DOI:10.1111/j.1600-0625.2010.01116.x
www.blackwellpublishing.com/EXD

Leishmaniasis, contact hypersensitivity and graft-versus-host disease: understanding the role of dendritic cell subsets in balancing skin immunity and tolerance

Kordula Kautz-Neu[1], Ralf G. Meyer[2], Björn E. Clausen[3] and Esther von Stebut[1]

[1]Department of Dermatology, University Medical Center of the Johannes Gutenberg University Mainz, Mainz, Germany;
[2]Department of Medicine 3, Hematology and Oncology, University Medical Center of the Johannes Gutenberg University Mainz, Mainz, Germany;
[3]Department of Immunology, Erasmus MC, University Medical Center, Rotterdam, The Netherlands
Correspondence: Dr Esther von Stebut, Department of Dermatology, University Medicine, Johannes Gutenberg University, Langenbeckstrasse 1, 55131 Mainz, Germany, Tel.: +49-6131-175731, Fax: +49-6131-173470, e-mail: vonstebu@uni-mainz.de

Accepted for publication 7 April 2010

Abstract: Dendritic cells (DC) are key elements of the immune system. In peripheral tissues, they function as sentinels taking up and processing antigens. After migration to the draining lymph nodes, the DC either present antigenic peptides by themselves or transfer them to lymph node–resident DC. The skin is the primary interface between the body and the environment and host's various DC subsets, including dermal DC (dDC) and Langerhans cells (LC). Because of their anatomical position in the epidermis, LC are believed to be responsible for induction of adaptive cutaneous immune responses. The functions of LC and dDC in the skin immune system *in vivo* are manifold, and it is still discussed controversially whether the differentiation of T-cell subtypes (e.g. effector T cells and regulatory T cells) may be initiated by distinct DC subtypes. As skin DC are able to promote or downmodulate immune responses, we chose different skin diseases (cutaneous leishmaniasis, contact hypersensitivity, UV radiation-induced suppression, and graft-versus-host disease) to describe the biological interactions between different DC subtypes and T cells that lead to the development of efficient or unwanted immune responses. A detailed knowledge about the immune modulatory capacity of different cutaneous DC subsets might be helpful to specifically target these cells through the skin during therapeutic interventions.

Please cite this paper as: Leishmaniasis, contact hypersensitivity and graft-versus-host disease: understanding the role of dendritic cell subsets in balancing skin immunity and tolerance. Experimental Dermatology 2010; **19**: 760–771.

Introduction

Dendritic cells (DC) are potent regulators of adaptive immunity. They process and present antigens either taken up from the extracellular space or produced intracellularly. When DC present antigens on major histocompatibility complex (MHC) class I or II molecules, the expression of costimulatory surface molecules and cytokines leads to either priming and activation or silencing of antigen-specific T cells.

Growing insight into DC biology led to the discovery of various subgroups of these professional antigen-presenting cells (APC). They vary in phenotype and function depending on their location in the lymphoid or non-lymphoid tissues as well as on their appearance in the steady state or during inflammatory situations. DC are constantly taking up antigen and subsequently carry it to the draining lymph nodes (LN). This traffic is markedly increased during inflammation. The immune regulatory capacity of DC located in the tissues of the inner and outer body surfaces, namely the skin and mucosa, is of particular importance to mount sufficient defense against pathogens but also to avoid unwanted immune responses against foreign, yet harmless antigens including commensal bacteria.

Skin dendritic cell subtypes

Dendritic cells are derived from haematopoietic stem cells in the bone marrow (BM) (1,2) and are specialized to induce and regulate T-cell immunity and tolerance (3). Situated in peripheral tissues and lymphoid organs, DC are uniquely able to detect and capture pathogens. Although they share many common features, multiple subtypes of DC with distinct immune functions have been identified in recent years (4,5).

In the mouse, several types of DC have been described (6), which have been shown to differentially promote Th1 or Th2 responses according to their origin and state of

Table 1. Different DC subtypes in mice

Plasmacytoid DC (pDC)	
$CD11c^+B220^+Gr\text{-}1^+$ (113)	
Conventional DC (cDC)	
Lymphoid tissue–resident DC	Migratory DC
$CD8^+CD4^{neg}$ cDC	Langerhans cells (LC)
$CD8^{neg}CD4^{neg}$ cDC	Dermal DC (dDC) ($Langerin^+$ or
$CD8^{neg}CD4^+$ cDC	$Langerin^{neg}$ dDC)

maturation (4,7) (Table 1). Several factors, e.g. antigen uptake itself as well as proinflammatory cytokines, can induce DC maturation and promote a proinflammatory phenotype. DC take up antigen via different groups of receptor families, such as Fc receptors for antigen–antibody complexes, pattern-recognition receptors such as Toll-like receptors (TLR), which enable DC to recognize a wide range of microbial stimuli (8), and C-type lectin receptors for binding of glycoproteins (9).

The DC system can be divided into type-1 interferon-producing plasmacytoid DC (pDC) (10) and conventional DCs (cDC) (11). cDC involved in defense mechanisms against pathogens comprise all DC subsets located in antigen-capture areas related to epithelia surfaces (such as the skin) and secondary lymphoid organs (LN, spleen, bronchial- and gut-associated lymphoid tissues) (5). These can be further subdivided into lymphoid tissue-resident DC and migratory DC that act as sentinels in the periphery. For example, the skin harbours Langerhans cells (LC) in the epidermis and dermal DC (dDC).

Langerhans cells have long been thought to play a pivotal role in the initiation of immune responses by acquiring antigens that are encountered in the skin (12). They represent a unique DC subset localized in basal and suprabasal layers of the epidermis and in stratified mucosal epithelia (13). Langerin (CD207) is a C-type lectin predominantly expressed by LC (14,15), but Langerin expression was also found on some murine $CD8\alpha^+$ LN DC (16,17). In addition, a new subset of $Langerin^+$ dDC that is independent from epidermal LC has recently been identified (18–20). Recently, it was described that next to LC in transit from the epidermis, the dermis contains two more subsets of Langerin-positive dDC (distinguished from each other by differential expression of CD103) and two subsets of Langerin-negative dDC that differ in their expression of CD11b (21). Both $Langerin^+$ DC subsets constitute no more than ~3% of all dDC, $CD207^{neg}CD11b^+$ dDC represent the majority of all dDC (~66%), whereas $CD207^{neg}CD11b^{neg}$ dDC are less frequent (~16%). Thus, murine skin contains at least five phenotypically distinct DC populations, i.e. epidermal LC, two $Langerin^+$ and two $Langerin^{neg}$ dDC subsets, which may also differ functionally, e.g. in their ability to (cross-)present antigen (21–23).

Dendritic cells reside in the skin in an immature state. Upon antigen encounter together with inflammatory mediators, they undergo maturation enabling their migration to skin-draining LN. It was demonstrated that after epicutaneous application of tetramethylrhodamine (TRITC), both LC and dDC home to the skin-draining LN (16). Antigen-bearing LC preferentially migrate to the inner paracortex, whereas antigen-loaded dDC accumulated in the outer paracortex of the LN. Furthermore, Kissenpfennig et al. (16) showed that migration of LC to the skin-draining LN is delayed in comparison with dDC. While dDC arrived in the LN within 2 days after epicutaneous antigen application, LC were only detected after 4 days. Nagao et al. (23) demonstrated that epidermal LC and Langerin-expressing dDC are unrelated and exhibit distinct functions *in vivo*: $Langerin^+$ dDC were required for optimal production of antigen-specific IgG2a/b primarily found during Th1 immune responses, whereas LC primarily induced Th2-associated IgG1.

Dermal DC can be distinguished from macrophages (MΦ) by their expression of MHC class II, CD11c and CD205. LC and dDC are both well equipped to capture environmental antigens, migrate to the draining LN and initiate specific T-cell immune responses. Consequently, both skin DC populations contribute to the transport of pathogens to the draining LN (16,24–26). Nevertheless, as observed in herpes simplex virus (HSV) infections, despite their important function in antigen delivery, MHC class I–restricted antigen presentation was initiated by non-migratory LN-resident $CD8^+$ DC, rather than by skin-derived DC (27). Thus, inter-DC antigen transfer may function to amplify antigen presentation in certain situations, i.e. infection with cytolytic virus. The observations in the HSV infection model, however, cannot be generalized. Upon lentiviral infection, which does not kill the cells, LC are perfectly capable of direct Ag presentation and T cell activation (28). In addition, Geijtenbeek and coworkers (29) have shown that $Langerin^+$ LC are important for protection from HIV infection.

Additionally, infection-induced inflammatory reactions caused by bacteria, viruses and parasites involve a strong increase in *de novo* generation of DC at the infection site and in draining LN (30–32). In these locations, $Ly\text{-}6C^+$ monocytes can differentiate into DC under inflammatory conditions (33). Experimental cutaneous leishmaniasis provided new insights into the *de novo* DC differentiation during infection *in vivo*. After *L. major* infection, two newly formed DC subsets were described in the popliteal LN, which were not found in the steady state (34). Here, dermal moncocyte–derived DC controlled the induction of protective immune responses against *Leishmania*, indicating that these newly generated DC were essential for T-cell immunity against this parasite (34).

