CellPress
Immunity
Review

Development and Function of Dendritic Cell Subsets

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http://dx.doi.org/10.1016/j.immuni.2014.04.016

Classical dendritic cells (cDCs) form a critical interface between innate and adaptive immunity. As myeloid immune cell sentinels, cDCs are specialized in the sensing of pathogen challenges and cancer. They translate the latter for T cells into peptide form. Moreover, cDCs provide additional critical information on the original antigen context to trigger a diverse spectrum of appropriate protective responses. Here we review recent progress in our understanding of cDC subsets in mice. We will discuss cDC subset ontogeny and transcription factor dependencies, as well as emerging functional specializations within the cDC compartment in lymphoid and nonlymphoid tissues.

Introduction

The vertebrate immune system evolved to react to infection and injury caused by bacteria, fungi, viruses, and immunogenic particles (collectively referred to here as antigens [Ags]) by mounting protective immune responses that improve survival. The highly diverse Ag receptor repertoire required for this broad and almost unlimited reactivity is encoded by T and B lymphocytes. Due to the randomness of the somatic rearrangements generating T and B cell receptors, the repertoire bears reactivity to non-self Ags, as well as self-proteins. Consequently, mechanisms must be in place to avoid fatal autoimmune reactions. Burnet's clonal selection theory proposed that Ag-specific lymphocytes are selected for self- or non-self Ags and undergo clonal expansion upon exposure to the latter (Burnet, 1957). However, early studies based on in vitro "Mishell-Dutton cultures" already postulated accessory adherent, nonlymphoid immune cells required for efficient lymphocyte activation (Hartmann et al., 1970), a notion later substantiated with the discovery of MHC restriction of T cell stimulation (Zinkernagel and Doherty, 1974). Although these accessory cells were first thought to be Metchninkoff's macrophages, studies in the early seventies identified among splenocytes so-called A or 3rd cells that promoted adaptive in vitro immune responses (Cosenza et al., 1971). About the same time, Ralph Steinman discovered in the mouse spleen a rare population of cells characterized by stellate morphology and extended veils (Steinman and Cohn, 1973). He named them dendritic cells (DCs) after the Greek word for tree (*dendron*), but their biological significance met considerable skepticism. It took the persistence of Steinman and his colleagues to provide, over the following years, further compelling evidence for the uniqueness of their novel cell type. In another milestone study they demonstrated that DCs, which prominently express both major histocompatibility complex class (MHC) I and II molecules (Nussenzweig et al., 1980; Steinman et al., 1979), were unrivaled stimulators of T cells in primary mixed leukocytes reactions (MLR) (Steinman and Witmer, 1978). In addition, Steinman showed that DCs could process protein Ag and initiate Ag-specific cellular immune responses (Nussenzweig et al., 1980). For his pioneering work in establishing DC function and biology, as well as his subsequent efforts toward the exploitation of these cells for vaccination, Ralph Steinman was honored in 2011 with the Nobel Prize in Physiology or Medicine.

MHC-II-expressing non-B cells have been identified in almost every tissue investigated, including the intestine, heart, and kidney, with the notable exception of the brain parenchyma. All DCs share the capability to efficiently uptake and process Ags for presentation to naive T cells. However, in the decades since Steinman's seminal discovery, DC subsets have emerged that considerably differ in ontogeny, localization, cytokine secretion pattern, and immunological function. In this review we summarize recent findings and scientific progress in our understanding of murine DC subset development and function. For information regarding human DC subsets, we refer the reader to other excellent recent reviews (Haniffa et al., 2013; Villadangos and Shortman, 2010). We will mainly, but not exclusively, focus on murine classical DC (cDC) subsets, by which we mean all DCs other than plasmacytoid DCs (pDCs) and monocyte-derived DCs (moDCs).

Unifying cDC Features: What Makes These Cells So Special?

Before we discuss individual cDC subsets, we briefly recapitulate some of the unique and potentially unifying features of these highly phagocytic sentinels that seem to have evolved to constantly sense and respond to their immediate environment and communicate with T cells.

