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Cutaneous leishmaniasis: Distinct functions of dendritic cells and macrophages in the interaction of the host immune system with *Leishmania major*

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ABSTRACT

Leishmaniasis is transmitted by sand flies leading to parasite inoculation into skin. In the mammalian host, the parasite primarily resides in skin macrophages (MΦ) and dendritic cells (DC). MΦ are silently invaded by the parasite eliciting a stress response, whereas DC become activated, release IL-12, and prime antigen-specific T cells. Here we review the basics of the immune response against this human pathogen and elucidate the role and function DC and MΦ for establishment of protective immunity against leishmaniasis. We focus on cell type-specific differences in parasite uptake, phagocyte activation and processing of parasite antigens to facilitate an understanding how their respective function may be modulated e.g. under therapeutic considerations.

1. Immune defense against cutaneous leishmaniasis

Infections with *Leishmania* spp. represent a serious health problem in large parts of the world. Leishmaniasis is endemic in 88 countries. More than 350 million individuals are at risk of leishmaniasis worldwide (Alvar et al., 2012; Herwaldt, 1999), and 12 million people are currently suffering from leishmaniasis. Leishmaniasis presents as a spectrum of manifestations ranging from cutaneous or mucocutaneous disease forms to visceral infections. In general, the pathological changes reflect the balance between parasite multiplication, the immune response of the patient and resulting degenerative changes. Even though lesion resolution and cure is mediated by antigen-specific T cells, a vaccine is not yet available. Therefore, a better understanding and detailed characterization of the mechanisms leading to the development of $CD4^+$/$CD8^+$ T cell-dependent protection via different types of antigen-presenting cells (APC) is crucially required.

Leishmaniasis is caused by the protozoan *Leishmania* that parasitizes in phagocytic cells (Sacks and Noben-Trauth, 2002). The disease is transmitted to the host by the bite of the phlebotomine sand fly. The early inflammatory events that take place in the skin and lymph nodes have been studied in the so-called “low dose” infection model in mice using only infectious stage parasites that are delivered intradermally into the ear skin of experimental mice (Belkaid et al., 2000), thereby mimicking natural transmission of the parasite (Fig. 1). After inoculation of infectious stage metacyclic promastigotes into the upper dermis, *Leishmania* are phagocytosed by skin-resident macrophages (MΦ) and primarily locate to the phagolysosomes (Sacks and Noben-Trauth, 2002). Herein, the parasites transform into the obligate intracellular amastigote life form and replicate (‘silent phase’ post infection) (Kaye and Scott, 2011). Within a short time span, polymorphonuclear neutrophils (PMN) are also recruited to the site of infection, where they engulf promastigotes providing them a temporary shelter (Fig. 1) (Kamhawi, 2006; Ribeiro-Gomes and Sacks, 2012; van Zandbergen et al., 2006). After ~3 weeks, an inflammatory wave with influx of PMN, inflammatory monocyte-derived MΦ and (later) also dendritic cells (DC) is observed (Kautz-Neu et al., 2011).

Subsequently, DC prime and activate antigen-specific T cells, thereby eliciting the adaptive immune response against *Leishmania*. At this stage, clinically apparent lesions are observed. Recently, at least 5 distinct DC subsets have been identified in skin, and they appear to have different functions for subsequent T cell-dependent immunity. As such, epidermal Langerhans cells induce parasite-specific regulatory T cells, whereas dermal DC promote generation of protection by inducing Th1/Tc1-dependent immunity (Ashok and Acha-Orbea, 2014; Kautz-

Abbreviations: APC, antigen presenting cells; MΦ, macrophages; DC, dendritic cells; ER, endoplasmic reticulum; PMN, polymorphonuclear neutrophil; PV, parasitophorous vacuole; CR, complement receptor; LACK, *Leishmania* homoloque of receptors for activated C kinase; MINCLE, macrophage inducible C-type lectin; iNOS, inducible nitric oxide synthase; NO, nitric oxide; MOI, multiplicity of infection; mTOR, mammalian target of rapamycin; IFR, interferon regulatory factor; IFIT, interferon induced protein with tetratricopeptide repeats; LC3, microtubule-associated protein1 light chain 3; LPS, lipophosphoglycan

