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Origin and functional heterogeneity of non-lymphoid tissue dendritic cells in mice

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Summary: Dendritic cells (DCs) have been extensively studied in mice lymphoid organs, but less is known about the origin and the mechanisms that regulate DC development and function in non-lymphoid tissues. Here, we discuss recent evidence establishing the contribution of the DC-restricted lineage to the non-lymphoid tissue DC pool and discuss the mechanisms that control the homeostasis of non-lymphoid tissue DCs. We also review recent results underlining the functional specialization of tissue DCs and discuss the potential implications of these findings in tissue immunity and in the development of novel vaccine strategies.

Keywords: *dendritic cells, CD103$^+$ dendritic cells, non-lymphoid tissue, homeostasis, functional specialization*

Introduction

Dendritic cells (DCs) are a heterogeneous population of cells that localize in most tissue including lymphoid and non-lymphoid organs in the steady state. The main role of tissue DCs is the induction of specific immunity against invading pathogens (1, 2). Three types of DCs have been described in mice and humans. Plasmacytoid DCs represent a population of DCs that accumulate mainly in lymphoid organs in the steady state and whose major function is to secrete very high amounts of interferon-α (IFNα) in response to viral infections prior to differentiating into mature DCs able to prime T cells against viral antigens (3). Monocyte-derived DCs accumulate mainly in inflamed tissue (4). By opposition to plasmacytoid DCs and monocyte-derived DCs, tissue-resident steady-state DCs are called 'classical' DCs (cDCs). This review focuses mainly on the origin, developmental cues, and function of non-lymphoid tissue-resident cDCs.

Phenotypic characterization of non-lymphoid tissue-resident cDCs

Non-lymphoid tissue-resident cDCs are present in most tissues in the steady state. DCs that populate stratified epithelia are often called Langerhans cells (LCs), whereas DCs in connective

tissues are called interstitial DCs. Because these terms do not provide details on the origin or function of the cells, we chose to refer to non-lymphoid tissue DCs only by the name of the tissue in which these cells reside.

Phenotypically, DCs have a forward and side scatter that fall in the monocyte gate, they lack lineage markers, express both the hematopoietic marker CD45 and the integrin CD11c and constitutively express major histocompatibility complex class II (MHCII). Several studies have now established that this definition is too broad and includes distinct DC populations with different origins and functions.

The cutaneous DC network

Cutaneous DCs are present throughout the skin in the epidermis and the dermis (5). DCs in the epidermis are also known as LCs, whereas dermal DCs belong to a broader subset of interstitial DCs (5). Through their extended dendrites, LCs form a continuous cellular network to survey foreign antigens that breach the skin thus providing the first immunological barrier to the external environment. Epidermal LCs account for 3–5% of all nucleated cells in the epidermis of mice and human, with approximately 700 LCs/mm^2 that are arranged in a network occupying the interstices between neighboring keratinocytes (5). LCs are the only hematopoietic cells in the epidermis in quiescent human skin, whereas mice have an additional population of epidermal γδ T cells (6).

Human and murine LCs are easily identified in the epidermis based on the expression of CD45 and MHCII molecules. They also constitutively express the lectin receptor langerin along with its associated Birbeck granules. Human and murine LCs express the adhesion molecules E-cadherin and the epithelial-cell adhesion molecule (EpCAM), which anchor LCs to neighboring keratinocytes (7, 8) and the lectin CD205 implicated in antigen capture and antigen processing (9, 10). Human but not murine LCs express high levels of CD1a (previously named OKT6; 11), a member of the group 1 CD1 protein (CD1a, CD1b, and CD1c), which has the capacity to present microbial lipid antigens to T cells (12). Some studies refer to all DC populations present in stratified epithelia as LCs, including those present in the cornea (13), oral mucosa (14), tonsils (15), pharynx, upper esophagus (16), vagina, and ectocervix (16). Stratified epithelia DCs have been called LCs based on their localization and the expression of langerin, but it remains to be examined whether these cells are ontogenically related to LCs. One key feature of epidermal LCs is their ability to repopulate locally independently of circulating precursors (17). This key homeostatic property distinguishes them not only from other cutaneous DCs but also from DCs in other stratified epithelia locations (18) suggesting that epidermal LCs represent a unique population. We therefore propose restricting the term LCs to epidermal DCs.

Dermal DCs have been much less studied than LCs owing to the difficulty in isolating these cells. DCs in the dermis include dermal-resident DCs and migratory LCs on their way to the draining lymph nodes (LNs; 5). Up until recently, human and murine dermal-resident DCs were thought to form a homogenous population easily distinguishable from migratory LCs based on the absence of langerin expression (19). There is now clear evidence that at least in mice, dermal-resident DCs contain an additional DC population, independent of LCs that also express langerin (20–23).

The classical dermal langerin$^-$ DCs represent the majority of the dermal DC pool, they lack langerin expression, and express high levels of the integrin CD11b and several macrophage markers such as F4/80, CX3CR1, and SIRPα (24). The recently identified langerin$^+$ DC population represents 20% of the total dermal DC pool. In contrast to LCs, dermal langerin$^+$ DCs express the integrin αEβ7 (also called CD103) (25), they lack the adhesion molecules E-cadherin and Epcam, and express low levels of the integrin CD11b (Fig. 1). CD103$^+$ DCs do not express the chemokine receptor CX3CR1, F4/80, and SIRPα (24). For simplicity, the two dermal-resident DC populations are referred to as CD103$^+$ DCs and CD11b$^+$ DCs throughout this review. CD103 is not an essential molecule for DC development, nor it is a specific marker of DCs. CD103 is also expressed on epithelial T cells (25), and CD103$^{-/-}$ mice do not have major defects in DC development (J. Helft and M. Merad, unpublished data). CD103$^+$CD11b$^-$ langerin$^+$ DCs are also present in lymphoid organs where they co-express CD8αα antigen on the cell surface (J. Helft and M. Merad, unpublished data). In addition to these two major DC subsets, MHCII$^+$CD11c$^+$CD103$^-$langerin$^-$CD11b$^-$ cells have also been identified in the dermis and shown to migrate to the draining LN in the inflamed setting (23, 24).

The lung cDC network

Lung MHCII$^+$CD11c$^+$ cells form a rich network of cells that accumulates in the airways epithelia and the lung parenchyma (26). DCs that accumulate in the stratified epithelia of the large airways resemble LCs (18, 26). They are organized in a tight network of cells (26) and express high levels of langerin (27, 28) and the integrin CD11b (A.J. Bonito, F. Ginhoux, and M. Merad, unpublished data). The lung parenchyma contains

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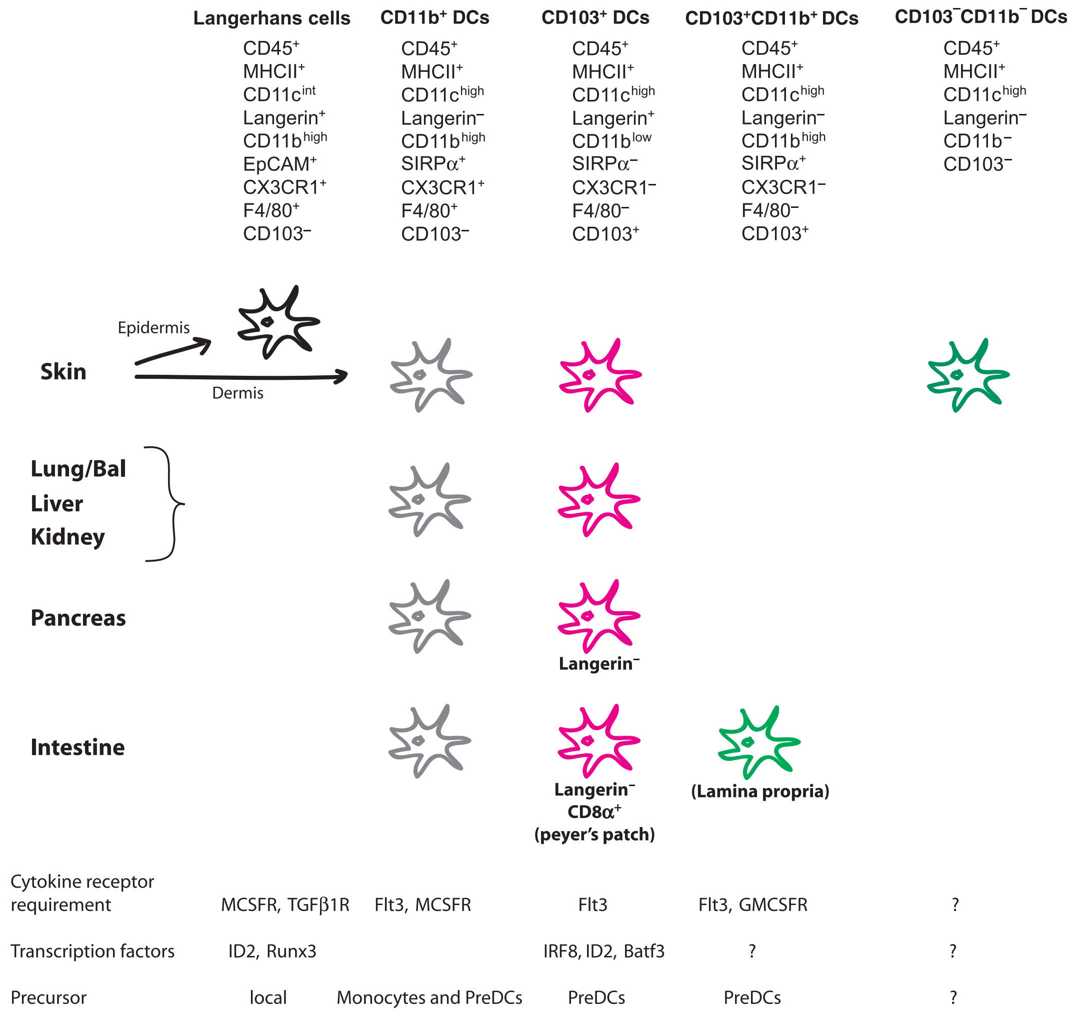

Fig. 1. Characterization of tissue-resident dendritic cells (DCs). Two DC compartments with distinct cell surface phenotype and immune function have been identified in most non-lymphoid tissues. The nature of the bone marrow precursor that gives rise to each DC subset, the growth factor receptor requirements, as well as the transcription factors that control their development are summarized here. Additional DC populations can be found in the skin and intestine. In the skin, embryonically derived DCs also called Langerhans cells populate the epidermis. In the intestine, a population of CD103$^+$ DCs with a distinct phenotype and regulatory program than most non-lymphoid tissue CD103$^+$ DCs populates the *lamina propria*.

two DC populations that accumulate near the small airway epithelia. Phenotypically, these cells resemble those present in the dermis and include CD103$^+$ DCs and CD11b$^+$ DCs (29) (Fig. 1). Although in most tissues the proportion of CD103$^+$ DCs among total DCs rarely exceeds 20%, they are present in higher numbers in the lung where they represent up to 40% of the total lung DC pool.