Most of our current knowledge of cutaneous DC subsets is derived from the analysis of steady state and inflammatory conditions in murine skin. However, as described in detail later, a number of critical findings have already been confirmed for human DC subsets. For example, in addition to epidermal LC, Haniffa et al. (35) recently described three principal subsets of $CD45^+$ $HLA\text{-}DR^+$ cells in the dermis. The dDC population consists of a $CD1a^+$ $CD14^{neg}$ and a $CD1a^{neg}$ $CD14^+$ subset, whereas $CD1a^{neg}$ $CD14^+$ $FXIIIa^+$ cells were identified to be MΦ.

Mouse models for inducible *in vivo* ablation of dendritic cells

The developmental interrelations and differential *in vivo* functions of the numerous phenotypically distinct DC subsets are still incompletely understood. New experimental approaches such as constitutive or inducible cell lineage ablation enable the investigation into specific cell function *in vivo* (36). In mice, toxigenic depletion methods using diphtheria toxin (DT) have become a widely used strategy and were first described by Saito et al. (37). The cytotoxicity of DT is dependent on receptor-mediated endocytosis (38). Following receptor binding by the B subunit of DT and internalization, the toxic A chain (DTA) catalyses ADP ribosylation of the eukaryotic elongation factor 2, resulting in inhibition of translation, which finally leads to apoptotic cell death (39,40). Two main strategies are applied to deplete cells by DT. One method involves expression of the human DT receptor (DTR) in the target cell population, rendering naturally DT-resistant mouse cells susceptible to DT killing (37,41). Here, DT needs to be injected systemically or locally into mice to trigger cell death. Another approach is based on the endogenous expression of DTA (42,43).

The DT/DTR system has been successfully used to achieve conditional ablation of DC in CD11c-DTR mice (44). Jung et al. generated mice that carried a transgene encoding a human DTR-green fluorescent protein (GFP) fusion protein under control of the murine CD11c promoter. Murine CD11c expression is largely restricted to the DC compartment. In CD11c-DTR mice, a single i.p. injection of DT leads to depletion of all $CD11c^{high}$ cells for 2 days after which the DC compartment is gradually and completely restored by day 6 after DT (44). Interestingly, LC are not depleted in these mice. However, some other immunologically relevant cells such as CD8 T cells, splenic MΦ populations and mast cells were also affected to varying degree by DT treatment (45,46).

To understand the *in vivo* dynamics and functions of $Langerin^+$ DC in general and LC in particular, knockin mice expressing the same DTR-eGFP cDNA under control of the endogenous *langerin* promoter were generated (47). Another group made knockin mice by targeting an IRES-DTR-eGFP cassette into the 3′-untranslated region of *langerin* (16). In both of these Langerin-DTR strains, LC, $Langerin^+$ dDC, and $Langerin^+CD8\alpha^+$ DC can be efficiently depleted from skin, LN, spleen and thymus following a single intraperitoneal (i.p.) injection of DT (16,47). Repopulation of the epidermis is slow with LC starting to reappear only at 2–4 weeks after DT injection (47), whereas $Langerin^+$ dDC start to reappear in the skin already 4 days following DT treatment, demonstrating that this DC subset is capable of replenishing the connective tissue significantly faster than LC the epidermis.

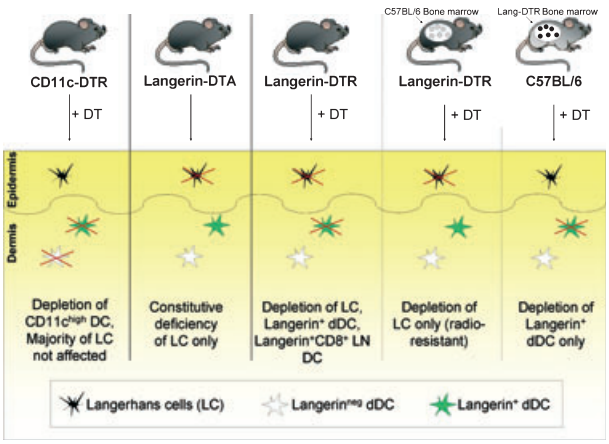

Figure 1. Overview of the different murine DC ablation models. Mouse models were generated for the inducible depletion of either $CD11c^{high}$ DC (44) or $Langerin^+$ DC (16,47) using the DTR/DT system. Alternatively, Langerin-DTA mice exhibit a constitutive Langerhans cells (LC) deficiency throughout life (48). Separation of effects mediated by either LC or $Langerin^+$ dDC can be achieved by timed DT treatments that allow for the depletion of all $Langerin^+$ DC (DT injected on day 1 and 2 prior to immunization) or selective depletion of only LC (DT administered from day 7 to 10). Although not ideal (only about 50% reconstitution of $Langerin^+$ dDC), this approach is frequently used in the light of the major time and effort required to generate wild-type bone marrow x Langerin-DTR chimeras.

On the other hand, a bacterial artificial chromosome (BAC) transgenic mouse strain was generated by inserting DTA into the 3′-untranslated region of the human *langerin* gene (48). In contrast to the two inducible Langerin-DTR models, these Langerin-DTA mice constitutively lack LC. Importantly, only LC and no other $Langerin^+$ DC are absent in these mice, likely reflecting that regulation of expression of the human promotor does not entirely correlate with murine *langerin* (Fig. 1).

Bone marrow (BM) reconstitution studies with congenic strains (CD45.1 and CD45.2) provided evidence that $Langerin^+$ dDC and LC develop independently and exhibit distinct repopulation kinetics and migratory properties (18,20). Before syngeneic BM transplantation, the mice need to be conditioned by e.g. (sub-)lethal irradiation to enable engraftment with donor BM. Epidermal LC are

radio-resistant, and after irradiation and BM transplantation, LC remain of host origin throughout life in the steady state (49). In contrast, all other DC subtypes are radiosensitive and replaced by donor-derived cells. As a result, it is now possible to selectively deplete either LC or Langerin$^+$ dDC and Langerin$^+$CD8α^+ DC depending on the chosen chimeras (host: Langerin-DTR, donor: C57BL/6 wt, or *vice versa*, Fig. 1).

Thus, selective cell depletion allows for an analysis of the function of a distinct DC subtype during pathogenic infections or other skin diseases with the aim of improving current therapeutic approaches. In the future, modern genome/proteome analysis might reveal even better molecular targets for the selective depletion of different DC subsets.

Cutaneous leishmaniasis

Leishmaniasis is a parasitic disease transmitted by the bite of a sand fly (Fig. 2), which affects ~12 million people worldwide. The infectious metacyclic promastigotes, representing the flagellated life form of the parasite, are transmitted to the host skin from the salivary gland of sand flies. As shown recently by intravital microscopy, neutrophils constitute the first wave of inflammatory cells migrating to the site of infection (50). Neutrophils efficiently capture *Leishmania major* parasites early after needle inoculation or sand fly transmission. By 6–7 days after the sand fly bite, the majority of cells harbouring parasites were MΦ, which acquired viable parasites that had been released from neutrophils undergoing apoptosis. Thus, in addition to complement receptor 3–mediated uptake of parasites by MΦ, this indirect phagocytosis of parasites by neutrophils and subsequent transfer of parasites to MΦ contributes to the silent entry of parasites into MΦ and parasite replication (51). In addition, DC might be involved in the very early events after infection as well. Using an intradermal infection model and intravital microscopy, Ng et al. (52) found that already within minutes after infection, dDC incorporated multiple *L. major* parasites into cytosolic vacuoles independently of neutrophils.

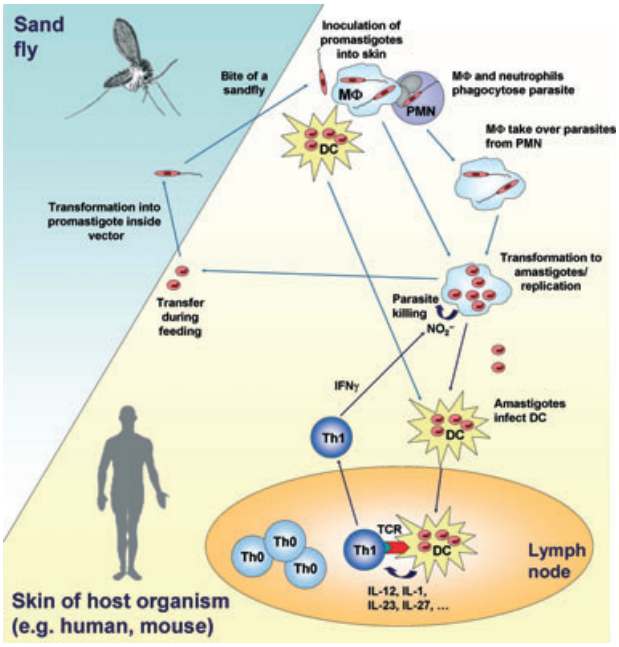

Figure 2. Life cycle of *L. major*. *Leishmania* parasites are inoculated into the skin upon the bite of a sand fly or in a mouse model via needle inoculation. This leads to a rapid influx of neutrophils to the wound site, which phagocytose *L. major* promastigotes. In the following, macrophages phagocytose infected neutrophils or they take up viable parasites released from apoptotic neutrophils. Inside the macrophages, *L. major* promastigotes transform into the obligate intracellular amastigote life form. Free amastigotes are released from lysed macrophages and are able to infect DC. Infected DC migrate to the draining lymph nodes (LN) and activate naïve T cells. *L. major*-resistant strains like C57BL/6 develop a protective Th1 response after infection. The life cycle is completed upon re-infection of sand flies after feeding at infected skin sites.