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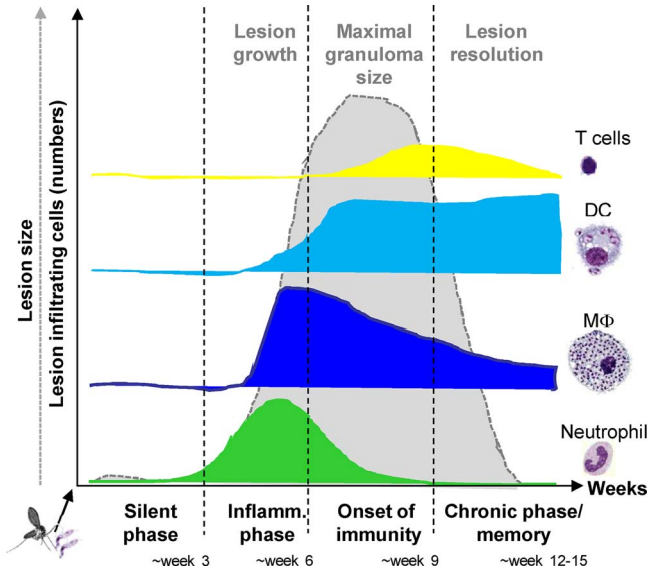

Fig 1. Lesion evolution in leishmaniasis.
Parasites are inoculated into the skin as flagellated promastigote life form by sand flies. After phagocytosis by skin-resident macrophages (MΦ), parasites transform inside MΦ into obligate intracellular amastigotes and replicate (silent phase). Amastigotes released from lysed MΦ are subsequently internalized by other phagocytic cells, e.g. neutrophils and dendritic cells (DC). At the onset of inflammation after several weeks, immigration of first neutrophils, followed by inflammatory MΦ is observed. Later, recruitment of inflammatory DC precedes immigration of antigen-specific T cells into lesions. T cell-derived IFNγ finally induces lesion resolution by facilitating parasite killing.

Neu et al., 2011).

Finally, IFNγ-producing, antigen-specific $CD4^+$ Th1 and $CD8^+$ Tc1 cells induce lesion resolution by activating infected MΦ to induce NO and eliminate the parasites. This process was recently shown to be dependent on Hypoxia-inducible factor-1α (HIF-1α), which accumulates in mammalian host organisms during infection and supports antimicrobial defense (Schatz et al., 2016). Without treatment, the entire process takes ~18 months in immunocompetent humans and ~3 months in resistant C57BL/6 mice. After healing, life-long immunity is observed against the same *Leishmania* subspecies.

2. Origin and function of DC and macrophages

Ontogenetically, MΦ and DC are closely related myeloid cells and represent the most potent phagocytic cells of the body. Historically, these cells were grouped together because monocytes were considered to be precursors of MΦ and DC. MΦ are characterized as larger cells with prominent nucleus, which express CD11b and are $F4/80^{high}$. In contrast, DC exhibit their characteristic protrusions, have a somewhat smaller nucleus and are $CD11b^+$ and $CD11c^{high}$/MHC class $II^{intermediate/high}$. Recently, a new nomenclature was proposed that separates MΦ from monocyte-derived cells and defines three different subtypes of DC (Guilliams et al., 2014). After internalization (e.g. by endocytosis or phagocytosis) of the parasite, both MΦ and DC are capable of efficient antigen processing and presentation to T cells (Doebel et al., 2017) on MHC class I and II molecules.

However, even though MΦ and DC are both professional APC, their biological behavior after contact with *Leishmania* parasites differs dramatically. Whereas MΦ represent the primary host cell for the parasite and are ultimately responsible for their elimination from the host, DC appear to be the cells mainly responsible for T cell priming and the induction of protective immunity. Notably, the multifaceted interactions between *Leishmania* and the host APCs critically determine the final outcome of the disease (Liu and Uzonna, 2012).