The intestinal cDC network

MHCII$^+$CD11c$^+$ cells are present throughout the intestine where they reside in the *lamina propria* facing the lumen (30, 31) and in the muscular layers and the serosa facing the peritoneum (32). DCs also accumulate in intestinal lymphoid tissues that include the Peyer's patches, isolated lymphoid follicles, and the mesenteric LNs (33). Although the phenotype of DCs in the Peyer's patches and mesenteric LNs has been the subject of several reviews (33, 34), the diversity of *lamina propria* DCs is only starting to be unraveled. Here, we will review mainly recent developments in *lamina propria* DC biology.

Lamina propria DCs that express the cell surface molecule CD103 or CX3CR1 play key roles in mucosal immunity. CD103$^+$ DCs were shown to have a superior ability to induce the expression of gut homing molecules on T lymphocytes and to drive the peripheral generation of Foxp3$^+$ T-regulatory cells (29, 35, 36), whereas the expression of CX3CR1 by DCs is thought to control the projection of dendrites through the epithelial cell layer and the sampling of luminal antigens (37). However, it remained unclear whether CD103 and CX3CR1

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molecules were expressed by similar or distinct DC populations. We recently found that MHCII$^+$CD11c^{hi} DCs accumulate mainly in the intraepithelial and *lamina propria* cell fractions and were absent from the muscularis and the serosa. MHCII$^+$CD11c$^+$ DCs included three populations best characterized as CD103$^+$CD11b$^-$, CD103$^+$CD11b$^+$, and CD103$^-$CD11b$^+$ DCs (38) (*Figs 1 and 2*). CD103$^+$CD11b$^-$ DCs were enriched in the Peyer's patches, and the majority of these cells also co-expressed CD8αα on the cell surface, whereas CD103$^+$CD11b$^+$ and CD103$^-$CD11b$^+$ DCs accumulated mainly in the *lamina propria*. Similar to other tissues, *lamina propria* CD103$^+$ DCs in the small bowel represented no more than 20% of the total DC pool. CX3CR1 was expressed exclusively on CD103$^-$CD11b$^+$ DCs and was absent from CD103$^+$CD11b$^-$ DCs and CD103$^+$CD11b$^+$ DCs (38). In contrast to the mucosa, the muscularis and serosa layers contained only one homogeneous DC population characterized as MHCII^{hi}CD11c^{lo}CD103$^-$CD11b$^+$CX$_3$CR1$^+$ (38). Similar populations were found in the colon, although the number of CD103$^+$CD11b$^-$ DCs was higher in the colon than in the small bowel. Altogether our results suggest that most CD103$^+$ DCs in non-lymphoid tissues express low levels of CD11b and lack CX3CR1, with the exception of the *lamina propria* CD103$^+$ DCs that express high levels of CD11b but still lack CX3CR1. CD103$^+$CD11b$^-$ DCs are also present in the gut, but they accumulate mainly in the lymphoid tissue and similar to the CD103$^+$ DC subset of lymphoid organ-resident CD8$^+$ DCs, they express the CD8αα homodimer and the integrin CD103 and lack the integrin CD11b (38).

cDCs in distant tissues

MHCII$^+$CD11c$^+$ cells are present in most tissues in the steady state with the exception of the brain parenchyma. Liver, kidney, and pancreatic islet DCs resemble those present in the dermis and the lung parenchyma and include CD103$^+$ and CD11b$^+$ DCs (24) (*Fig. 1*). In all these tissues, the relative number of CD103$^+$ DCs is much lower than that of CD11b$^+$ DCs and rarely exceeds 20% of the total tissue DC pool. The phenotype of these DC compartments is similar to the phenotype of the dermal DC subsets with the exception of langerin, which is expressed at variable levels among tissue DCs and is absent from pancreatic islet DCs (24). In addition to these two major DC subsets, MHCII$^+$CD11c$^+$ CD103$^-$langerin$^-$CD11b$^-$ cells (24) can also be found in these tissues and a better characterization of this population both at the protein and mRNA levels is currently being done in the laboratory.

Tissue-migratory DCs

DCs are thought to migrate to the draining LN at a very low rate in the steady state (39–41) and their rate of migration is increased during inflammation (42). The chemokine receptor CCR7 controls DC migration to the draining LN (43, reviewed in 44). CCR7-deficient mice LNs lack mainly tissue-migratory DCs but not lymphoid-resident DCs (44).

Transgenic mice with enforced production of melanin granules in the skin have been used to assess steady-state migration of cutaneous DCs. Because melanin granules are not degradable they can be used to trace antigen trafficking to the LN in these mice. Interestingly, melanin granules were absent from the LN of mice deficient in transforming growth factor-β1 (TGF-β1) but not in mice deficient in Fms-like thyrosine kinase 3 ligand (Flt3L; 40). As LCs represent the only cutaneous DC subset that is dependent on TGF-β1 (8) and independent of Flt3L (see next), these results suggest that LCs are the main population that transport melanin to the draining LN in the steady state. To assess the steady-state migration of DCs in other tissues, we used *LysM-Cre* × *Rosa26-floxstopfloxEGFP* mice in which *Cre* activity removes a stop cassette upstream of the floxed reporter and induces irreversible expression of enhanced green fluorescence protein (eGFP) in lysozyme M (LysM)-positive cells and their progeny (45). Lysozyme is expressed by granulocytes, monocytes, and a subset of DCs. The hypothesis underlying this study was that eGFP$^+$ DCs in non-lymphoid tissues should not give rise to DCs expressing lower eGFP levels in the draining LN, as eGFP expression is irreversible in these mice. Therefore, this model can be used only to trace the steady-state migration of lysozyme eGFP$^+$ DCs but not lysozyme eGFP$^-$ DCs (45). In the *lamina propria*, 90% of CD103$^-$CD11b$^+$ *lamina propria* DCs are eGFP$^+$ in *LysM-Cre* × *Rosa26-floxstopfloxEGFP* mice, whereas less than 20% DCs express eGFP in the mesenteric LN suggesting that CD103$^-$CD11b$^+$ *lamina propria* DCs are unlikely to migrate to the mesenteric LN in the steady state (38). The migration of *lamina propria* CD103$^+$CD11b$^-$ DCs could not be assessed in this model because they express low levels of lysozyme/eGFP.

In contrast in the inflamed setting, tissue DC migration to the draining LN increases many fold (45). Interestingly in the skin, cutaneous DC subsets differentially migrate to the draining LN in response to contact sensitizing agents. Migration of CD11b$^+$ and CD103$^+$ dermal DCs peaked after 1 day, followed by LCs at 4 days post-skin sensitization (23, 46) although in a herpes simplex virus (HSV) skin infection model, epidermal LCs emigrated from the epidermis at earlier time points (47). Similarly in the gut, CD103$^+$CD11b$^+$ *lamina*

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propria are the first DC population to arrive in the mesenteric LN upon *Salmonella* infection (38) (see next section). The functional implications of the differential migration of tissue DC populations remain to be examined.

Turnover of non-lymphoid tissue DCs

Tissue cDCs sample antigens and migrate constantly through afferent lymphatics to the T-cell areas of LNs, a process that increases many fold in response to inflammatory signals (reviewed in 44). Although DC efflux from tissues to the tissue-draining LN is difficult to quantify accurately, it is clear that tissue DC homeostasis requires constant replacement with new cells. Results from studies assessing bromodeoxyuridine (BrdU) labeling, cell cycle analysis, and rate of DC replacement in bone marrow chimera mice and parabiotic mice have helped characterize DC turnover in tissue. Although most of these studies have been performed in lymphoid tissue-resident DCs, recent studies have started to examine the turnover of DCs in non-lymphoid tissue.

BrdU labels actively proliferating cells and cells that derive from proliferating precursors. As DCs are constantly recruited from circulating bone marrow-derived circulating precursors, we measured BrdU labeling at early time points after injection to identify DC proliferation in non-lymphoid tissues. We found that 12 h after BrdU injection, 5–10% of DCs in the dermis and lung, and 10–20% of DCs in the liver and kidney were labeled with BrdU. The relative numbers of BrdU-labeled CD103$^+$ DCs and CD11b$^+$ DCs were similar except in the kidney where the CD11b$^+$ DC subset was labeled to a lower level suggesting that both DC subsets in tissues are dividing in the steady state (24).

Although the rate at which tissue-resident DCs are replaced by hematopoietic precursors in bone marrow chimera mice is often used as a measure of DC turnover in mice, these measures reflect only DC turnover after radiation-induced injuries as bone marrow reconstitution is usually performed in irradiated animals. Kidney and heart cDCs are replaced in 2–4 weeks after lethal irradiation and bone marrow reconstitution, whereas DC repopulation in the vagina (16), airway epithelia (18), and the intestine is more rapid and occurs in 7–13 days (M. Bogunovic and M. Merad, unpublished data).