Once phagocytosed by skin-resident MΦ, promastigotes differentiate into the non-flagellated amastigote form that replicates within the acidified phagolysosomes (53). After several divisions, the amastigotes (eventually) rupture the cell, are released and rapidly taken up by other MΦ or DC. Some parasites remain in the skin and cause cutaneous lesions, while others visceralize (directly or by being 'shuttled' within cells) to the spleen, liver and BM. The life cycle is completed when a sand fly ingests amastigotes, which then transform back to promastigotes (Fig. 2).

Control of parasite replication is mediated by activation of leishmanicidal mechanisms in infected MΦ that are dependent on interferon (IFN)γ production by Th1 or Tc1 cells. IFNγ induces the expression of inducible nitric oxide synthase (iNOS) by phagocytic cells, leading to the production of nitric oxide (NO). Finally, IFNγ-mediated NO production is required for parasite killing (54–56). DC play an essential role in initiating protective Th1 responses in *Leishmania* infection. Recently, it was shown that a population of inflammatory DC (CD11b$^+$ CD11c$^+$ Ly-6C$^+$ MHC class II$^+$) were the main producers of iNOS *in vivo* during the course of *L. major* infection, and the induction of iNOS in these cells required the development of a local Th1 environment (57). Disease progression depends on both the host's immune response and the particular strain of *Leishmania* causing the disease.

Murine models of cutaneous leishmaniasis have been used to study immune responses following needle inoculation of *L. major*. Generally, supra-physiological high doses of *L. major* stationary-phase promastigotes (2×10^5 to 2×10^7) have been injected subcutaneously or intradermally into footpads of mice. To study experimental cutaneous leishmaniasis in a system that most closely mimics

natural transmission, a low-dose infection model has been established using intradermal inoculation of small numbers (10–1000) of metacyclic promastigotes. This low-dose infection model allowed for identification of additional factors relevant for resulting immune responses (58–60).

In murine leishmaniasis, BALB/c mice respond to infection with preferential production of Th2 type cytokines, in particular IL-4 and IL-10, which are associated with disease progression and susceptibility. In contrast, C57BL/6 mice are resistant to *L. major* and manifest a self-healing local inflammation with an IL-12-driven Th1 response, elevated IFNγ production, MΦ activation and *Leishmania* killing. *L. major* infection of C57BL/6 mice is considered a relevant model for human self-limiting cutaneous leishmaniasis. In contrast to MΦ, skin DC function as the critical APC initiating the primary immune response against *L. major*. Here, skin DC play an important role as bridge from innate to adaptive immune responses as they prime naïve T cells. When DC take up *Leishmania* parasites, they acquire a mature phenotype by upregulation of MHC class I and II, increase their expression of co-stimulatory molecules, release IL-12 and transport the parasites from infected skin to the draining LN for presentation to T cells (61). *In vitro*, DC predominantly take up the amastigote form of the parasite, even though only promastigotes are inoculated into the skin by the bite of a sand fly. This effect might explain why infected DC were not found until after several days/weeks and at later stages of infections (58). DC were shown to transport amastigotes from lesions to the draining LN and to harbour long-term persistent parasites in otherwise immune animals. Additionally, DC pulsed with *Leishmania* antigen protected BALB/c mice from infection (62,63).

The immunological basis of protection against *Leishmania* parasites has been a focus of research for a number of years. The knowledge described herein has been obtained by studying murine experimental leishmaniasis, but also from experimental infections of humans. Even though these human studies were limited, it was shown that the basic mechanisms described in mice are also valid for humans (64–67). This especially holds true for T-cell subsets found in skin lesions, but not in peripheral tissues. This means that even though mixed Th1/Th2 responses against *Leishmania* can be found in the blood of infected patients, which did not correlate with the course of disease (64), a clear association between elevated levels of lesional IFNγ and healing was observed (65). Comparable observations were made when studying infections with *Mycobacteria leprae*, as tuberculoid (Th1-associated, restricted bacterial growth) and lepromatous leprosy (Th2-dominated, disseminated disease) have been described (68). Finally, treatment of persisting leishmaniasis in a child with recombinant IFNγ led to complete resolution of disease (66). However, the role of the various DC subsets for the induction of protective immunity against *Leishmania* in humans is not clear yet.

Even though lifelong immunity develops once an infection has healed, a vaccine against primary infection with *Leishmania* does not exist at present. One critical issue is how an appropriate antigen is presented to the immune system, and how DC direct diverse key functions like anergy, tolerance, initiation and regulation of the adaptive immune response as well as the development of memory responses (3). Thus, a more detailed understanding of the complexity of the skin DC system will improve the use of DC as tools for immune intervention strategies and enhance otherwise insufficient immune responses against this important human pathogen (69).

Role of different skin DC subsets for control of *L. major* infections

Prior studies demonstrated that infected DC are the critical APC responsible for T-cell priming in *Leishmania* infections (25,63,70,71). Because of their position in the epidermis, LC have been thought to play a vital role in the initiation and control of skin diseases. By labelling of LC with a fluorescent cell linker and *in vivo* tracking, LC were shown to transport *L. major* from the skin to the draining LN (62). Although both LC and dDC can home to the draining LN, they may have different capacities in engulfing promastigotes/amastigotes of the parasite and distinct functions in experimental leishmaniasis (24,25). More recent studies have suggested that DC other than LC may be crucial to initiate protective anti-*Leishmania* immunity (25) (Table 2).

To address the question which cells harbour and transport parasites to the draining LN, Baldwin et al. (71) systematically examined the parasite load in the different DC subpopulations isolated from draining LN of susceptible BALB/c and resistant C57BL/6 mice (Fig. 3). Although parasites were already detected in the LN, a few hours after infection with 10^3 metacyclic promastigotes, none of the skin DC immigrants harboured any parasites until week 3, indicating that DC are not responsible for transport of the parasites from the skin to the draining LN early after infection with physiologically relevant low-dose inocula. Interestingly, in both *Leishmania*-resistant C57BL/6 and *Leishmania*-susceptible BALB/c mice, double-negative ($CD8^{neg}Dec205^{neg}$) DC, $CD8^{high}Dec205^{int}$ cells, $CD8^{int}Dec205^{int}$ dDC and $CD8^{int}$ $Dec205^{high}$ LC from the LN contained comparable numbers of parasites 3 weeks postinfection, whereas pDC were not significantly parasitized. In addition, the majority of DC emigrating from infected ears at different time points were LC followed by dDC. As it is still unclear whether LC efficiently phagocytose parasites in the skin and subsequently migrate to

Table 2. Phenotype of mouse and human skin DC subsets

	Dermal mo-DC	LC	Langerin^{+} dDC	Langerin^{neg} dDC
Murine				
Tissue	Dermis, LN	Epidermis	Dermis	Dermis
Contribution during	Ag presentation (34)	Ag presentation (62)	Ag presentation (24)	Ag presentation (24)
L. major infection	Induction of protective Th1 immunity (34)	Tolerance? (72) Suppression (unpublished data)	CD8 T-cell priming (114)	Induction of protective Th1 immunity (24)
Marker expression	CD8α			
	CD11b	CD11b		CD11b
	CD11c	CD11c	CD11c	CD11c
	MHC class II	MHC class II	MHC class II	MHC class II
	?		CD103	
	DEC205 (CD205)	DEC205 (CD205)	?	DEC205 (CD205)
	?	Langerin (CD207)	Langerin (CD207)	
Human				
Contribution to immunity	Production of:			
	TNFα			
	iNOS			
	IL-12/23p40			
Marker expression	–	CD1a	Unknown, but likely separation in:	
	CD1c	–	CD1a^{+}/CD14^{neg}	
	CD11c	–	CD1a^{neg}/CD14^{+} (35)	
	HLA-DR	HLA-DR		
	–	Langerin (CD207)		

Table adapted and modified from (22,115,116).
Mo-DC, monocyte-derived DC; LC, Langerhans cells; dDC, dermal DC; iNOS, inducible nitric oxide synthase; n.d., not determined; LN, lymph nodes.

draining LN, infection of LC might also occur in draining LN.