Antigen Processing and Presentation

cDCs are specialized in Ag processing and can—probably opposed to most other immune and nonimmune cells—efficiently present endogenous and exogenous Ag in both MHC-I and -II contexts. The unconventional presentation of exogenous noncytosolic Ags on MHC-I by cDCs relies on "cross-presentation" (Bevan, 1976): a phenomenon critical for immunity against viruses and intracellular bacteria (Rock, 2003). The detailed machinery enabling the unique Ags' transport from endosome to cytosol is under intense investigation with different pathways being discussed (Joffre et al., 2012). Unconventional presentation of endogenous cytosolic Ags on MHC-II by cDCs relies in contrast on autophagy (Paludan et al., 2005). Accordingly, this pathway is impaired in ATG5-deficient cDCs, although their Ag cross-presentation remains intact (Lee et al., 2010). As opposed to macrophages, cDCs degrade their engulfed cargo slowly and can control lysosomal degradation potentially to preserve peptides for T cell recognition (Savina et al., 2006). This activity is influenced by the maturation status of the DC, with

lipopolysaccharide (LPS) exposure enhancing lysosomal acidification and Ag proteolysis (Trombetta et al., 2003). Toll-like receptor (TLR) ligand exposure also stimulates Ag macro-pinocytosis, ensuring efficient MHC-peptide (MHCp) formation under inflammatory conditions (West et al., 2004). Interestingly, the glycolytic rate of DCs is also affected by TLR stimulation triggering a circuit that ensures the de novo synthesis of fatty acids critical for proper DC activation (Everts et al., 2014).

Migration

cDCs are strategically positioned at body barriers and also organ entry ports, such as the splenic marginal zone. To ensure stimulation of naive T cells, cDCs require efficient directional migration toward T cell zones either within their respective lymphoid organ of residence or toward remote tissue-draining lymph nodes (LNs). Peripheral cDC migration via afferent lymphatics is CCR7 dependent (Förster et al., 1999) and cDCs utilize CCL19 and CCL21, the same chemokine cues as migrating T cells that enter the LN. Interestingly, mobilization of cDCs can involve, in addition to the chemokine receptor switch, autocrine desensitization by chemokine expression (Dieu et al., 1998). Immobilized CCL21 on, or stored in, lymphatic endothelium plays a critical role in facilitating chemotaxis and arrest of migrating tissue DCs to enter the afferent lymphatics (Tal et al., 2011). Complementary roles in cDC migration have been suggested for other chemokine receptors and S1P1/S1P3 signaling, but CCR7 seems to be the major player. Integrins, on the other hand, are dispensable for the migration of cDCs to LNs under physiological conditions (Lämmermann et al., 2008) but are needed for optimal migration during contact sensitization (Johnson et al., 2006). Intratissue migration of lymphoid organ-resident cDCs, although seemingly also CCR7 dependent, remains less well understood. Recruitment of splenic CD11b$^+$ cDCs to the bridging channels of the marginal zone is controlled by the chemotactic receptor EBI1 (Gatto et al., 2013). A similar scenario was suggested for cDC movement in the Peyer's patch, i.e., the CCR6-CCL20 axis controlling migration toward the mucosal surfaces, whereas the CCR7-CCL19 axis is important for CD8$^+$ DC localization to the T cell region (Iwasaki and Kelsall, 2000). The highly specific expression of the chemokine receptor XCR1 on cross-presenting DCs (Dorner et al., 2009) orchestrates their intratissue positioning in the thymus (Lei et al., 2011). The MHC-II-associated invariant chain (CD74) also seems to be involved in the coordination of DC migration, because CD74-deficient DCs display increased motility, whereas DCs overexpressing CD74 due to absence of cathepsin S migrate at lower speed (Faure-André et al., 2008).

Specialization in T Cell Crosstalk

cDCs have unrivaled potential to stimulate T cells in an MLR in vitro (Steinman and Witmer, 1978). Indeed, studies of mice lacking cDCs, after their constitutive or conditionally ablation, have confirmed the central role of cDCs in the initiation of naive T cell responses (Birnberg et al., 2008; Jung et al., 2002) and the effective restimulation of memory T cells. Importantly though, T cell encounter of MHCp complexes on cDCs has, depending on its context, distinct outcomes. Productive, protective T cell responses, including proliferation, T helper (Th) cell polarization, and memory formation, are believed to rely on three distinct stimuli: cognate MHCp encounter, costimulatory signals provided by B7 family members, and instructing cytokines. All three signals can be derived from DCs for productive T cell priming to occur. Furthermore, these signals seem to have to come from the same DC, because only pathogen-exposed DCs (not inflammation or cytokine-stimulated DCs) can direct full Th cell differentiation (Spörri and Reis e Sousa, 2005). Direct recognition of pathogen-associated Ag by DCs, therefore, seems critical for the initiation of protective T cell responses, suggesting that inflammatory mediators can amplify, but not initiate, adaptive immunity. Such a scenario ensures that T cells read the original context of the cognate Ag, for instance its association with pathogen- or danger-associated molecular patterns. Indeed, it has been proposed that DCs might even maintain the distinction between innocuous Ags and the one received in TLR-ligand context on the single-cell level by segregating their cargo (Blander and Medzhitov, 2006), although this remains to be confirmed in an in vivo setup.