Parabiotic mice provide a valuable assessment of the physiological turnover of DCs in non-lymphoid tissues. Parabionts are surgically attached mice that share the same blood circulation but separate organs for long periods of time (48). The level of tissue DC chimerism in parabionts depends on DC turnover in tissue, but is also dependent on the half-life of DC precursors in the blood. Circulating myeloid precursors are thought to have a shorter lifespan in circulation than lymphoid precursors, leading to a reduced mixing of myeloid cells compared with lymphoid cells in parabionts (49). Instead, measurement of the decay of parabiont-derived DCs in the lung, liver, kidney and spleen after parabiosis separation provides a better assessment of DC lifespan in non-lymphoid tissues. The rate of DC replacement by blood precursors occurs in 12–14 days in most tissues including the spleen, LN, liver, and kidney with the exception of the lung. Among lung DCs, most CD11b$^+$ DCs were lost in 30 days after separation (15 days half-life), whereas only 50% of parabiont-derived lung CD103$^+$ DCs were lost at that time (30 days half-life) (24). These results suggest that DCs in non-lymphoid tissues undergo a limited number of divisions and must be continually replaced by blood-borne precursors.

In contrast, epidermal LC lifespan differs fundamentally from that of other cDCs (reviewed in 5). Approximately, 2–3% of LCs are constantly cycling in the steady state and LCs are maintained locally independently of circulating precursors throughout life (17). Although not representative of steady-state turnover, it is interesting that in patients who received allogeneic hematopoietic cell transplantation, recipient LCs can be identified unequivocally in the epidermis more than 1 year after transplantation (50). Donor LCs have also been shown to persist for years in a recipient of a human limb graft (51) suggesting that human LCs like their murine counterparts repopulate locally in the steady state.

Origin of non-lymphoid tissue-resident cDCs

The DC-restricted lineage

With the exception of epidermal LCs, DCs have a limited lifespan in tissue and must be continually replaced by circulating blood DC precursors (17, 49). The identity of the circulating precursors that contribute to steady-state DC replenishment has been difficult to establish. Recent results from several laboratories revealed the presence of a DC-restricted lineage that originates from the bone marrow and gives rise to DCs in most tissues.

Functionally specialized hematopoietic cells in peripheral tissue are thought to derive from bone marrow hematopoietic progenitors that have progressively lost their developmental potential to other cell types (52). Current data suggest the earliest lineage potential decision a developing multipotent progenitor population must make is whether to become a lymphoid or myeloid cell type, and once it does, this decision is permanent. Early committed myeloid and lymphoid pro-

genitors have been identified in mice and men (52). Common myeloid progenitors (CMPs) give rise to granulocytes, erythrocytes, megakaryocytes, and monocytes, whereas common lymphoid progenitors give rise mainly to lymphocytes. In addition to these committed progenitors, more recent studies reveal overlapping and alternative graded stages of early lineage commitment (53).

The successive commitment steps in DC differentiation have recently been elucidated (*Fig.* 2). Studies suggest that DCs differentiate from a myeloid precursor that has lost the potential to give rise to granulocytes, erythrocytes, and megakaryocytes but maintains its capacity to differentiate into monocytes, macrophages, DCs, and plasmacytoid DCs. This precursor called the macrophage/DC precursor (MDP) gives rise to a common DC precursor (CDP) which subsequently produces cDC-restricted precursors called pre-cDCs and plasmacytoid DCs in the bone marrow but has lost the potential to give rise to monocytes (49, 54–61). Pre-cDCs leave the bone marrow and circulate through the blood to home to lymphoid organs where they differentiate into lymphoid tissue-resident DCs (*Fig.* 2). Each of these differentiation steps corresponds to distinct immuno-phenotypes that can be captured using flow cytometry analysis. Because adoptive transfer of purified progenitors *in vivo* in congenic mice remains the main model to assess the differentiation potential of hematopoietic restricted progenitors, accurate phenotypic markers are critical to define these progenitor populations. For example, MDPs were first described as lineage negative cells expressing the chemokine receptor CX3CR1, the receptor for the macrophage colony-stimulating factor receptor (M-CSFR also called CD115), and c-kit and were thought to give rise upon adoptive transfer to macrophages and lymphoid organ-resident cDCs but not to plasmacytoid DCs. Subsequent studies showed that lineage^{-}ckit^{hi}CX3CR1^{+}

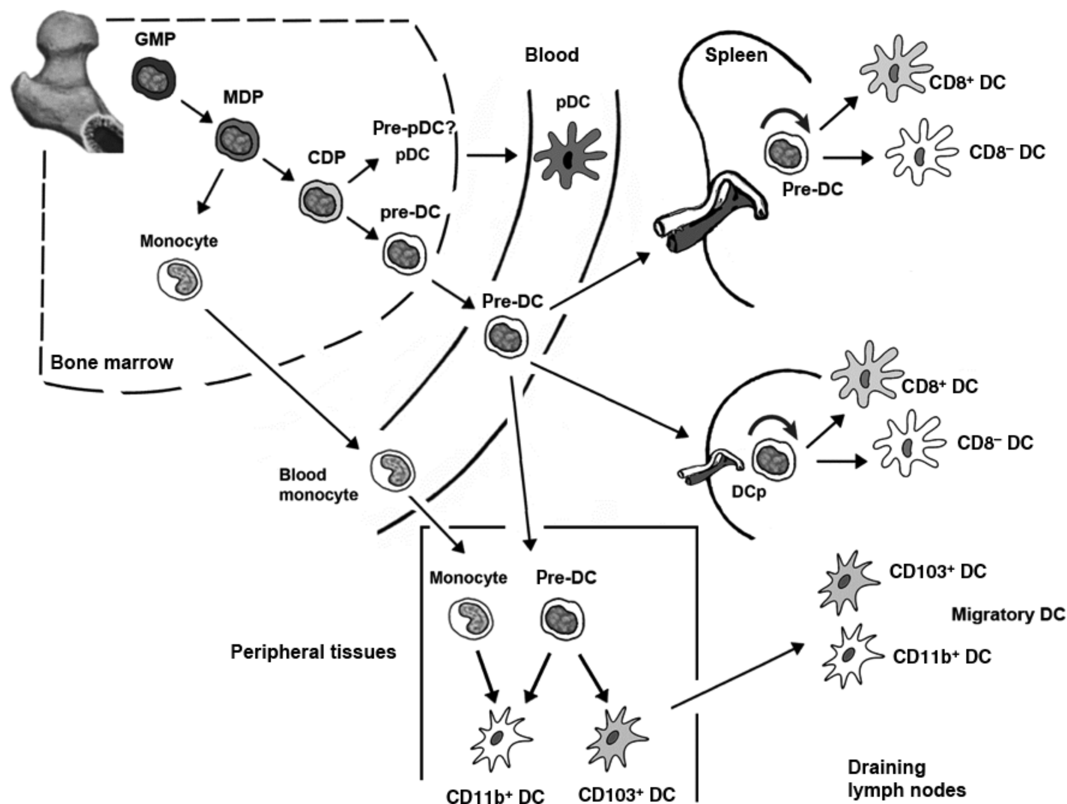

Fig. 2. Origin of non-lymphoid tissue dendritic cells (DCs). The successive commitment steps in DC differentiation have recently been elucidated. These studies suggest that DCs differentiate from a myeloid precursor that has lost the potential to give rise to granulocytes, erythrocytes, and megakaryocytes but maintains its capacity to differentiate into monocytes, macrophages, DCs, and plasmacytoid DCs. This precursor called the macrophage/DC precursor (MDP) gives rise to a common DC precursor (CDP), which subsequently produces classical DC (cDC)-restricted precursors called (pre-cDCs) and plasmacytoid DCs in the bone marrow but has lost the potential to give rise to monocytes. Pre-cDCs leave the bone marrow and circulate through the blood to home to lymphoid organs where they differentiate into lymphoid tissue-resident CD8α^{+} and CD8α^{-} DCs. Pre-cDCs also home to non-lymphoid tissue to give rise to DCs. In the intestine, pre-cDCs give rise exclusively to *lamina propria* CD103^{+}CD11b^{+} DCs and Peyer's patches CD103^{+}CD11b^{-} DCs but fail to give rise to CD103^{-}CD11b^{+} DCs, whereas monocytes give rise exclusively to CD103^{-}CD11b^{+} DCs. In other tissues, pre-cDCs give rise preferentially to CD103^{+} DCs, although they can also differentiate into CD11b^{+} DCs, whereas monocytes give rise mainly to CD11b^{+}.

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M-CSFR^{+}-expressing the receptor for Flt3 can also give rise to plasmacytoid DCs in addition to monocytes, macrophages, and cDCs (58). This revised phenotypic definition is now used to define MDP *in vivo* (*Table* 1). Based on these results, we conclude that a DC-restricted lineage is present in the bone marrow and separates from a monocyte/macrophage lineage at the level of the MDP.

Common lymphoid precursors are very efficient at giving rise to thymic DCs but less efficient than the common myeloid precursor at giving rise to spleen and LN DCs upon adoptive transfer in irradiated animals (62). Common lymphoid precursors can also differentiate directly into DCs upon stimulation with Toll-like receptor (TLR) ligands *in vitro* bypassing the usual cellular differentiation steps (63). These data suggest that although myeloid progenitors are the main contributors of non-thymic DCs, lymphoid precursors can also acquire the ability to differentiate into non-thymic DCs in response to injury signals.