In contrast, it was demonstrated that *L. major* antigen was transported to the draining LN by dDC, and not LC, using a high-dose infection model (3×10^6 metacyclic promastigotes). In addition, only the CD8α^{neg} dDC were able to present endogenous *L. major* antigen to CFSE-positive CD4^{+} T cells and induced proliferation (24). Whereas antigen-specific effector T cells are primed by dDC in the outer paracortex of the LN, LC may be responsible for the maintenance of peripheral tolerance and in the induction of regulatory T cells that exhibit immunosuppressive properties (72). Iezzi et al. (73) postulated that skin-derived DC migration is not required for initiation of *L. major*-specific T-cell responses. They demonstrated that the initiation of antigen-specific immune responses is mediated by a population of CD11c^{high}CD11b^{high}CD8^{neg}Langerin^{neg} DC, which reside in LN and acquire soluble antigens transported through the lymph after high-dose infection (2×10^6 metacyclic promastigotes). León et al. described two de novo formed DC subsets in the popliteal LN during *L. major* high-dose infection. These DC included both dermal and LN monocyte–derived DC. Using fluorescent parasites, antibodies and T cells that recognized *L. major* antigens presented on MHC molecules, they identified only the monocyte-derived DC to be involved in capture and presentation of *L. major* (34).

Plasmacytoid DC produce large amounts of type-1 interferons after stimulation with viral or other microbial infections. IFNα released by pDC has an inhibitory effect on viral replication, but also contributes to the activation of natural killer (NK), T and B cells (74,75). pDC possess the capacity to mediate resistance against *L. major* high-dose infection in susceptible BALB/c mice (76). Activation with CpG induced the release of high amounts of IFN-α from pDC after 48 h in culture. Although Remer et al. documented that a single treatment with *L. major* lysate–pulsed pDC is sufficient to induce T cell-mediated protection, this was not accompanied by a polarized Th1 cytokine profile.

Taken together, advances have been made in the characterization of the different DC subpopulations which may direct T-cell responses and influence disease progression in leishmaniasis. New experimental settings, e.g. the *in vivo* depletion of distinct DC subtypes will provide better insights into the complex DC network in orchestrating the anti-*Leishmania* immune response.

Function of LC and other skin DC in contact hypersensitivity

Similar to the situation in leishmaniasis, DC and LC have been shown to be critically involved in the induction and elicitation of contact hypersensitivity (CHS) in humans and

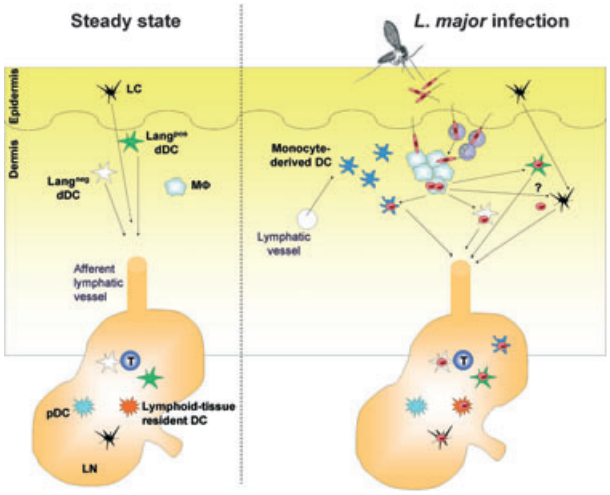

Figure 3. Different dendritic cell subtypes may be involved in the induction and maintenance of adaptive T cell-dependent immunity against *L. major*. In the steady state, migratory DC (Langerhans cells, LC; dermal Langerin$^+$ and Langerin^{neg} DC, Lang$^{+/neg}$ dDC) in the skin migrate to the lymph node (LN) and acquire a mature phenotype. LN-resident DC remain immature in the absence of an activating stimulus. During *Leishmania* infection, *L. major* promastigotes are internalized by skin-resident dermal macrophages (MΦ), in which they transform to non-flagellated amastigotes. Monocyte-derived DC are not found in the steady state and immigrate into skin as a consequence of infection and inflammation. Within days/weeks, the various skin DC take up *L. major* amastigotes released from lysed MΦ and migrate to the draining LN. In the LN, *L. major* antigen may be processed by LN-resident DC as well. Consequently, *L. major* antigen-specific T cells with a Th1-like phenotype are activated and proliferate.

mice. CHS is a common skin disease, presenting clinically as allergic contact dermatitis. Millions of individuals suffer from allergic contact dermatitis, e.g., against nickel.

Experimentally, CHS is induced by application of a sensitizing dose of hapten (e.g. dinitro-fluorobenzene (DNFB), oxazolone – both comparably weak sensitizers – or the strong hapten trinitrochlorobenzene (TNCB)) onto the skin, which leads to DC activation, transport of haptenized self-proteins to the draining LN and antigen presentation to naïve T cells. Upon re-exposure to the same hapten (experimentally applied at a site different from the sensitization area, i.e. the ears), recruitment of hapten-specific T cells leads to local inflammation usually measured as ear swelling reaction. It was previously demonstrated that the T cells responsible for elicitation of CHS responses are primarily antigen-specific CD8$^+$ and – to some extent – IFNγ-producing CD4$^+$ Th1 cells or Thy (thymocyte differentiation antigen)-1$^+$ NK cells, whereas Th2 as well as Treg contribute to control of inflammation (77,78). The Treg involved in the regulation/suppression of CHS are primarily CD4$^+$CD25$^+$ cells and act via production of IL-10 (77).

Following primary exposure to the contact allergen in the skin, LC/dDC take up haptenized proteins and migrate to the regional LN, where they prime naïve hapten-specific T cells during the asymptomatic sensitization phase. LC/dDC activation and mobilization is mediated by proinflammatory cytokines [tumor necrosis factor (TNF) TNFα and IL-1β produced e.g. by 'irritated'/stimulated keratinocytes and/or LC/dDC] (78). LC/dDC activation correlates with induction of cytokine secretion (IL-1β, IL-6, IL-12), enhanced surface expression of MHC class I and II, and costimulatory molecules. Although according to the classical paradigm they were considered critical inducers of CHS, LC appear to be dispensable for T-cell sensitization in CHS, and dDC may also contribute to priming of naïve T cells after contact with hapten.

Taking advantage of the mouse models described previously, the role of LC when compared to other skin DC subsets in CHS has recently been re-examined (22,79). Using the two inducible Langerin-DTR mice and application of DT immediately before hapten sensitization, it was demonstrated that ear-swelling responses were either unaltered (16) or diminished but not absent (47), suggesting that LC and Langerin$^+$ dDC are not absolutely required for T-cell priming and induction of CHS. In contrast, mice constitutively lacking only LC developed enhanced CHS, suggesting that LC may exert a regulatory function and limit contact allergy (48).

Apart from variations in the experimental design of these initial studies, the controversial results may rely on the presence/absence of Langerin$^+$ dDC, which seem to participate in the induction of CHS (22). This hypothesis is supported by a recent study using platelet-activating factor (PAF)-deficient mice (80). In these mice, migration of hapten-pulsed LC was markedly inhibited, whereas recruitment of antigen-loaded dDC to draining LN was unaltered. As a result, CHS responses were comparable to those in wild-type mice, but not enhanced as in LC-deficient Langerin-DTA mice (48). Moreover, based on the faster repopulation kinetics of dermal Langerin$^+$ DC, timed toxin treatments can be used to achieve selective depletion of only LC in Langerin-DTR mice. Specifically, applying the hapten onto the skin 1–2 or 7–14 days after DT will lead to sensitization in the absence of, respectively, all Langerin$^+$ (skin) DC and LC alone (Fig. 1). In initial experiments using this strategy, CHS was diminished when all Langerin$^+$ DC were depleted and indistinguishable from wild type (but again not enhanced) when only LC were lacking (19). From this it was concluded that the magnitude of a CHS reaction is a function of the Langerin$^+$ dDC subset. However, more recent experiments demonstrate that hapten dose and access to the dermis are the critical parameters that determine whether CHS is similar or reduced when compared to wild type. At a low hapten dose, ear swelling is decreased both when all Langerin$^+$ DC and when LC are depleted. In contrast, upon painting of a higher concentra-

tion of hapten onto the skin, CHS is diminished only when all Langerin$^+$ skin DC are missing (81). These data suggest functional redundancy of the different skin DC subsets in CHS. In conclusion, the degree of ear swelling is determined by the efficiency of LC/dDC antigen transport and naïve T-cell priming during hapten sensitization, rather than a function of a particular skin DC subset (82).