T cell encounter of MHCp on DCs that lack costimulatory molecules contributes to peripheral tolerance (Hawiger et al., 2001; Probst et al., 2003). This notion is supported by the intimate interaction of steady-state T cells and DCs under physiological conditions (Scheinecker et al., 2002). Moreover, DCs can also actively silence T cells by expressing molecules, such as Programmed cell death 1 ligand 1 (PD-L1), which deliver inhibitory signals (Carter et al., 2002). Further evidence for the central role of DCs as critical "hubs" for T cell activation stems from the fact that they are under constant control of thymic and inducible T regulatory (Treg) cells. Relief of this "Treg cell brake" is sufficient to unleash autoreactive cytotoxic T lymphocytes (CTLs) and cause autoimmunity (Feuerer et al., 2009). Interestingly, removal of Treg cells and thus control of DCs also reveals otherwise latent antitumor immunity, as it contributes to the clinical efficacy of costimulation blockades (Marabelle et al., 2013; Vom Berg et al., 2013). Of note, the crosstalk between T cells and DCs is bidirectional; CD40L-expressing T cells critically promote DC "maturation" (Elgueta et al., 2009). Moreover, T cells, as well as innate immune cells, can also shape the cDC compartment by production of the DC poietin Fms-related tyrosine kinase 3 ligand (Flt3L) (Saito et al., 2013; Guermonprez et al., 2013).

Classical DCs

cDCs can be divided into at least two main subsets characterized by either CD8α and CD103 or CD11b expression. Both subpopulations can be found in lymphoid tissue, including spleen, LN, and bone marrow (BM), as well as most nonlymphoid tissue.

CD8α^+ and CD103$^+$ cDCs

Heterogeneity within the DC population was first demonstrated by both the Shortman and Steinman groups, including the discovery of a CD8α-expressing DC subset in murine lymphoid organs (Crowley et al., 1989; Vremec et al., 1992). An equivalent population also exists in nonlymphoid tissues, although these cells do not express CD8 but are instead identified by the CD103 integrin marker ($\alpha_E\beta7$) (Bursch et al., 2007; del Rio et al., 2007) (see below). CD8α^+ and CD103$^+$ cDCs are to date the best-characterized cDC subset, both phenotypically and by gene expression signature (Edelson et al., 2010), and they also appear to be conserved through evolution (Crozat et al., 2010). Indeed, transcriptome profiling allowed the alignment of CD8α^+ lymphoid organ and CD103$^+$ nonlymphoid tissue cDCs