Recent studies in the laboratory established that in addition to blood and lymphoid organs, pre-cDCs are also present in non-lymphoid tissues, such as the liver, kidney, and lung suggesting that pre-cDCs not only home to lymphoid organs but also to non-lymphoid tissues (24). To examine the contribution of DC-restricted precursors to non-lymphoid tissue DCs, we adoptively transferred purified CDP and pre-cDCs intravenously into naïve congenic mice and looked for their DC progeny 7 days later. CDP and pre-cDCs gave rise preferentially to liver and kidney CD103^{+} DCs but were also able to give rise to CD11b^{+} DCs in these tissues (24). Although the preferential differentiation of DC-restricted progenitors into CD103^{+} DCs could reflect true developmental bias, we cannot rule out that *ex vivo* manipulation of DC progenitors also affected their differentiation potential.

We were unable to recover CDP or pre-cDCs progeny in the lung, likely because the half-life of lung DCs exceeds the half-life of circulating DC precursors as discussed before and we are currently examining whether CDP and pre-cDCs give rise to lung DCs in mice that have been depleted of their lung DC content. In the intestine, CDP and pre-cDCs gave rise exclusively to CD103^{+}CD11b^{+} DCs in the *lamina propria* and to CD103^{+}CD11b^{-} DCs in the Peyer's patches but did not give rise to CD103^{-}CD11b^{+} *lamina propria* DCs, restricting the contribution of the DC-restricted lineage only to mucosal CD103^{+} DCs (38).

Monocyte contribution to non-lymphoid tissue DCs in the steady state

Monocytes were initially described as circulating precursors for tissue macrophages. Two monocyte subsets can be found in mouse blood. These monocytes differ in the expression of the molecule Ly6C as well as molecules involved in leukocyte migration and homing (64). Ly6C^{hi} monocytes are CCR2^{+}CX3CR1^{low}CD62L^{+}, whereas Ly6C^{-} monocytes are CCR2^{-}CX3CR1^{hi}CD62L^{-} (64). Interestingly, Ly6c^{+} monocytes are recruited to inflamed tissues, whereas the Ly6C^{-} subset appears to be recruited to non-inflammatory sites by a CX3CR1-dependent mechanism (64). These subsets correspond to the CD14^{+}CD16^{-} and CD14^{-}CD16^{+} human monocyte subpopulations, respectively (65).

The discovery of the culture conditions that leads to the differentiation of human circulating monocytes into DCs *in vitro* (66) has been largely used in the field to study the cues that regulate DC differentiation and maturation processes. Monocyte-derived DCs have also been used to promote tissue immunity in patients (67). However, the exact contribution of monocytes to tissue-resident DCs in the steady state remains a difficult question to address experimentally. Genetic tagging of monocytes is difficult owing to the lack of specific markers. Systemic injection of latex beads has been used to follow the

***Table 1.* Phenotype of the classical dendritic cell (cDC) progenitors in mice**

DC precursor	MDP (Fogg *et al.*)	MDP (Varol *et al.*)	MDPΔ (Waskow *et al.*)	MDP (Liu *et al.*)	CDP (Onai *et al.*; Naik *et al.*)	CDP (Liu *et al.*)	Pre-cDCs (Liu *et al.*)
Lineage markers	–	–	–	–	–	–	–
CD11c	–	–	–	–	–	–	+
MHC II	–	–	–	–	–	–	–
c-kit	+	+	∓	High	Low	Low	ND
CX3CR1	+	+	+	+	ND	+	+
CD115	∓ (mRNA+)	+	+	+	+	+	ND
Flt3/CD135	∓ (mRNA+)	ND	∓	+	+	+	High
References	(56)	(61)	(178)	(58)	(59, 60)	(58)	(58)

MDP, macrophage/DC precursor; CDP, common DC precursor; MHC, major histocompatibility complex; Flt3, Fms-like thyrosine kinase 3; ND, not determined.

progeny of the two monocyte subsets *in vivo* (68). Using this technique, Ly6/c^{+} monocytes were found to give rise to lung CD103^{+} DCs, whereas Ly6c1^{lo} monocytes differentiate mainly into lung CD103^{-} DCs (69). Adoptive transfer experiments have also been used to trace monocyte differential potential *in vivo*. In contrast to DC-restricted precursors, monocytes do not expand after adoptive transfer and monocyte-derived cells may never reach threshold detection levels which may explain why several studies have failed to detect monocyte-derived DCs in this model. We have also failed to detect a substantial contribution of adoptively transferred monocytes to lung, kidney, liver, and *lamina propria* DCs in the steady state (24). To increase the sensitivity of the transfer, we and others have injected monocytes into transgenic mice expressing the diphtheria toxin receptor (DTR) under the CD11c promoter (CD11c^{-} DTR mice) and used DT injection to eliminate tissue-resident CD11c^{+} cells (70). In DT-treated mice, adoptively transferred monocytes gave rise to *lamina propria* CD103^{-}CD11b^{+} DCs but failed to give rise to CD103^{+} DCs (38, 71). We are currently examining whether monocytes can also give rise to DCs in other tissues in DT-treated mice and whether similar to mucosal DCs they give rise specifically to CD103^{-} DCs.

The LC exception

In contrast to most DC populations, LCs are maintained locally independently of circulating precursors in the steady state (17). These results were established in two separate models. First, in parabiotic mice, LCs did not mix in the epidermis up to a year after parabiosis, whereas approximately 20–30% dermal and lymphoid organ DCs were mixed in 4 months post-parabiosis. In lethally irradiated mice, LCs remained of host origin more than 18 months post-transplant, whereas lymphoid organ DCs were almost entirely replaced by 3–4 weeks post-transplant.

Induction of minor skin injuries such as X-ray irradiation or skin sensitization in congenic bone marrow chimeric mice leads to partial elimination of LCs followed by their complete recovery in 1–4 weeks after tissue injury. LC recovery occurs independently of circulating precursors, as most LCs remained of host origin in these mice (17). Recent data in the laboratory suggest that LC repopulation in these mice occurs through local proliferation of differentiated LCs and we are currently examining the mechanisms that control LC proliferation and LC homeostasis in adult skin. In contrast, in major inflammatory injuries such as graft versus host disease (72) and exposure to ultraviolet (UV) light (17), LCs are repopulated by circulating precursors in a M-CSFR-dependent manner (73). LC repopulation can also be of mixed origin and derives from local and circulating precursors in a model of mild cutaneous graft versus host disease (72) and in HSV-infected skin that leads to limited LC depletion in the infected dermatome (47). LC repopulation by circulating precursors is dependent on the chemokine receptor CCR2 and CCR2 ligands including the macrophage chemokine proteins MCP-1 and MCP-3 that are secreted at much higher levels in UV-exposed skin compared with sensitized or X-ray-exposed skin (17, 72).

Origin of DCs in inflamed tissues

Depending on the degree of inflammation, tissue-resident DCs are either absent or reduced from injured sites and replaced by newly recruited blood-derived DCs. Monocytes represent an important source of blood-derived DCs in inflamed tissue and participate in tissue immunity (65).

In the skin for example, monocyte-derived DCs accumulate in the dermis of inflamed skin and play a role in tissue immunity (74, 75). In contrast, in the epidermis the replacement of LCs by circulating monocytes is dependent on the degree of inflammation as we have shown that embryonically derived LCs are replaced by monocyte-derived cells upon exposure to UV light, but not after mild injuries such as exposure to skin sensitizers (17).

The exact half-life of monocyte-derived DCs in inflamed tissue remains to be established but it is likely that inflammatory DCs are replaced by resident DCs once inflammation subsides. The mechanisms that control the repopulation of tissue-resident DCs during the repair process remain to be examined.

Cytokines that control DC homeostasis in non-lymphoid tissues

The cytokine Flt3L and its receptor (Flt3) play a key role in the development of DCs in mice and humans. Flt3L is ubiquitously secreted by multiple tissue stroma and endothelial cells and by activated T cells (76, 77), whereas the expression of the Flt3 receptor is restricted to the DC lineage. Bioactive levels of Flt3L are measurable in the serum in the steady state and increase upon inflammation and hematopoietic stress, such as irradiation-induced cytopenia. Flt3L as a single cytokine is sufficient to drive the differentiation of mouse and human hematopoietic bone marrow progenitors into DCs *in vitro* (78). Loss of Flt3 expression in hematopoietic progenitors correlates with the loss of DC differentiation

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potential (79), whereas enforcement of Flt3 expression on progenitors lacking Flt3 expression rescues their ability to differentiate into DCs (80). Consistently, mice that are deficient in Flt3 and Flt3L have reduced numbers of plasmacytoid DCs and cDCs in lymphoid organs (81). Intriguingly, reduction of lymphoid organ DC is more severe in mice that lack Flt3L compared with those lacking Flt3 suggesting the presence of an alternative Flt3L receptor at least in mice. Injection or conditional expression of Flt3L in mice leads to massive expansion of cDCs in lymphoid and non-lymphoid organs such as the intestine, the pancreas, and the liver (79, 82–86). Injection of Flt3L in humans also leads to massive expansion of blood cDCs (87–89).

We recently examined the role of Flt3 and Flt3L in the development of non-lymphoid tissue DCs. We found that pre-cDCs were strongly reduced from the lung, liver, and kidney of mice that lack Flt3L (24). $CD103^+$ DCs were absent from the dermis, lung, liver, pancreatic islet, kidney, *lamina propria*, and Peyer's patches of mice lacking Flt3 or its ligand, whereas $CD103^-CD11b^+$ DCs were reduced but at a lower levels in these mice (24). In contrast, epidermal LCs developed normally in Flt3- and Flt3L-deficient mice (24). We also found that Flt3L administration in the steady state induces DCs to proliferate in non-lymphoid tissues suggesting that similar to what has been found for lymphoid organ DCs, Flt3L plays a role not only in DC development but also in the homeostasis of differentiated DCs in the steady state.