In contrast to CHS, delayed-type hypersensitivity (DTH) reactions are primarily elicited by antigen-specific CD4$^+$ T cells. The experimental models for studying DTH responses vary tremendously between different research laboratories. In the majority of studies, DTH is elicited by intradermal or even subcutaneous injection of antigen in the presence of adjuvant. In others, epidermal expression of a neo-self antigen such as ovalbumin (OVA) is achieved genetically in transgenic mice (83). While initial experiments suggested functional redundancy of LC and dDC in activating reactions towards epidermally restricted OVA, two authors demonstrated that keratinocyte-derived OVA was cross-presented to T cells by Langerin$^+$ dDC, and not LC (21,84). However, it is still premature to draw definite conclusions about the physiological role of the different skin DC subsets from these studies. A comprehensive overview of this complex area of research is beyond the scope of this review and has recently been elegantly discussed elsewhere (83).

Requirement of LC in UV radiation induced immunosuppression

Based on the findings in the CHS model, a relevant question is whether a similar redundancy of skin DC function may also exist for UV radiation (UVR)-induced immunosuppression, which has long been considered to be exclusively mediated by LC. Middle wavelength range UVR (UVB, 290–320 nm) can be a risk to human health by inducing skin cancer, but also suppresses adaptive immunity and therefore can be used therapeutically to treat autoimmune skin diseases (e.g. psoriasis). Experimentally, UVR-mediated immunosuppression was demonstrated by inhibition of CHS. Hapten sensitization via UVR-treated skin induces antigen-specific tolerance (85). As UVR exposure induced LC depletion from the epidermis, attenuated ear swelling reactions were initially explained by inhibition of sensitization and inefficient T-cell priming. Later, it was demonstrated that immunological unresponsiveness is systemic. CHS could not be elicited when the same hapten was painted at a later time point onto non-UVR exposed skin maintaining a physiological number of LC. In addition, hapten-specific tolerance could be adoptively transferred by spleen or LN cells (86) and was mediated by UVR-induced CD4$^+$CD25$^+$ Treg (87) secreting high amounts of IL-10 (88). Development of UVR Treg required migration of DNA-damaged, but still viable LC to the local LN (89,90), which demonstrated that the capacity of epidermal LC to induce Treg was dependent on receptor activator of NF-κB (RANK) interaction with its ligand (RANKL, also known as TRANCE). RANKL/TRANCE was induced on keratinocytes by UVR and is known to increase DC survival by upregulating expression of Bcl-X_L (91). This mechanism may be operating in UVR-damaged LC to delay their death until after they have reached the draining LN.

However, the conclusion that LC are responsible for UVR-induced Treg and suppression of CHS was based on the identification of Langerin$^+$ skin DC in the nodes, which as we know in the meantime may also include Langerin$^+$ dDC. Taking advantage of timed toxin treatments of Langerin-DTR mice, it was recently shown that UVR-induced inhibition of CHS and induction of Treg did not occur in the absence of epidermal LC (92). These findings strongly support a critical requirement of LC in UVR-mediated immunosuppression. In contrast, in a similar recent study using LC-depleted Langerin-DTR mice, it was found that LC are dispensable for UVR-induced suppression of CHS (93). However, probably the UVR dose in these experiments was too low, because against published literature (85,94,95) the authors claimed that LC were not depleted by UVR. Moreover, the development of Treg following UVR was not determined in this study.

Taken together, a continuous downtuning of the adaptive skin immune system may be beneficial to limit unwanted reactions to e.g. commensal bacteria and this may be achieved by physiological doses of UVR. LC appear to be an essential part of this regulatory system (92), in contrast to their redundant function in CHS.

Langerhans cells and graft-versus-host disease

Allogeneic haematopoietic stem cell transplantation is a curative therapy for many haematologic malignancies and some non-malignant diseases (96). For malignant tumors, the major remission-maintaining therapeutic effect of stem cell transplantation is carried out by donor-derived T cells (97). On the other hand, T cells of donor origin can also cause damage to healthy host-tissue resulting in graft-versus-host disease (GVHD). GVHD is the major cause of non-relapse mortality following stem cell transplantation (98). In acute GVHD, cytotoxic CD8$^+$ T cells are the main effectors of the underlying immune reaction leading to inflammation of the skin, gut and liver. In contrast, chronic GVHD resembles the features of chronic autoimmune disease like, e.g., systemic sclerosis and is mediated by multiple effector mechanisms.

Current understanding holds that GVHD pathophysiology consists of three steps (99,100). First, tissue damage is

caused by the preparative (chemo-) radiotherapy (the conditioning regimen) prior to stem cell transplantation. Secondly, the local inflammation caused by tissue damage – especially of the intestinal mucosa – leads to the activation of resident DC in mucosa-associated lymphatic tissue. The translocation of bacterial products such as lipopolysaccharides (LPS) via the bloodstream also activates host APC in other organs (101). Activated donor-derived T cells are subsequently primed against host antigens in secondary lymphoid organs. In the third step (effector phase) of GVHD, multiple cells and soluble mediators of the innate as well as the adaptive immune system cause severe tissue damage and eventually death.

The essential role of APC for GVHD has been proven by transplantation of β2-microglobulin knockout ($\beta 2m^{-/-}$) mice with haematopoietic stem cells derived from wild-type animals and *vice versa*. GVHD developed only in the presence of MHC class I–positive APC – even in $\beta 2m^{-/-}$ mice lacking MHC class I on tissue cells (102). Consequently, the GVHD-preventing effect of KIR (killer cell immunoglobulin-like receptor)-mismatched donor NK cells in haploidentical transplantation (103) is most likely associated with the NK cells' DC-depleting capacity. In contrast to the initiation phase, donor DC are more detrimental than host DC during later stages of GVHD (104). As in leishmaniasis, the GVHD-promoting T-cell response is primed in secondary lymphoid organs and blocking T-cell homing to secondary lymphoid organs in splenectomized mice prevents the initiation and development of GVHD (105).

After allogeneic stem cell transplantation, APC in the peripheral blood of the patient are rapidly replaced by cells derived from the haematopoietic cell transplant (106). LC of the epidermis, however, persist in patients for months after stem cell transplantation (107). One reason for this phenomenon is the resistance of LC to gamma irradiation used in many conditioning regimen. In addition, local depletion of LC by irritants leads to proliferation of LC precursors in the skin without recruitment of blood-derived progenitors (49). In humans, depletion of epidermal LC by GVHD-causing T cells has been demonstrated in the early 1980s (108) and in fact depletion of T cells from the stem cell graft in murine transplantation models leads to persistence of host LC (109). In this model, a secondary challenge with donor T cells predominantly induced GVHD in those mice with persisting host LC (Fig. 4). In addition, GVHD can be prevented by depleting LC with UV radiation prior to T-cell challenge. The influence of T-cell depletion on the persistence of host LC in humans is less well documented than in mice. However, the density of LC at the time of stem cell transplantation depends on the intensity of the preceding conditioning regimen (110,111).

In different models of GVHD, the role of LC in comparison with that of other skin-derived DC is still

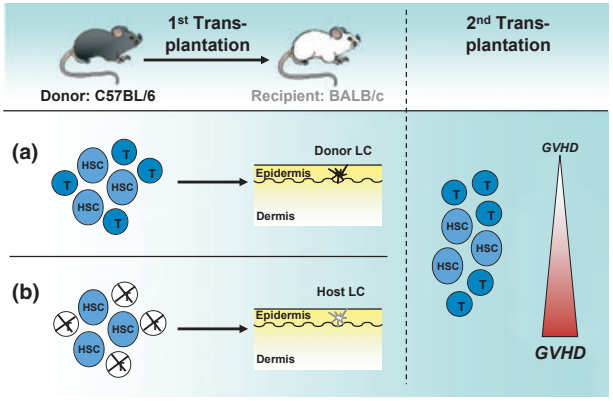

Figure 4. Persistence of host Langerhans cells (LC) is associated with skin GVHD. Lethally irradiated BALB/c mice are reconstituted with either an unaltered (T-cell replete) bone marrow graft (a) or with a T cell-depleted graft (b) from C57BL/6 mice. LC remained of host origin after transplantation with T cell-depleted allogenic haematopoetic stems cells (HSC) but are largely replaced by donor-derived LC after transplantation with a T-cell replete full marrow graft. A second transplantation of a C57BL/6 T-cell replete full marrow graft only results in GVHD and infiltrating donor T cells in the skin of mice with persistent LC (109).

controversial. When skin allografts from either Langerin-DTA mice or wild types were transplanted onto MHC-mismatched recipients, the presence of LC did not seem to be required for rejection (112). This is in contrast to the critical role of LC in transplant rejection reported by Merad et al. (109), and it is currently not clear whether these incongruous results are because of generally enhanced T-cell reactions in Langerin-DTA mice (48) not observed with any other LC-deficient mouse model (16,19,47). Moreover, the kinetics of replacement of skin DC after stem cell transplantation supports the critical role of LC in mediating GVHD, because all dDC populations are rapidly replaced by donor cells. In humans, the kinetics of replacement of dermal APC by transplant-derived cells has been studied extensively (35). The $CD1a^+$ dDC as well as the $FXIIIa^{neg}/CD14^+$ dDC are replaced by donor cells within the first 40 days following transplantation – faster than the replacement of LC (110). Dermal MΦ can be distinguished from $CD14^+$ dDC by their auto-fluorescence and the expression of FXIIIa. They remain of host origin in relevant percentages even beyond the first year after transplantation. It is unlikely, however, that dermal MΦ contribute to the stimulation of donor T cells because they are not depleted during skin GVHD (35). In contrast, LC as well as dDC are rapidly replaced by donor-derived cells after any episode of acute GVHD (108). This advocates for their capacity to serve as targets for infiltrating donor-derived T cells.

