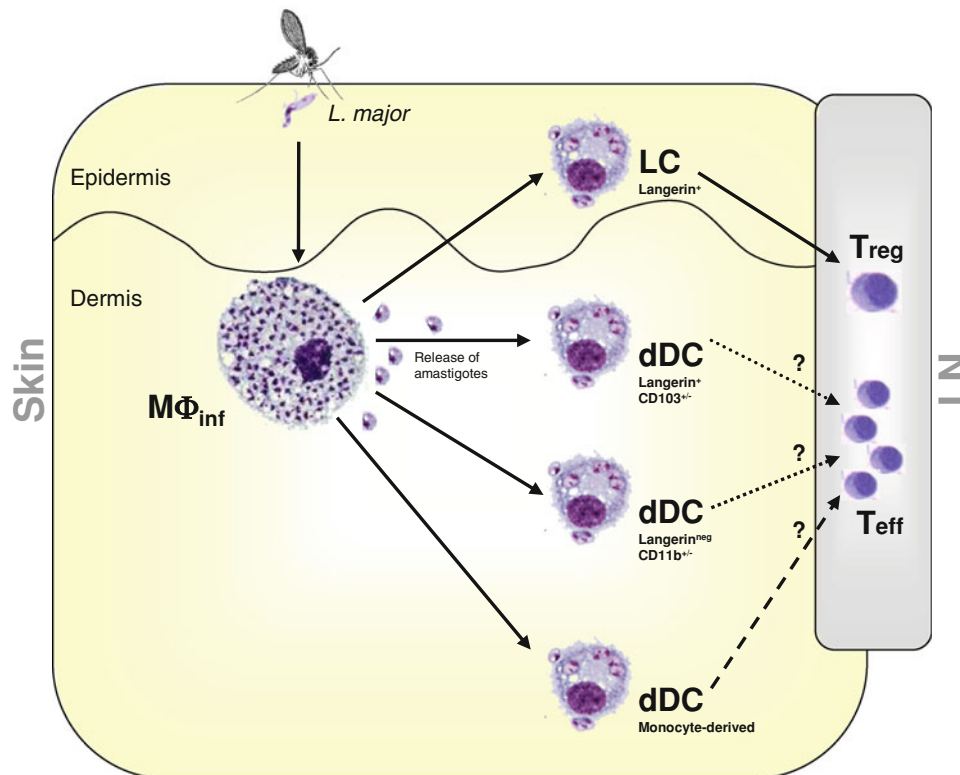


Fig. 3 Various skin dendritic cell subsets with different roles for disease outcome. *L. major*-infected skin contains at least 6 different subsets of DC: epidermal LC, dermal Langerin⁺ DC, dermal Langerin^{neg} DC and monocyte-derived inflammatory DC. All subsets have been shown to be infected with *L. major* in vivo and migrate to the draining LN. Recently, we have shown that—in contrast to all other DC subsets—epidermal LC regulate anti-*Leishmania* immunity

by inducing regulatory T cells (Treg). These Treg promote parasite persistence and counterbalance the parasite-eliminating, IFN γ -producing effector T cells (Teff). Whether the various dermal DC (dDC) perform differing tasks during the initiation or maintenance of a protective T cell response in *L. major* infections is under investigation. The identification of “the protective” DC subset is important for vaccine development

present so far to answer the question whether this DC population must be infected (with live parasites) or not.

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