Granulocyte macrophage colony stimulating factor (GM-CSF) is a key cytokine for the differentiation of hematopoietic progenitors (90, 91) and monocytes (66) into DCs *in vitro* in mice and humans. Injection of GM-CSF is used in clinical studies to attract or generate DCs at disease sites (92, 93). GM-CSF does not play a role in the development of DCs in lymphoid organs in the steady state (94) but may play a role in these tissues during inflammation (95). These results suggested that GM-CSF mainly controlled the development of inflammatory DCs. Recently, several studies including studies from our group revealed the role of the GM-CSF receptor in the development of steady-state DCs in non-lymphoid tissues. One study showed that absence of the GM-CSF receptor compromises the development of $CD11b^+$ DCs but not $CD11b^-$ DCs in the *lamina propria* (71), whereas our study showed that among $CD11b^+$ *lamina propria* DCs GM-CSF receptor controls only the development of $CD103^+CD11b^+$ DCs but not $CD11b^+CX3CR1^+$ DCs (38). In the skin, GM-CSF was shown to play a role in the development of dermal $CD11b^+$ DCs but not $CD11b^-$ DCs (96). However, the role of GM-CSF in DC development in sterile tissues remains to be established.

M-CSF is a key cytokine for macrophage development. Mice that lack M-CSF or its receptor, M-CSFR, lack several macrophage populations and develop osteopetrosis owing to the absence of osteoclasts (97, 98). M-CSF is secreted by endothelia, stroma cells, osteoblasts, and macrophages, and is detectable in the steady-state serum and increases upon inflammation (99). M-CSFR reporter mice in which GFP is expressed under the M-CSFR promoter revealed that GFP is expressed by most lymphoid organ DCs, but the exact correlation of GFP levels and the protein expression in these mice remains unclear (100). Although initially thought to be dispensable for DC development (101, 102), data from our laboratory established that M-CSFR is required for LC development and mice that lack M-CSFR also lack epidermal LCs (73). Importantly, in addition to LCs, we recently showed that M-CSFR plays a role in the development of $CD103^-CD11b^+$ DCs in several tissues including the dermis, lung, liver, kidney, and intestine, but is dispensable for the development of $CD103^+$ DCs in these tissues.

In addition to M-CSF, TGF-β1 is a non-redundant cytokine for LC development *in vivo* in mice (8, 103) and for LC differentiation *in vitro* from human hematopoietic progenitors (104, 105). In the skin, keratinocytes are a source of TGF-β1 and it has been assumed that exogenous TGF-β1 was critical for LC development (103). Recent data, however, have challenged this view as mice in which the absence of TGF-β1 secretion is restricted to LCs cannot form epidermal LCs (106) suggesting that an autocrine source of TGF-β1 controls LC development.

These data suggest that the development of non-lymphoid tissue DCs is regulated by a cytokine network that includes at least Flt3L, M-CSF, GM-CSF, and TGF-β1. Flt3L plays an instructive role in the commitment of hematopoietic progenitors into the DC-restricted lineage and plays an additional role in the homeostasis of tissue $CD103^+$ DCs. M-CSF regulates the development of $CD103^-CD11b^+$ DCs in non-lymphoid tissues and controls together with TGF-β1, the homeostasis of epidermal LCs. GM-CSF controls the development of dermal $CD11b^+$ DCs and intestinal $CD103^+$ DCs but does not play a role in the differentiation of intestinal $CD103^-$ DCs.

Transcription factors in the development of non-lymphoid tissue DCs

Several transcription factors have been shown to control the development of all lymphoid organ DC populations *in vivo*. For example, STAT3, a transcription factor downstream Flt3 signaling and the E-twenty six (ETS) family of DNA-binding proteins member PU.1 controls the development of lymphoid

organ CD8α^- and CD8α^+ cDCs (107–109) and likely plays a key role in the development of non-lymphoid tissue DCs. We have recently reviewed elsewhere the role of transcription factors in the development of lymphoid organ cDCs *in vitro* and *in vivo* (110). Here, we chose to focus on those transcription factors that were shown to control the development of non-lymphoid tissue DC populations.

Interferon-regulatory factor (IRF) belongs to the family of IRF proteins [formerly also called PU.1 interaction partner (Pip) or interferon consensus sequence-binding protein (ICSBP)]. IRF-2-deficient mice have reduced lymphoid organ CD8α^- DCs and slightly reduced epidermal LCs (111); IRF-4-deficient mice have reduced lymphoid organ CD8α^- DCs and slightly reduced plasmacytoid DCs; whereas CD8α^+ DCs are unaffected (112, 113). IRF-8-deficient mice have reduced plasmacytoid DCs and lymphoid organ CD8α^+ DCs but intact lymphoid organ CD8α^- DCs. IRF-8-deficient mice also have reduced epidermal LCs (114–117), whereas mice that carry the BXH2 IRF8 mutation have a defect in the development of CD8α^+ DCs but not lymphoid organ CD8α^- DCs or plasmacytoid DCs (118). We have recently examined whether the development of non-lymphoid tissue DCs was compromised in mice carrying the BXH2 IRF-8 mice. Although we have not observed the reported reduction in LC number in these mice, IRF-8$^{-/-}$ LCs have much reduced MHCII expression levels compared with IRF8$^{+/+}$ LCs (Ginhoux and Merad, unpublished data). Strikingly, IRF8$^{-/-}$ mice and mice that carry the IRF8 BXH2 mutation completely lacked CD103$^+$ DCs but had wildtype levels of CD11b$^+$ DCs in the dermis, lung, liver, and kidney (24). Altogether, these results suggest that IRF8 controls the differentiation of CD103$^+$CD11b$^-$ DCs in most tissues as well as the development of lymphoid organ CD8α^+ DCs, but does not control the development of CD8α^- lymphoid organ DCs, epidermal LCs, and the development of CD103$^-$CD11b$^+$ DCs in non-lymphoid tissues.

The inhibitor of DNA-binding protein-2 (Id-2) plays a role in the differentiation of several hematopoietic lineages and mice that lack Id2 have a severe reduction of lymphoid organ CD8α^+ DCs, and lack LCs (119). The LC deficiency is thought to be a result of a defect in TGF-β signaling (119) although this remains to be clearly established. We recently discovered that similar to IRF8$^{-/-}$ mice, Id2$^{-/-}$ mice also lack CD103$^+$ DCs in the dermis, lung, liver, and kidney, whereas CD11b$^+$ DCs were not affected in these mice. Similar to IRF8$^{-/-}$ mice, *lamina propria* CD03$^+$CD11b$^+$ and CX3CR1$^+$ DCs were not affected in Id2$^{-/-}$ mice, whereas CD103$^+$CD11b$^-$ Peyer's patches DCs were completely absent in these mice (24).

Using expression profile studies, the transcription factor Batf3 was found to be highly expressed in DCs compared with other hematopoietic and non-hematopoietic cells. Batf3-deficient mice have a selective loss of spleen CD8α^+ DCs and dermal CD103$^+$CD11b$^-$ DCs, whereas lymphoid organ CD8α^- DCs and dermal CD11b$^+$ DCs remain unaffected in these mice (120). Whether Batf3 also controls the development of CD103$^+$ DCs in other tissues remains to be examined.

Runx3, a member of the runx domain family of transcription factors, mediates TGF-β responses. The absence of Runx3 in mature DCs results in the loss of TGF-β-mediated inhibition of maturation, therefore leading to DC activation and inflammation. Importantly, lack of appropriate TGF-β-induced Runx3 signaling also leads to a defect in LC development (119, 121).

These results establish that lymphoid organ CD8α^+ DCs and non-lymphoid tissue CD103$^+$CD11b$^-$ DCs are controlled by a similar group of transcription factors that include IRF8, Batf3, and Id2, whereas Id2 and Runx3 also control the development of epidermal LCs. How and at what stage during the development these factors control non-lymphoid tissue DC homeostasis remains to be established.

Functional specialization of tissue-resident non-lymphoid tissue cDCs

Several studies have established the functional diversity of DC populations in the spleen. CD4$^+$ and CD8α^+ splenic DC subsets express different Toll-like receptors, lectin receptors, phagocytic receptors (122–124) and distinct antigen-processing and presentation machinery (123–126). Splenic CD4$^+$ DCs interact preferentially with CD4$^+$ T cells, whereas CD8α^+ DCs are specialized in cross-presentation of cell-associated antigens to CD8$^+$ T cells (127, 128). In contrast to lymphoid organ DCs, the heterogeneity of DCs in non-lymphoid tissue has only been recently established. Next, we review recent studies that suggest that similar to lymphoid organ DCs, non-lymphoid tissue DCs are functionally specialized and play different roles in tissue immunity.

Functional specialization of lung DC subsets

Lung CD103$^+$ DCs and CD11b$^+$ DCs express distinct Toll-like receptor, cytokine receptor, and chemokine receptor profile in the steady state (29, 129). The CD11b$^+$ DC subset was shown to be a major secretory cell type and express high levels of chemokines and cytokine mRNA in the steady state, whereas CD103$^+$ DCs secrete low levels of chemokines with the exception of CCL22, is a chemokine

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important in the recruitment of T-regulatory cells and Th2 cells (129).

Lung CD103$^+$ DCs have also been shown to have a lower capacity to capture soluble ovalbumin (OVA) compared with lung CD11b$^+$ DCs (130, 131). Despite lower ability for phagocytosis, purified lung CD103$^+$ DCs were much more efficient in the steady state at presenting antigens to OVA-specific T cell receptor (TCR) transgenic CD8$^+$ T cells (OT-I) but not to OVA-specific TCR transgenic CD4$^+$ T cells (OT-II), whereas CD11b$^+$ DCs presented antigens to OT-II but not to OT-I cells (131).

As CD103$^+$ DCs are developmentally related to CD8α^+ DCs, it is possible that they are also better at cross-presenting OVA to CD8$^+$ T cells. Interestingly, the lectin CLEC9A is recently shown to be required for the cross-presentation of necrotic cell-associated antigens to CD8$^+$ T cells (124). This lectin is expressed by splenic CD8α^+ but not CD4$^+$ DCs (124) and also by a population of circulating blood DCs in humans (132). We recently discovered that CLEC9A is expressed specifically on lung CD103$^+$ DCs but not CD11b$^+$ DCs again emphasizing the similarity between non-lymphoid tissue CD103$^+$ and lymphoid organ CD8α^+ DCs (J. Helft, C. Reis e Sousa, and M. Merad, unpublished data).