In the present review, we aim to characterize the specific differences in the interaction of *L. major* with MΦ and DC, which render them attractive targets for immunomodulating strategies. Therefore, specific targeting of DC or MΦ in leishmaniasis aiming to enhance or modulate their natural function may facilitate the development of improved therapeutic strategies.

3. Parasite internalization

Early parasite/APC interactions have been studied by many groups *in vitro*. DC preferentially take up amastigote life forms of *L. major* as compared to metacyclic promastigote (Blank et al., 1993; Locksley et al., 1988; von Stebut et al., 1998; Woelbing et al., 2006). In parallel, a thorough side-by-side comparison of human MΦ and DC subsets revealed similar results (Zahn et al., 2010). Parasite internalization was both time and dose-dependent as expected. MΦ take up parasites within a few hours reaching a plateau beginning at 6 h post infection and display a high uptake efficiency leading to ~60-80% infected cells (MOI 3). In contrast, DC internalized *L. major* significantly slower and less efficiently (max. 30% infected cells, MOI 3, 18 h) (Table 1). The difference between cell-specific parasite uptake efficiency is less prominent at higher MOI, however, the physiological relevance of these findings with regard to the situation in the skin microenvironment is unclear to date. It appears more likely that after inoculation of 10–100 parasites by the sand fly and efficient complement-mediated parasite elimination of roughly 90% of inoculated parasites (Domínguez et al., 2003), only very few viable parasites get in contact with skin-resident APC *in vivo* (Fig. 2)

The different uptake kinetics suggested that DC and MΦ utilize different receptors and/or uptake mechanisms for parasite internalization. Metacyclic promastigotes evade complete destruction by complement via lipophosphoglycan (LPG), which interferes with insertion of the membrane attack complex, and via deactivation of the classical and alternative pathways (Hermoso et al., 1991; Puentes et al., 1990; Sacks and Sher, 2002). Furthermore, the leishmanial membrane-resident protease gp63 cleaves C3b to inactive C3bi, which binds to CR3, thereby mediating entry into MΦ (Brittingham and Mosser, 1996). This in turn prevents the parasites from being killed by phagocytes or plasma components. In addition, it appears that – compared to other MΦ populations – dermal MΦ lack a respiratory burst machinery, suggesting that *Leishmania* promastigotes taken up by dermal MΦ have a better chance to transform into amastigotes (Locksley et al., 1988).

Prior work suggested that CR3 is involved in parasite uptake by DC (Blank et al., 1993). $CD18^{-/-}$ DC, however, did not exhibit a deficiency in parasite internalization indicating that complement may be involved in parasite adherence to the cell surface prior to ingestion (Woelbing et al., 2006). Parasite update by DCs requires parasite-reactive IgG and involves FcγRI and FcγRIII (Fig. 3). Both receptors can fully be substituted for each other (Woelbing et al., 2006). *In vivo*, DC infiltration of *L. major*-infected skin lesions coincided with the appearance of antibodies in sera (Woelbing et al., 2006). Infected B cell-deficient mice as well as $FcγR^{-/-}$ mice (C57BL/6 background) showed similarly increased disease susceptibility as assessed by lesion volumes and parasite burdens (Woelbing et al., 2006). While B cell-deficient mice displayed impaired T cell priming and dramatically reduced IFNγ production, these deficits could be circumvented by infection with IgG-opsonized parasites. In both mouse strains, infected skin contained fewer parasite-infected DCs *in vivo*. These data demonstrated that DC and MΦ use different receptors to recognize and ingest *L. major* (FcR vs. CR3) with different outcomes, and indicated that B cell-derived, parasite-reactive IgG and DC FcγRI/III are essential for the optimal development of a protective immune response.

It remains an open question how the initial B cell response to the parasite itself develops in the absence of B cell priming by infected DC. So called natural antibodies may facilitate early parasite internalization by DC.