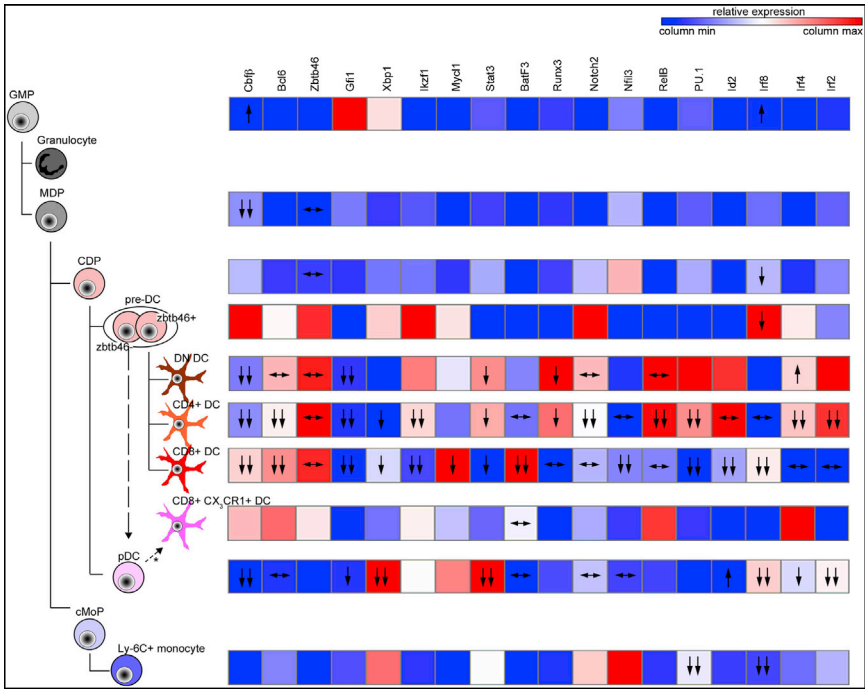

Figure 1. Development of cDC Subsets and Profiles of Key Transcription Factor Profiles

DCs arise from common monocyte-DC precursors (MDPs) in the BM, which differentiate into common monocyte progenitors (cMoPs) or common DC precursors (CDPs). CDPs can give rise to pDCs or pre-DCs that display considerable heterogeneity. Zbtb46-expressing pre-DCs are committed cDC precursors, which reach lymphoid and nonlymphoid tissues via the circulation. The heat map depicts the expression of selected key DC transcription factors throughout the commitment. Arrows within the heat map fields indicate phenotype of transcription-factor-deficient mice in different populations. The down arrow (↓) indicates a reduction in mutant mice, up arrow (↑) an increase, and horizontal arrow (↔) unchanged numbers of the cells. Asterisk indicates reduced expression of E2-2 may favor the conversion of pDCs into $CD8^+CX_3CR1^+$ splenic DCs.

(Miller et al., 2012). Moreover, it led to the discovery of the human equivalent of $CD8\alpha^+$ and $CD103^+$ mouse DCs (Villadangos and Shortman, 2010) and the identification of the chemokine receptor XCR1 as a potential unifying marker of this DC subset in both human and mice (Bachem et al., 2010; Crozat et al., 2010; Dorner et al., 2009).

Splenic $CD8\alpha^+$ cDCs are functionally specialized in cross-presenting exogenous Ags on MHC-I molecules to $CD8^+$ T cells (den Haan et al., 2000). Of note, the entry of exogenous protein to the cytosol also renders these cells uniquely sensitive to ablation by an extracellular cytochrome c (CytC) challenge (Lin et al., 2008). Cross-presentation activity has also been shown for skin-derived $CD103^+$ cDCs (Bedoui et al., 2009). $CD8\alpha^+$ cDCs furthermore present glycolipid Ags in CD1d context and can activate and polarize iNKT toward the production of T helper 1 (Th1) or Th2 cytokines (Arora et al., 2014). Specifically, discrete glycolipid Ag structures were reported to modulate expression of costimulatory and coinhibitory molecules and to thereby influence the antigen-presenting cell (APC) potential of $CD8\alpha^+$ cDCs (Arora et al., 2014). Stimulation of TLR signaling on the $CD8\alpha^+$ and $CD103^+$ cDC lineage induces prominent secretion of "bioactive" IL12p70 (Reis e Sousa et al., 1997). It is noteworthy that, in contrast to macrophages, secretion of IL-12 production by $CD8\alpha^+$ cDCs requires c-Rel (Grumont et al., 2001).

Development of $CD8\alpha^+$ cDCs and their nonlymphoid tissue equivalent, the $CD103^+$ ($CD11b^-$) cDCs, is orchestrated by the same transcription factors: inhibitor of DNA binding 2 (Id2), interferon regulatory factor 8 (IRF8), basic leucine zipper ATF-like 3 transcription factor (BATF3), and the nuclear factor interleukin 3 regulated (NFIL3). Deletion of either of these genes leads to a severe developmental defect of $CD8\alpha^+$ DCs, as well as $CD103^+$ nonlymphoid tissue cDCs, but not $CD11b^+$ cDCs (Figure 1; Aliberti et al., 2003; Ginhoux et al., 2009; Hacker et al., 2003; Hildner et al., 2008; Kashiwada et al., 2011). The hierarchy and sequential involvement of these specific transcription factors within the $CD8\alpha$ and CD103 cDC lineage is emerging (Murphy, 2013). IRF8 is obligatory for the development of Id2-expressing DC precursors and BATF3 is induced at later maturation stages of $CD8\alpha$ and CD103 cDCs (Hildner et al., 2008). Indeed, $CD8\alpha^+$ cDC development can be observed in BATF3-deficient mice infected with intracellular pathogens or treated with IL-12, a bypass mediated through compensatory BATF and BATF2 expression (Tussiwand et al., 2012). Furthermore, short-term development of $CD8\alpha$ and CD103 cDCs has been observed in the absence of Id2, NFIL3, and BATF3 expression, which collectively suggests that IRF8 is the master regulator of $CD8\alpha$ and CD103 cDC lineage development (Seillet et al., 2013). In addition to its regulation of development, IRF8 was recently shown to regulate expression of the transcription factor L-MYC, which controls mature $CD8\alpha^+$ DC proliferation and functionality (KC et al., 2014).