Ex vivo assays in which specific DC populations isolated at different times after microbial infections are cultured with either polyclonal or antigen-specific transgenic T cells have largely been used to compare the ability of tissue-migratory DCs and resident lymphoid organ DCs to present viral antigens to CD4$^+$ and CD8$^+$ T cells in the LN. Although this assay does not take into account differences in susceptibility to pathogens, microbial-induced cellular damage as well as differences in migratory abilities between different DC populations, it still provides information on which DC subset has the potential to present viral antigens to LN T cells. Upon lung infection with influenza or HSV infection, the migratory CD11b^{low} DC subset (presumably CD103$^+$ DCs) purified from the lung-draining LN was more efficient at priming antigen-specific TCR transgenic naïve CD8$^+$ T cells (133, 134) but not memory CD8$^+$ T cells (135), whereas both CD103$^+$ and CD11b$^+$ DCs were efficient at priming viral antigen-specific TCR-transgenic CD4$^+$ T cells (134). These results suggest that although both CD103$^+$ DCs and CD11b$^+$ DCs can acquire viral antigens, CD103$^+$ DCs are better equipped to prime antiviral CD8$^+$ T-cell response (134).

Transgenic mice models expressing the DTR under the mouse langerin promoter have been used recently to specifically deplete langerin$^+$ cells upon DT injection and to assess the role of langerin$^+$ DCs *in vivo*. In the lung CD103$^+$ DCs express langerin and DT administration to langerin-DTR transgenic mice eliminates lung CD103$^+$ DCs but spares lung CD11b$^+$ DCs (136). Upon infection with influenza virus, langerin-DTR mice that received DT develop more severe lung infection symptoms compared with control mice (136). However, DT administration in langerin-DTR mice also partially depletes LN-resident CD8α^+ DCs as a subset of these cells express langerin (J. Helft and M. Merad, unpublished data) making it difficult to distinguish the contribution of lung CD103$^+$ DCs and LN CD8α^+ DCs to the induction of lung immunity to influenza virus in this model. Altogether, these studies suggest the superior ability of lung CD103$^+$ DCs to present viral and innocuous antigens to CD8$^+$ T cells. However, the exact contribution of the lung CD103$^+$ DC subset to lung immunity *in vivo* and the exact mechanisms underlying their greater ability to prime CD8$^+$ T cells remain to be established.

In contrast to CD103$^+$ DCs, CD11b$^+$ DCs are difficult to distinguish from monocyte-derived DCs that rapidly accumulate in inflamed tissues. Therefore, studies on the role of CD11b$^+$ DCs in inflamed lungs can reflect the role of tissue-resident DCs or newly recruited monocyte-derived DCs or both. In a mouse model of airway allergic inflammation, airway CD11b$^+$ but not CD11b$^-$ DCs were able to present the allergen for weeks after antigen exposure and played a major role in the activation of antigen-specific Th2 cells locally in the lung (137). In mice infected with *Mycobacteria tuberculosis*, lung CD11b$^+$ but not CD11b$^-$ DCs were responsible for the secretion of interleukin-12, a cytokine that is required for maintaining protective Th1 immunity against the *Mycobacterium* (138). In mice infected with influenza virus, pulmonary CD11b$^+$ DCs were also required for the recruitment of circulating DCs and plasmacytoid DCs to the lung and for the amplification of CD8$^+$ T-cell response (139), a role also shown for monocyte-derived DCs (140).

Role of cutaneous DCs in skin anti-microbial immunity

Epidermal LCs were thought to be the main DC population to control skin immunity. However, the recent identification of the diversity of the dermal DC compartment (5) (*Fig. 1*) together with the discovery that upon skin infection with HSV LCs are unable to present viral antigens to T cells in the draining LN (141) reignited a series of studies on the role of cutaneous DCs in skin immunity. In this initial study, purified CD8α^+ DCs isolated from skin-draining LNs but not skin-migratory DCs were able to prime naïve viral-specific TCR-transgenic T cells in an *ex vivo* DC/T cell co-culture assay, leading to the hypothesis that the main role of skin-migratory DCs is to transport and deliver antigens to LN CD8α^+ DCs. In contrast, upon injection of a lentiviral vector into the skin,

cutaneous migratory DCs were able to present antigens to T cells (142). Because HSV is highly cytolytic while lentivirus is not, it has been proposed that the inability of LCs to present viral antigens could be owing to virus-induced cellular damage.

Skin infection with HSV-1 leads to two phases of acute viral replication in the skin: a primary infection that is limited to the site of scarification and a secondary viral recrudescence phase involving the entire innervated dermatome. Using *ex vivo* assays the same group showed that although lymphoid organ $CD8\alpha^+$ DCs are uniquely able to activate antigen-specific naïve TCR-transgenic $CD8^+$ T cells during the first wave of infection (141), dermal $CD103^+$ DCs but not dermal $CD11b^+$ nor LCs were able to present antigens to naïve TCR-transgenic $CD8^+$ T cells *ex vivo* (143). In contrast, all migratory DCs including LCs, dermal $CD11b^+$ DCs and dermal $CD103^+$ DCs were able to present viral antigens to $CD4^+$ T cells in *ex vivo* culture assays. These results suggest that although the three cutaneous DC populations acquired viral antigens only, $CD103^+$ DCs were able to present viral antigens to $CD8^+$ T cells. It remains unclear as to whether $CD103^+$ DC interaction with $CD8^+$ T cells *ex vivo* results from direct presentation of viral antigens or cross-presentation of infected epithelial cells. In addition, the exact contribution of $CD103^+$ DCs to HSV immunity *in vivo* remains to be established.

The preferential ability of dermal $CD103^+$ DCs to interact with $CD8^+$ T cells, observed in the second wave of HSV infection and in lung immunization has also been observed in a model of cutaneous leishmaniasis infection model. In this model, depletion of $CD103^+$ dermal DCs using the langerin-DTR transgenic mice impaired the priming of $CD8^+$ T cells, whereas the $CD4^+$ T-cell response remained intact (144). However, it remains possible that lymphoid organ $CD8\alpha^+$ DCs also participated in the induction of a $CD8^+$ T-cell response in this model as a proportion of $CD8\alpha^+$ DCs in lymphoid organs are also depleted upon DT administration in langerin-DTR mice, as discussed before.

Similarly, $Batf3^{-/-}$ mice, which lack lymphoid organ $CD8\alpha^+$ DCs but not $CD4^+$ DCs, are unable to mount an efficient antiviral $CD8^+$ T-cell response against subcutaneous infection with West Nile virus. Failure to mount antiviral responses in this model has been attributed to the absence of lymphoid organ $CD8\alpha^+$ DCs (120). However, dermal $CD103^+$ DCs are also absent from these mice and the exact contribution of dermal $CD103^+$ DCs and LN-resident $CD8\alpha^+$ DCs in the control of cutaneous West Nile virus infection remains to be examined (120).

In contrast to dermal $CD103^+$ DCs, dermal $CD11b^+$ DCs play a critical role in the local expansion of effector and regulatory T cells (145). Upon skin immunization with incomplete Freud adjuvant to mimic chronic skin inflammation, dermal $CD11b^+$ DCs were shown to control the induction of cytokine production by $CD4^+$ effector and regulatory T cells that have infiltrated the inflamed skin, despite the fact that all DC subsets including LCs and $CD103^+$ DCs were able to present MHCII/peptide complexes at their surface (145). Whether these dermal $CD11b^+$ DCs derive from circulating monocytes or whether they represent tissue-resident DCs that present antigens for prolonged periods of time as found in chronic inflamed lung (137) remains to be examined.

These data suggest that $CD103^+$ DCs play an important role in the priming of $CD8^+$ T cells, whereas $CD11b^+$ DCs play a key role at the site of the infection. However, these results remain to be confirmed in vivo through the use of tools allowing the conditional deletion of specific DC subsets.

Role of cutaneous DCs in allergic contact dermatitis

Induction of contact hypersensitivity responses (CHS) to haptens is commonly used as a mouse model for allergic contact dermatitis. Two mouse models have recently been used to revisit the role of LCs in CHS. These models include the langerin-DTR transgenic mouse expressing DTR under the mouse langerin promoter (46, 146) and the langerin-DTA mice, which express the DT A under the human langerin promoter (147). Studies using the langerin-DTR mouse model have shown that CHS is either slightly reduced or unaffected after DT administration (46, 146). Subsequent studies have shown that two cutaneous DC populations can lead to CHS. These include the LCs when hapten administration is restricted to the epidermis or the dermal $CD103^+$ DCs when the hapten diffuses to the dermis (148, 149). In contrast to these results, the use of the langerin-DTA transgenic mouse revealed that CHS is increased in the absence of LCs, revealing a potential tolerogenic role for LCs in the skin (147). Important differences exist between these two mice models. In the langerin-DTA model, LCs are absent from birth but dermal $CD103^+$ DCs remain unaffected because the human langerin promoter is not expressed in these cells. In contrast, in the langerin-DTR model both LCs and dermal $CD103^+$ DCs are conditionally depleted upon DT administration (20–22). To distinguish the contribution of LCs and dermal $CD103^+$ DCs in the studies discussed before (148, 149), immunization was administered either 1 or 7 days after DT administration as dermal DC repopulate much faster than LCs after DT administration. These studies found that immunization 7 days after DT lead to a decreased CHS response when hapten administration was restricted to the epidermis suggesting a role for LCs in the

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induction of T effector cell response against epidermal antigens (148, 149). However, it now appears that the repopulation of dermal CD103$^+$ DCs is slower than initially anticipated and it is possible that incomplete CD103$^+$ DC repopulation 7 days after DT treatment rather than LC ablation interfered with the induction of a strong CHS response in these studies (148, 149). The development of a conditional LC mouse model in which DTR is controlled by the human langerin promoter, should help settle the contribution of LCs in CHS.