In summary, host skin DC are critical for inducing acute GVHD. In particular, epidermal LC persist longer than

dDC after stem cell transplantation and are depleted by donor T cells, suggesting a crucial role of LC in cutaneous GVHD. The priming of donor T cells seems to be dependent on their homing to secondary lymphoid organs, where they encounter allo-antigens presented by skin-derived DC. Therefore, approaches to either deplete host DC from GVHD target organs or to block their homing receptors and co-stimulatory molecules may provide new strategies to prevent GVHD. However, the hierarchy of different recipient-derived skin DC subsets in their ability to induce acute GVHD needs further investigation.

Conclusions

There are still controversial and unresolved issues surrounding skin DC–mediated immune responses. The majority of knowledge is derived from studies in murine experimental models; however, critical aspects such as the persistence of host LC and the correlation to induction of GVHD have been shown to be relevant for humans as well. As DC are critical regulators of skin immune responses, they are attractive targets for immunotherapeutic approaches. Thus, it is important to understand the precise contribution of a particular (skin) DC subset during immune responses against various antigens, including pathogens, haptens or alloantigens. Building on this knowledge, future targeted immune intervention strategies will need to circumvent these cells or exploit DC/LC to, respectively, prevent deleterious and elicit efficient protective immune reactions.

Acknowledgements

This work was supported by grants from the DFG (SFB490, GK1043 and Ste 833/6-1 to E.v.S.) and NWO (DN 93-525 to B.E.C.), by the MAIFOR program of the University of Mainz (to K.K.-N.) and grant 07/07 of the Deutsche José Carreras Leukämiestiftung (to RGM). B.E.C. is a fellow of the Netherlands Organization for Scientific Research (NWO, VIDI 917-76-365) and the Landsteiner Stichting voor Bloedtransfusie Research (LSBR, 0414F).

References

1 Katz S I, Tamaki K, Sachs D H. Epidermal Langerhans cells are derived from cells originating in bone marrow. Nature 1979: **282**: 324–326.
2 Frelinger J G, Hood L, Hill S, Frelinger J A. Mouse epidermal Ia molecules have a bone marrow origin. Nature 1979: **282**: 321–323.
3 Banchereau J, Steinman R M. Dendritic cells and the control of immunity. Nature 1998: **392**: 245–252.
4 Shortman K, Liu Y J. Mouse and human dendritic cell subtypes. Nat Rev Immunol 2002: **2**: 151–161.
5 Shortman K, Naik S H. Steady-state and inflammatory dendritic-cell development. Nat Rev Immunol 2007: **7**: 19–30.
6 Henri S, Vremec D, Kamath A *et al.* The dendritic cell populations of mouse lymph nodes. J Immunol 2001: **167**: 741–748.
7 Boonstra A, Asselin-Paturel C, Gilliet M *et al.* Flexibility of mouse classical and plasmacytoid-derived dendritic cells in directing T helper type 1 and 2 cell development: dependency on antigen dose and differential toll-like receptor ligation. J Exp Med 2003: **197**: 101–109.
8 Takeda K, Akira S. TLR signaling pathways. Semin Immunol 2004: **16**: 3–9.
9 Geijtenbeek T B, van Vliet S J, Engering A, 't Hart B A, van Kooyk Y. Self- and nonself-recognition by C-type lectins on dendritic cells. Annu Rev Immunol 2004: **22**: 33–54.
10 Asselin-Paturel C, Boonstra A, Dalod M *et al.* Mouse type I IFN-producing cells are immature APCs with plasmacytoid morphology. Nat Immunol 2001: **2**: 1144–1150.
11 Wu L, Dakic A. Development of dendritic cell system. Cell Mol Immunol 2004: **1**: 112–118.
12 Schuler G, Steinman R M. Murine epidermal Langerhans cells mature into potent immunostimulatory dendritic cells in vitro. J Exp Med 1985: **161**: 526–546.
13 Romani N, Holzmann S, Tripp C H, Koch F, Stoitzner P. Langerhans cells – dendritic cells of the epidermis. APMIS 2003: **111**: 725–740.
14 Valladeau J, Clair-Moninot V, Dezutter-Dambuyant C *et al.* Identification of mouse langerin/CD207 in Langerhans cells and some dendritic cells of lymphoid tissues. J Immunol 2002: **168**: 782–792.
15 Takahara K, Omatsu Y, Yashima Y *et al.* Identification and expression of mouse Langerin (CD207) in dendritic cells. Int Immunol 2002: **14**: 433–444.
16 Kissenpfennig A, Henri S, Dubois B *et al.* Dynamics and function of Langerhans cells in vivo: dermal dendritic cells colonize lymph node areas distinct from slower migrating Langerhans cells. Immunity 2005: **22**: 643–654.
17 Douillard P, Stoitzner P, Tripp C H *et al.* Mouse lymphoid tissue contains distinct subsets of langerin/CD207 dendritic cells, only one of which represents epidermal-derived Langerhans cells. J Invest Dermatol 2005: **125**: 983–994.
18 Ginhoux F, Collin M P, Bogunovic M *et al.* Blood-derived dermal langerin$^+$ dendritic cells survey the skin in the steady state. J Exp Med 2007: **204**: 3133–3146.
19 Bursch L S, Wang L, Igyarto B *et al.* Identification of a novel population of Langerin$^+$ dendritic cells. J Exp Med 2007: **204**: 3147–3156.
20 Poulin L F, Henri S, de Bovis B, Devilard E, Kissenpfennig A, Malissen B. The dermis contains langerin$^+$ dendritic cells that develop and function independently of epidermal Langerhans cells. J Exp Med 2007: **204**: 3119–3131.
21 Henri S, Poulin L F, Tamoutounour S *et al.* CD207$^+$ CD103$^+$ dermal dendritic cells cross-present keratinocyte-derived antigens irrespective of the presence of Langerhans cells. J Exp Med 2010: **207**: 189–206.
22 Kaplan D H, Kissenpfennig A, Clausen B E. Insights into Langerhans cell function from Langerhans cell ablation models. Eur J Immunol 2008: **38**: 2369–2376.
23 Nagao K, Ginhoux F, Leitner W W *et al.* Murine epidermal Langerhans cells and langerin-expressing dermal dendritic cells are unrelated and exhibit distinct functions. Proc Natl Acad Sci U S A 2009: **106**: 3312–3317.
24 Ritter U, Meissner A, Scheidig C, Korner H. CD8 alpha- and Langerin-negative dendritic cells, but not Langerhans cells, act as principal antigen-presenting cells in leishmaniasis. Eur J Immunol 2004: **34**: 1542–1550.
25 Misslitz A C, Bonhagen K, Harbecke D, Lippuner C, Kamradt T, Aebischer T. Two waves of antigen-containing dendritic cells in vivo in experimental *Leishmania major* infection. Eur J Immunol 2004: **34**: 715–725.
26 Itano A A, McSorley S J, Reinhardt R L *et al.* Distinct dendritic cell populations sequentially present antigen to CD4 T cells and stimulate different aspects of cell-mediated immunity. Immunity 2003: **19**: 47–57.
27 Allan R S, Waithman J, Bedoui S *et al.* Migratory dendritic cells transfer antigen to a lymph node-resident dendritic cell population for efficient CTL priming. Immunity 2006: **25**: 153–162.
28 He Y, Zhang J, Donahue C, Falo L D Jr. Skin-derived dendritic cells induce potent CD8(+) T cell immunity in recombinant lentivector-mediated genetic immunization. Immunity 2006: **24**: 643–656.
29 Cunningham A L, Carbone F, Geijtenbeek T B. Langerhans cells and viral immunity. Eur J Immunol 2008: **38**: 2377–2385.
30 Martin P, Ruiz S R, del Hoyo G M *et al.* Dramatic increase in lymph node dendritic cell number during infection by the mouse mammary tumor virus occurs by a CD62L-dependent blood-borne DC recruitment. Blood 2002: **99**: 1282–1288.
31 Serbina N V, Salazar-Mather T P, Biron C A, Kuziel W A, Pamer E G. TNF/iNOS-producing dendritic cells mediate innate immune defense against bacterial infection. Immunity 2003: **19**: 59–70.
32 Leon B, Ardavin C. Monocyte migration to inflamed skin and lymph nodes is differentially controlled by L-selectin and PSGL-1. Blood 2008: **111**: 3126–3130.
33 Leon B, Ardavin C. Monocyte-derived dendritic cells in innate and adaptive immunity. Immunol Cell Biol 2008: **86**: 320–324.
34 Leon B, Lopez-Bravo M, Ardavin C. Monocyte-derived dendritic cells formed at the infection site control the induction of protective T helper 1 responses against Leishmania. Immunity 2007: **26**: 519–531.
35 Haniffa M, Ginhoux F, Wang X N *et al.* Differential rates of replacement of human dermal dendritic cells and macrophages during hematopoietic stem cell transplantation. J Exp Med 2009: **206**: 371–385.
36 O'Kane C J, Moffat K G. Selective cell ablation and genetic surgery. Curr Opin Genet Dev 1992: **2**: 602–607.
37 Saito M, Iwawaki T, Taya C *et al.* Diphtheria toxin receptor-mediated conditional and targeted cell ablation in transgenic mice. Nat Biotechnol 2001: **19**: 746–750.
38 Naglich J G, Metherall J E, Russell D W, Eidels L. Expression cloning of a diphtheria toxin receptor: identity with a heparin-binding EGF-like growth factor precursor. Cell 1992: **69**: 1051–1061.
39 Collier R J. Understanding the mode of action of diphtheria toxin: a perspective on progress during the 20th century. Toxicon 2001: **39**: 1793–1803.