Role of cutaneous DCs in tolerance

In transgenic mice expressing membrane-bound OVA under a keratinocyte promoter, LCs and dermal DCs were found to cross-present keratinocyte-associated OVA to CD8$^+$ T cells. Importantly in this model, LCs and to a lesser degree dermal DCs lead to the deletional tolerance of CD8$^+$-specific T cells, whereas the contribution of CD8α^+-resident LN DCs in this process appeared to be limited (150). More recently, a study from the same group infirmed the role of LCs in the presentation of self-antigens by showing that dermal CD103$^+$ DCs are the only cutaneous DC population able to present OVA to OVA-specific TCR-transgenic T cells in this system (143). The discrepancy between the two studies remains to be explained by the authors but it is possible that improved characterization of the cutaneous DC populations that exist in the skin have helped identify the role of CD103$^+$ DCs in this model. It is unclear, however, whether CD103$^+$ DCs can also lead to deletional tolerance of CD8$^+$ T cells.

The proportion of regulatory T cells among total CD4$^+$ T cells is higher in the dermis in the steady state compared with the blood (F. Ginhoux, J. Helft, and M. Merad, unpublished data). The contribution of cutaneous DC populations if any in the homeostasis of T-regulatory cells has still not been clearly established. However, it is important to note that Batf3$^{-/-}$ mice that lack dermal CD103$^+$ DCs and LN CD8α^+ DCs do not develop overt autoimmunity suggesting that cross-presentation of tissue antigens may not be required to maintain tissue integrity in mice (120).

Functional specialization of *lamina propria* DC subsets

Several studies have established that mouse and human *lamina propria* DCs directly sample luminal antigens by extending dendrites through the epithelial cell monolayer into the lumen without compromising the epithelial cell integrity (37, 151, 152). The ability of *lamina propria* DCs to penetrate the epithelium is thought to provide a mechanism by which apoptotic epithelial cells (151, 153), commensals, and enteric pathogens (37, 154) can be captured and transported to the mesenteric LN. In mice, the formation of DC transepithelial projections is dependent on Toll-like receptor signaling by epithelial cells (152) and these projections accumulate in the proximal (152) and terminal ileum (37). DC projections in the terminal ileum are dependent on the expression of the fractalkine (CX3CL1) receptor (CX3CR1) by intestinal DCs (37).

In the rat, DCs that constitutively transport apoptotic cells to the draining LN express CD103 (153). Consistently, we found that CD103$^+$ DCs that lack CX3CR1 expression are the main DC population to constitutively migrate to the mesenteric LN (38) (Fig. 3). Although CD103$^+$ DCs lack CX3CR1 precluding them from sampling antigens through CX3CL1-dependent intraepithelial protrusions (37, 155), they might use several other mechanisms to acquire luminal antigens. Expression of the integrin CD103 by these cells, through its interaction with the epithelial cell adhesion molecule E-cadherin (25), may promote DC homing to the epithelia facilitating the sampling of cell-associated antigens (Fig. 3). The ability of CD103$^+$ to form transepithelial projections may explain the presence of CX3CR1-independent projection in mice proximal ileum (152). Another possibility is that CD103$^+$ DCs acquire antigens directly through Peyer's patch-independent M cells (156) or through the cross-presentation of CD103$^-$CD11b$^+$CX3CR1$^+$ *lamina propria* DC-associated antigens.

Role of *lamina propria* DCs in *Salmonella* infection

Salmonella typhimurium (*Salmonella*) is a facultative intracellular pathogenic bacterium that causes typhoid-like disease in mice. Entero-invasive *Salmonella* efficiently infiltrate M cells located in the follicle-associated epithelium (FAE) that lines the Peyer's patches (157, 158). *Salmonella* can also infiltrate the gut through DC-derived transepithelial projections (37, 151). The development of non-invasive *Salmonella* strains and the use of genetically labeled DCs have revealed the key role for CX3CR1$^+$ DCs in the translocation of non-invasive *Salmonella* to the gut (37, 159).

Entry of *Salmonella* via M cells delivers bacteria directly into DCs located in the sub-epithelial dome of Peyer's patches (160), whereas capture of *Salmonella* by transepithelial DC projections delivers the *Salmonella* to the *lamina propria* and the mesenteric LN. These different entry routes have been shown to affect tissue immunity to *Salmonella*. Penetration through

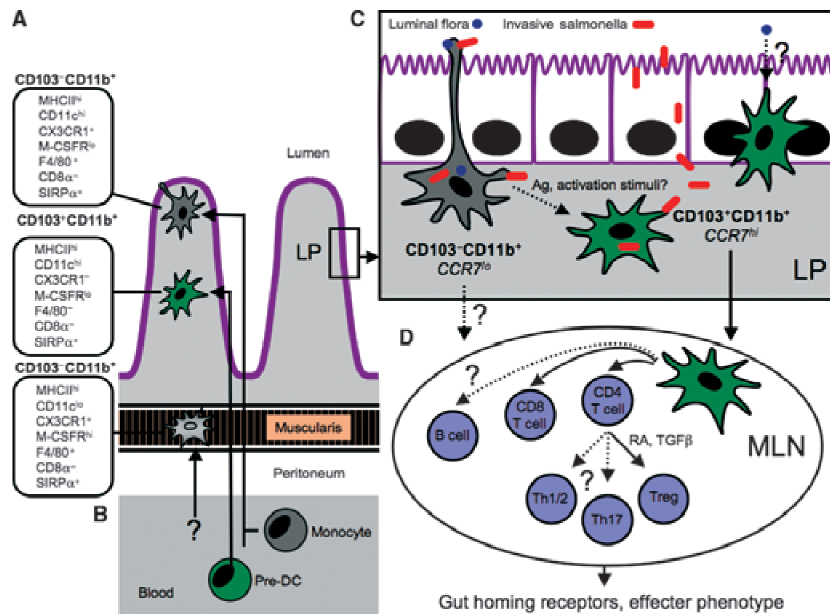

Fig. 3. Diversity of *lamina propria* (LP) dendritic cells (DCs). (A) Distribution and phenotype of DCs in the intestinal LP and muscularis layer. (B) LP $CD103^{+}CD11b^{+}$ DCs originate mainly from pre-DCs, whereas LP $CD103^{-}CD11b^{+}$ DCs derive from circulating monocytes. (C) In the ileum, $CD103^{-}CD11b^{+}$ DCs have been shown to sample luminal antigens by extending dendrites through the epithelial monolayer and into the lumen under the control of CX3CR1. $CD103^{+}CD11b^{+}$ DCs are found in the apical villi and in the intraepithelial cell fraction. They lack CX3CR1, and their ability to uptake luminal antigens remains to be analyzed. Although $CD103^{-}CD11b^{+}$ DCs can sample luminal antigens, they express low levels of CCR7 and likely do not constitutively migrate to the mesenteric lymph node (LN) in the steady state. (D) After oral infection with invasive *Salmonella*, both subsets uptake *Salmonella*, but $CD103^{+}CD11b^{+}$ DCs are the first to transport the pathogen to the draining LN. (E) LP and mesenteric LN $CD103^{+}$ DCs but not $CD103^{-}$ DCs promote *ex vivo* differentiation of naïve T cells into $Foxp3^{+}$ T-regulatory cells, which correlate with a greater ability to produce retinoic acid. The role of each LP DC subset in the induction in T- and B-cell effector function *in vivo* has not been examined.

M cells is required to induce anti-*Salmonella* immunoglobulin A (IgA) response, whereas *Salmonella* that have infiltrated through M cells or through transepithelial DC projections are equally capable of migrating to the mesenteric LN and induce systemic immunity (161).

Using a *Salmonella* oral infection model in streptomycin-pretreated mice to enforce pathogen invasion through the epithelia rather than Peyer's patches (162), we found that $CD103^{+}CD11b^{+}$ *lamina propria* DCs were the first DC population to transport the pathogen to the mesenteric LN. DC ability to transport antigens from the intestinal tract to the mesenteric LN participates in the systemic dissemination of *Salmonella* via the blood stream (163). This was most clearly shown in a model of *Salmonella* infection, in which the inability of DCs to sense the pathogen compromised its transport to the mesenteric LN and improved the infection outcome by reducing the systemic dissemination of the pathogen (164). Similarly, oral infection of Flt3-deficient mice, which lack $CD103^{+}$ but not $CX3CR1^{+}$ DCs in the *lamina propria*, have a reduced amount of *Salmonella* in the mesenteric LN to a degree comparable with that of CCR7-deficient mice (38). Altogether, these results suggest that upon *Salmonella* oral infection $CD103^{+}CD11b^{+}$ DCs play a key role in the early transport of pathogens to the draining LN. However, the exact role for $CD103^{+}$ and $CX3CR1^{+}$ *lamina propria* DCs in the outcome of *Salmonella* infection *in vivo* remains to be examined.

Role of *lamina propria* cDCs in IgA production

Secretory IgA produced by intestinal B cells play multiple protective roles including immune antigen exclusion by entrapping dietary antigens and microorganisms and preventing their entry in the gut (165). One striking feature in gut immunity is to produce massive amounts of non-inflammatory IgA antibodies (165). Peyer's patches and to a lesser extent, isolated follicles represent a major source for the production of IgA (reviewed in 166). However, production of IgA in extrafollicular sites has also been observed in human and mice (167). B-cell production of IgA in extrafollicular sites is controlled in part by *lamina propria* DCs shown to have B-cell licensing functions implicated in class switching (167). Naturally occurring *lamina propria* tumor necrosis factor (TNF) and inducible nitric oxide synthase (iNOS)-producing DCs also control IgA production in a TGF-β-dependent manner (168). The role of each *lamina propria* DC population in IgA production remains to be examined.

Role of *lamina propria* cDC subsets in tolerance

DCs constitutively transport commensal bacteria (154) and apoptotic epithelial cells to the mesenteric LN (153). Impairment in constitutive mucosal DC migration to the mesenteric LN in mice that lack CCR7 leads to impaired oral tolerance (169) strongly suggesting a role for mucosal DCs in the maintenance of intestinal integrity.

DCs from the *lamina propria* of the small intestine have been shown to be significantly better than splenic DCs at inducing the conversion of naïve Foxp3^{-} T cells into Foxp3^{+} T cells in the presence of exogenous TGF-β (170, 171). Intriguingly, mesenteric LN CD103^{+} DCs are much more efficient than mesenteric LN CD103^{-} DCs at inducing the conversion of naïve T cells into Foxp3^{+} T cells through TGF-β (171). The superior ability of CD103^{+} DCs to induce regulatory T cells in the gut is partially explained by their higher expression of the enzyme Raldh that controls the metabolization of vitamin A into retinoic acid, a dietary metabolite, required in the differentiation of mucosal Foxp3^{+} regulatory T cells (170, 171). Importantly, conditional loss of TGF-β-activating integrin $\alpha_v\beta_8$ by CD11c^{+} cells results in spontaneous autoimmune disease that selectively affects the gut (172) and DCs that lack $\alpha_v\beta_8$ fail to induce regulatory T cells *in vitro* (172).

In addition to their role in the induction of regulatory T cells, *lamina propria* and mesenteric LN CD103^{+} DCs are much more efficient than CD103^{-} DCs at inducing the expression of gut homing molecules on T lymphocytes (35, 36). Similar results were obtained in humans, in which mesenteric LN CD103^{+} DCs were found to have a higher ability to induce the expression of gut homing T cells on naïve T cells (36). The superior ability of CD103^{+} DCs to induce gut homing receptors on effector T cells may also be dependent on their superior ability to metabolize vitamin A as retinoic acids control the induction of gut homing receptors on T (173) and B cells (174).

Role of monocyte-derived DCs in tissue immunity

Upon inflammation, tissue-resident DCs are replaced by blood-born DCs that derive mainly from circulating monocytes (4). A subset of these cells produces TNF- and NO-mediated microbiocidal mediators. TNF- and NO-producing DCs were found in the spleen of mice infected with *Listeria monocytogenes* and shown to contribute to innate immunity against *Listeria* but were not involved in the induction of *Listeria*-specific T-cell responses (175). Monocyte-derived DCs were also found in the lungs of mice infected with influenza virus (140). Although, they were shown to promote deleterious lung inflammation in this model they also contributed to the amplification of local CD8^{+} T effector cells (140).

Upon administration of the pro-inflammatory haptens 2,4-dinitrofluorobenzene or measles virus nucleoprotein that have intrinsic adjuvant properties, monocyte-derived DCs accumulated in the buccal mucosa induced in a CCR6-dependent manner and were essential for the cross-priming of OVA-specific CD8^{+} T cells (75). Induction of antigen-specific CD4^{+} T-cell responses during skin *Salmonella* infection have also been claimed to rely on the recruitment of monocytes to the dermis, in a CCR6-dependent mechanism (176). In a mouse model of *Leishmania major* infection in which high dose of parasites were injected subcutaneously in the foot pad, monocyte-derived DCs that form in the dermis and subsequently migrate to the draining LN wrere required for the induction of protective Th1 responses against the parasite (74). Importantly, in HSV-infected skin, monocyte-derived DCs were also required for the reactivation of memory CD8^{+} T cell at the tissue site and for the control of the infection (177).

Division of labor for optimal immune response

Tissue-migratory DCs, tissue non-migratory DCs, lymphoid organ-resident DCs, and monocyte-derived DCs have all been shown to participate in the induction of tissue immunity. Although the exact contribution of each DC population needs to be examined in the same experimental model, it is likely that optimal immune responses result only from a successful collaboration between these DC populations. A potential scenario for an integrated role of each DC subset in the induction of a successful immune response is described in *Fig. 4*. Tissue-migratory DCs could be responsible for the transport of tissue antigens and the initiation of a CD8^{+} T-cell response before dying in the LN. Once dead, their antigen cargo is reprocessed by tissue-resident CD8α^{+} DCs that are specialized in the priming of CD8^{+} T cells but potentially also by CD4^{+} DCs specialized in the priming of CD4^{+} T cells. The reason for the specialized interaction between lymphoid organ DC subsets and specific T-cell subsets is unclear. However, the benefit of having a DC compartment removed from the site of injury able to relay tissue-migratory DC in T-cell presentation is likely critical for the induction of strong T-cell immunity to tissue antigens. Monocyte-derived DCs recruited to non-lymphoid tissues will subsequently replace tissue-migra-

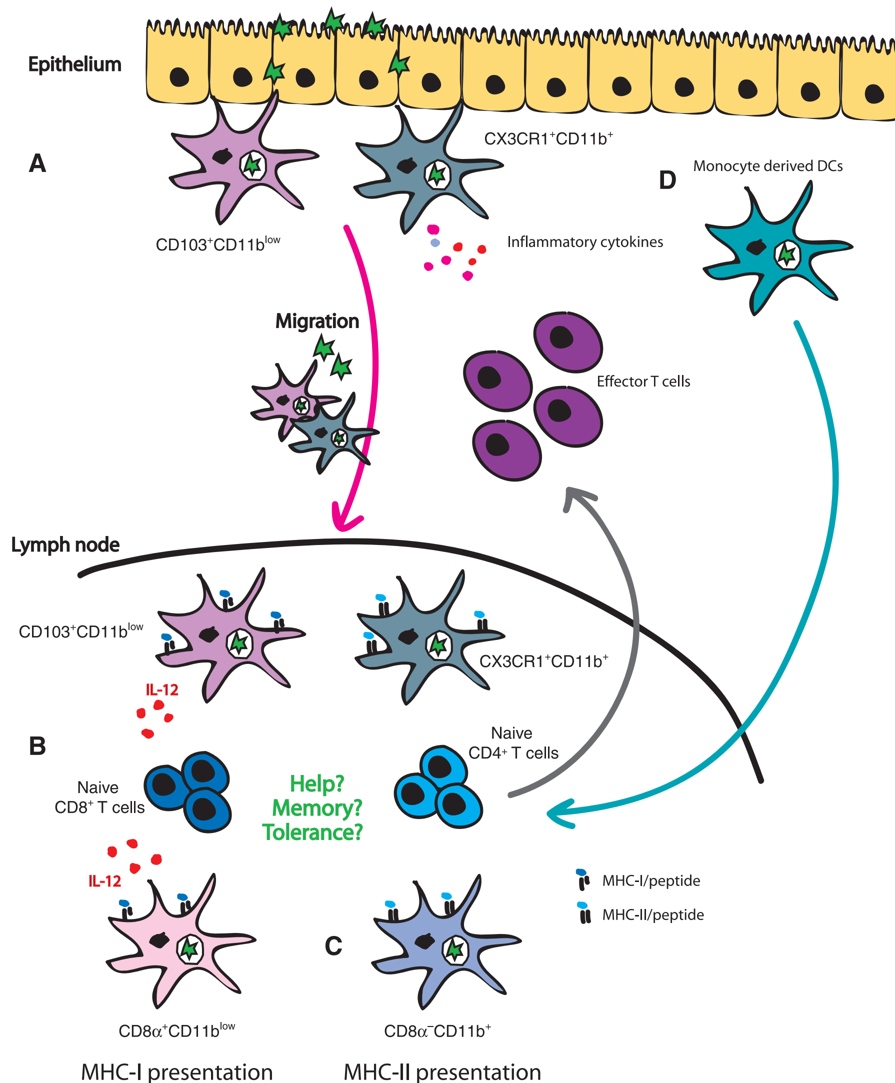

Fig. 4. Functional diversity of dendritic cell (DC) subsets. (A) Non-lymphoid tissue DCs in proximity with the epithelia capture self and pathogenic antigens prior to migrating to regional draining lymph node (LN) where they present tissue antigens to T lymphocytes. (B) In the LN, resident DCs also present tissue antigens to T cells. The mechanisms by which lymphoid organ DCs capture tissue antigens are not entirely established. (C) Naïve T cells should be able to sample antigens from several DC populations. How antigen presentation is coordinated between different DC subsets is still unclear. However, it is now clear that tissue-migratory $CD103^+$ DCs and lymphoid organ-resident $CD8\alpha^+$ DCs seem to be more efficient in the cross-presentation of extracellular antigens to $CD8^+$ T cells, whereas tissue-migratory $CD11b^+$ DCs and lymphoid organ-resident $CD8\alpha^-$ DCs are more efficient in the major histocompatibility complex class II presentation pathway. (D) $CD11b^+$ DCs play a key role in amplifying $CD4^+$ effector T cells and memory $CD8^+$ T cells that have infiltrated inflamed tissues. Whether these cells represent tissue-resident DCs that remain in the inflamed tissue or whether they represent newly recruited monocyte-derived DCs remains to be examined.

tory DCs, capture tissue antigens, and migrate to the LN where they also participate in the induction of a strong effector T-cell response. Monocyte-derived DCs or non-migratory tissue DCs that are present in inflamed tissues participate in the activation of effector or memory T cells that infiltrate the inflamed tissue. DCs located at the epithelial interface such as epidermal LCs, however, may not have the privilege to prime $CD8^+$ T-cell immune responses to avoid collateral damage at critical interfaces.

These results underline the sophistication of the DC network in the induction of tissue immunity and further emphasize the shift from an old lymphocyte-centric view to a more recent myeloid-centric view in the control of tissue immunity. We are only at the beginning of our understanding of the role of this intricate network of cells upon different types of injury. A better understanding of the contribution of antigen-presenting cells in the induction of tissue immunity will undoubtedly change the treatment and prevention of immune diseases.

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