40 Honjo T, Nishizuka Y, Kato I, Hayaishi O. Adenosine diphosphate ribosylation of aminoacyl transferase II and inhibition of protein synthesis by diphtheria toxin. J Biol Chem 1971: **246**: 4251–4260.

41 Buch T, Heppner F L, Tertilt C *et al.* A Cre-inducible diphtheria toxin receptor mediates cell lineage ablation after toxin administration. Nat Methods 2005: **2**: 419–426.

42 Breitman M L, Clapoff S, Rossant J *et al.* Genetic ablation: targeted expression of a toxin gene causes microphthalmia in transgenic mice. Science 1987: **238**: 1563–1565.

43 Palmiter R D, Behringer R R, Quaife C J, Maxwell F, Maxwell I H, Brinster R L. Cell lineage ablation in transgenic mice by cell-specific expression of a toxin gene. Cell 1987: **50**: 435–443.

44 Jung S, Unutmaz D, Wong P *et al.* In vivo depletion of CD11c(+) dendritic cells abrogates priming of CD8(+) T cells by exogenous cell-associated antigens. Immunity 2002: **17**: 211–220.

45 Bennett C L, Clausen B E. DC ablation in mice: promises, pitfalls, and challenges. Trends Immunol 2007: **28**: 525–531.

46 Probst H C, Tschannen K, Odermatt B, Schwendener R, Zinkernagel R M, Van Den B M. Histological analysis of CD11c-DTR/GFP mice after in vivo depletion of dendritic cells. Clin Exp Immunol 2005: **141**: 398–404.

47 Bennett C L, van Rijn E, Jung S *et al.* Inducible ablation of mouse Langerhans cells diminishes but fails to abrogate contact hypersensitivity. J Cell Biol 2005: **169**: 569–576.

48 Kaplan D H, Jenison M C, Saeland S, Shlomchik W D, Shlomchik M J. Epidermal langerhans cell-deficient mice develop enhanced contact hypersensitivity. Immunity 2005: **23**: 611–620.

49 Merad M, Manz M G, Karsunky H *et al.* Langerhans cells renew in the skin throughout life under steady-state conditions. Nat Immunol 2002: **3**: 1135–1141.

50 Peters N C, Egen J G, Secundino N *et al.* In vivo imaging reveals an essential role for neutrophils in leishmaniasis transmitted by sand flies. Science 2008: **321**: 970–974.

51 van Zandbergen G, Klinger M, Mueller A *et al.* Cutting edge: neutrophil granulocyte serves as a vector for *Leishmania* entry into macrophages. J Immunol 2004: **173**: 6521–6525.

52 Ng L G, Hsu A, Mandell M A *et al.* Migratory dermal dendritic cells act as rapid sensors of protozoan parasites. PLoS Pathog 2008: **4**: e1000222.

53 McConville M J, de Souza D, Saunders E, Likic V A, Naderer T. Living in a phagolysosome; metabolism of *Leishmania* amastigotes. Trends Parasitol 2007: **23**: 368–375.

54 Scott P, Artis D, Uzonna J, Zaph C. The development of effector and memory T cells in cutaneous leishmaniasis: the implications for vaccine development. Immunol Rev 2004: **201**: 318–338.

55 Sacks D, Noben-Trauth N. The immunology of susceptibility and resistance to *Leishmania major* in mice. Nat Rev Immunol 2002: **2**: 845–858.

56 Reiner S L, Locksley R M. The regulation of immunity to *Leishmania major*. Annu Rev Immunol 1995: **13**: 151–177.

57 De Trez C, Magez S, Akira S, Ryffel B, Carlier Y, Muraille E. iNOS-producing inflammatory dendritic cells constitute the major infected cell type during the chronic *Leishmania major* infection phase of C57BL/6 resistant mice. PLoS Pathog 2009: **5**: e1000494.

58 Belkaid Y, Mendez S, Lira R, Kadambi N, Milon G, Sacks D. A natural model of *Leishmania major* infection reveals a prolonged ''silent'' phase of parasite amplification in the skin before the onset of lesion formation and immunity. J Immunol 2000: **165**: 969–977.

59 Woelbing F, Kostka S L, Moelle K *et al.* Uptake of *Leishmania major* by dendritic cells is mediated by Fcgamma receptors and facilitates acquisition of protective immunity. J Exp Med 2006: **203**: 177–188.

60 Belkaid Y, Von Stebut E, Mendez S *et al.* $CD8^+$ T cells are required for primary immunity in C57BL/6 mice following low-dose, intradermal challenge with *Leishmania major*. J Immunol 2002: **168**: 3992–4000.

61 Moll H. The role of dendritic cells at the early stages of *Leishmania* infection. Adv Exp Med Biol 2000: **479**: 163–173.

62 Moll H, Fuchs H, Blank C, Rollinghoff M. Langerhans cells transport *Leishmania major* from the infected skin to the draining lymph node for presentation to antigen-specific T cells. Eur J Immunol 1993: **23**: 1595–1601.

63 Von Stebut E, Belkaid Y, Nguyen B V, Cushing M, Sacks D L, Udey M C. *Leishmania major*-infected murine langerhans cell-like dendritic cells from susceptible mice release IL-12 after infection and vaccinate against experimental cutaneous Leishmaniasis. Eur J Immunol 2000: **30**: 3498–3506.

64 Bottrel R L, Dutra W O, Martins F A *et al.* Flow cytometric determination of cellular sources and frequencies of key cytokine-producing lymphocytes directed against recombinant LACK and soluble *Leishmania* antigen in human cutaneous leishmaniasis. Infect Immun 2001: **69**: 3232–3239.

65 Bourreau E, Gardon J, Pradinaud R *et al.* Th2 responses predominate during the early phases of infection in patients with localized cutaneous leishmaniasis and precede the development of Th1 responses. Infect Immun 2003: **71**: 2244–2246.

66 Kolde G, Luger T, Sorg C, Sunderkotter C. Successful treatment of cutaneous leishmaniasis using systemic interferon-gamma. Dermatology 1996: **192**: 56–60.

67 Geiger B, Wenzel J, Hantschke M, Haase I, Stander S, Von Stebut E. Resolving lesions in human cutaneous leishmaniasis predominantly harbour chemokine receptor CXCR3-positive T helper 1/T cytotoxic type 1 cells. Br J Dermatol 2009.

68 Modlin R L. Th1-Th2 paradigm: insights from leprosy. J Invest Dermatol 1994: **102**: 828–832.

69 Dubsky P, Ueno H, Piqueras B, Connolly J, Banchereau J, Palucka A K. Human dendritic cell subsets for vaccination. J Clin Immunol 2005: **25**: 551–572.

70 Von Stebut E, Belkaid Y, Jakob T, Sacks D L, Udey M C. Uptake of *Leishmania major* amastigotes results in activation and interleukin 12 release from murine skin-derived dendritic cells: implications for the initiation of anti-*Leishmania* immunity. J Exp Med 1998: **188**: 1547–1552.

71 Baldwin T, Henri S, Curtis J *et al.* Dendritic cell populations in *Leishmania major*-infected skin and draining lymph nodes. Infect Immun 2004: **72**: 1991–2001.

72 Ritter U, Osterloh A. A new view on cutaneous dendritic cell subsets in experimental leishmaniasis. Med Microbiol Immunol (Berl) 2007: **196**: 51–59.

73 Iezzi G, Frohlich A, Ernst B *et al.* Lymph node resident rather than skin-derived dendritic cells initiate specific T cell responses after *Leishmania major* infection. J Immunol 2006: **177**: 1250–1256.

74 Liu Y J. IPC: professional type 1 interferon-producing cells and plasmacytoid dendritic cell precursors. Annu Rev Immunol 2005: **23**: 275–306.

75 McKenna K, Beignon A S, Bhardwaj N. Plasmacytoid dendritic cells: linking innate and adaptive immunity. J Virol 2005: **79**: 17–27.

76 Remer K A, Apetrei C, Schwarz T, Linden C, Moll H. Vaccination with plasmacytoid dendritic cells induces protection against infection with *Leishmania major* in mice. Eur J Immunol 2007: **37**: 2463–2473.

77 Saint-Mezard P, Berard F, Dubois B, Kaiserlian D, Nicolas J F. The role of $CD4^+$ and $CD8^+$ T cells in contact hypersensitivity and allergic contact dermatitis. Eur J Dermatol 2004: **14**: 131–138.

78 Martin S F, Jakob T. From innate to adaptive immune responses in contact hypersensitivity. Curr Opin Allergy Clin Immunol 2008: **8**: 289–293.

79 Bennett C L, Noordegraaf M, Martina C A, Clausen B E. Langerhans cells are required for efficient presentation of topically applied hapten to T cells. J Immunol 2007: **179**: 6830–6835.

80 Fukunaga A, Khaskhely N M, Sreevidya C S, Byrne S N, Ullrich S E. Dermal dendritic cells, and not Langerhans cells, play an essential role in inducing an immune response. J Immunol 2008: **180**: 3057–3064.

81 Noordegraaf M, Flacher V, Stoitzner P, Clausen B E. Functional redundancy of Langerhans cells and Langerin+ dermal dendritic cells in contact hypersensitivity. 2010: in press.

82 Clausen B E, Kel J M. Langerhans cells: critical regulators of skin immunity?. Immunol Cell Biol. 2010 May: **88**(4): 351–60.

83 Romani N, Clausen B E, Stoitzner P. Langerhans cells and more: langerin-expressing dendritic cell subsets in the skin. Immunol Rev 2010: **234**: 120–141.

84 Bedoui S, Whitney P G, Waithman J *et al.* Cross-presentation of viral and self antigens by skin-derived $CD103^+$ dendritic cells. Nat Immunol 2009: **10**: 488–495.

85 Toews G B, Bergstresser P R, Streilein J W. Epidermal Langerhans cell density determines whether contact hypersensitivity or unresponsiveness follows skin painting with DNFB. J Immunol 1980: **124**: 445–453.

86 Elmets C A, Bergstresser P R, Tigelaar R E, Wood P J, Streilein J W. Analysis of the mechanism of unresponsiveness produced by haptens painted on skin exposed to low dose ultraviolet radiation. J Exp Med 1983: **158**: 781–794.

87 Schwarz A, Maeda A, Wild M K *et al.* Ultraviolet radiation-induced regulatory T cells not only inhibit the induction but can suppress the effector phase of contact hypersensitivity. J Immunol 2004: **172**: 1036–1043.

88 Schwarz T. 25 years of UV-induced immunosuppression mediated by T cells-from disregarded T suppressor cells to highly respected regulatory T cells. Photochem Photobiol 2008: **84**: 10–18.

89 Schwarz A, Maeda A, Kernebeck K, van Steeg H, Beissert S, Schwarz T. Prevention of UV radiation-induced immunosuppression by IL-12 is dependent on DNA repair. J Exp Med 2005: **201**: 173–179.

90 Loser K, Mehling A, Loeser S *et al.* Epidermal RANKL controls regulatory T-cell numbers via activation of dendritic cells. Nat Med 2006: **12**: 1372–1379.

91 Wong B R, Josien R, Lee S Y *et al.* TRANCE (tumor necrosis factor [TNF]-related activation-induced cytokine), a new TNF family member predominantly expressed in T cells, is a dendritic cell-specific survival factor. J Exp Med 1997: **186**: 2075–2080.

92 Schwarz A, Noordegraaf M, Maeda A, Torii K, Clausen B E, Schwarz T. Langerhans Cells Are Required for UVR-Induced Immunosuppression. J Invest Dermatol 2010 May: **130**(5): 1419–1427.

93 Wang L, Jameson S C, Hogquist K A. Epidermal Langerhans cells are not required for UV-induced immunosuppression. J Immunol 2009: **183**: 5548–5553.

94 Aberer W, Schuler G, Stingl G, Honigsmann H, Wolff K. Ultraviolet light depletes surface markers of Langerhans cells. J Invest Dermatol 1981: **76**: 202–210.

95 Kolgen W, van Steeg H, van der Horst G T *et al.* Association of transcription-coupled repair but not global genome repair with ultraviolet-B-induced Langerhans cell depletion and local immunosuppression. J Invest Dermatol 2003: **121**: 751–756.

96 Appelbaum F R. Haematopoietic cell transplantation as immunotherapy. Nature 2001: **411**: 385–389.

97 Kolb H J. Graft-versus-leukemia effects of transplantation and donor lymphocytes. Blood 2008: **112**: 4371–4383.

98 Korngold R, Sprent J. Lethal graft-versus-host disease after bone marrow transplantation across minor histocompatibility barriers in mice. Prevention by removing mature T cells from marrow. J Exp Med 1978: **148**: 1687–1698.

99 Ferrara J L, Yanik G. Acute graft versus host disease: pathophysiology, risk factors, and prevention strategies. Clin Adv Hematol Oncol 2005: **3**: 415–419. 428

100 Sun Y, Tawara I, Toubai T, Reddy P. Pathophysiology of acute graft-versus-host disease: recent advances. Transl Res 2007: **150**: 197–214.

101 Hill G R, Crawford J M, Cooke K R, Brinson Y S, Pan L, Ferrara J L. Total body irradiation and acute graft-versus-host disease: the role of gastrointestinal damage and inflammatory cytokines. Blood 1997: **90**: 3204–3213.

102 Shlomchik W D, Couzens M S, Tang C B *et al.* Prevention of graft versus host disease by inactivation of host antigen-presenting cells. Science 1999: **285**: 412–415.

103 Ruggeri L, Capanni M, Urbani E *et al.* Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. Science 2002: **295**: 2097–2100.

104 Matte C C, Liu J, Cormier J *et al.* Donor APCs are required for maximal GVHD but not for GVL. Nat Med 2004: **10**: 987–992.

105 Beilhack A, Schulz S, Baker J *et al.* Prevention of acute graft-versus-host disease by blocking T-cell entry to secondary lymphoid organs. Blood 2008: **111**: 2919–2928.

106 Auffermann-Gretzinger S, Lossos I S, Vayntrub T A *et al.* Rapid establishment of dendritic cell chimerism in allogeneic hematopoietic cell transplant recipients. Blood 2002: **99**: 1442–1448.

107 Perreault C, Pelletier M, Belanger R *et al.* Persistence of host Langerhans cells following allogeneic bone marrow transplantation: possible relationship with acute graft-versus-host disease. Br J Haematol 1985: **60**: 253–260.

108 Suitters A J, Lampert I A. The loss of Ia$^+$ Langerhans' cells during graft-versus-host disease in rats. Transplantation 1983: **36**: 540–546.

109 Merad M, Hoffmann P, Ranheim E *et al.* Depletion of host Langerhans cells before transplantation of donor alloreactive T cells prevents skin graft-versus-host disease. Nat Med 2004: **10**: 510–517.

110 Collin M P, Hart D N, Jackson G H *et al.* The fate of human Langerhans cells in hematopoietic stem cell transplantation. J Exp Med 2006: **203**: 27–33.

111 Meyer R G, Britten C M, Wehler D *et al.* Prophylactic transfer of CD8-depleted donor lymphocytes after T-cell-depleted reduced-intensity transplantation. Blood 2007: **109**: 374–382.

112 Obhrai J S, Oberbarnscheidt M, Zhang N *et al.* Langerhans cells are not required for efficient skin graft rejection. J Invest Dermatol 2008: **128**: 1950–1955.

113 Nakano H, Yanagita M, Gunn M D. CD11c(+)B220(+)Gr-1(+) cells in mouse lymph nodes and spleen display characteristics of plasmacytoid dendritic cells. J Exp Med 2001: **194**: 1171–1178.

114 Brewig N, Kissenpfennig A, Malissen B *et al.* Priming of CD8$^+$ and CD4$^+$ T cells in experimental leishmaniasis is initiated by different dendritic cell subtypes. J Immunol 2009: **182**: 774–783.

115 Lopez-Bravo M, Ardavin C. In vivo induction of immune responses to pathogens by conventional dendritic cells. Immunity 2008: **29**: 343–351.

116 Zaba L C, Krueger J G, Lowes M A. Resident and ''inflammatory'' dendritic cells in human skin. J Invest Dermatol 2009: **129**: 302–308.