$CD11b^+$ cDCs

$CD11b^+$ cDCs are the most abundant cDCs in lymphoid organs except for the thymus and can also be found in nonlymphoid tissue. In contrast to $CD8\alpha$ and CD103 DCs, the population currently defined as $CD11b^+$ cDCs is heterogeneous and remains less well characterized. $CD11b^+$ cDCs can, for instance, be further segregated according to additional surface markers, such as CD4 and the Endothelial cell-selective adhesion molecule (ESAM) (Lewis et al., 2011); however, this subdivision does not yield homogeneous populations, as indicated by massive parallel single-cell transcriptome analysis (Jaitin et al., 2014).

Transcription factors that control general $CD11b^+$ cDC development include RelB (Wu et al., 1998), NOTCH2 (Lewis et al., 2011), RBP-J (Caton et al., 2007), IRF2 (Ichikawa et al., 2004), and IRF4 (Suzuki et al., 2004). Of note, IRF4 also controls functional aspects of $CD11b^+$ DCs, such as their MHC presentation (Vander Lugt et al., 2014) and migration (Bajaña et al., 2012; Gao et al., 2013). The significance of IRF4 for the $CD11b^+$ cDC development is still under debate (Murphy, 2013). However,

consistent with the notion that CD11b$^+$ cDCs are heterogeneous, deficiencies of IRF4 and NOTCH2 only partially impair this compartment and have differential penetrance in distinct tissues (Bajaña et al., 2012; Lewis et al., 2011). Moreover, in contrast to CD8 and CD103$^+$ cDCs, the hierarchy of transcription factor required for CD11b$^+$ cDC development is unknown and the pronounced heterogeneity of this compartment considerably complicates the interpretation of existing data.

When compared to CD8α^+ DCs or pDCs, CD11b$^+$ cDCs do show a unifying signature (Robbins et al., 2008; Bar-On et al., 2010). Interestingly, comparative transcriptional profiling revealed that CD4$^-$Esam^{lo}CD11b$^+$ cDCs, but not CD4$^+$ Esam^{hi}CD11b$^+$ cDCs, harbor a monocytic signature including expression of cytokine (*Csf1r*, *Csf3r*) and chemokine (*Ccr2*) receptors, as well as lysozyme (Lewis et al., 2011). Yet, CD4$^-$Esam^{lo} cDCs do not seem to derive from monocytes (Lewis et al., 2011). This finding further highlights the heterogeneity of the CD11b$^+$ cDC compartment, whose in-depth understanding will probably require novel approaches unbiased by historically defined surface markers, including the definition of the epigenomic landscape of cDCs, single-cell transcriptome analysis, and the further elucidation of DC origins by fate mapping.

Given their heterogeneity, it is not surprising that the assignment of specific functions to CD11b$^+$ DCs has remained challenging. In fact, at least in lymphoid organs, CD11b$^+$ DCs are still mostly defined by the absence of activities associated with CD8α^+ DCs. This includes their inefficiency to cross-present and produce specific cytokines, such as IL-12. It has however been noted that CD11b$^+$ cDCs are, as compared to CD8α^+ cDCs, superior in the induction of CD4$^+$ T cell immunity, potentially because of their prominent expression of MHC-II presentation machinery (Dudziak et al., 2007; Lewis et al., 2011). This specialization of CD11b$^+$ cDCs was recently attributed to their expression of the transcription factor IRF4 (Vander Lugt et al., 2014). CD11b$^+$ DCs can also be characterized by their production of cytokines, such as IL-6 (Persson et al., 2013) and IL-23 (Schlitzer et al., 2013). Furthermore, splenic CD11b$^+$ cDCs were shown to be prominent producers of proinflammatory chemokines after TLR ligand exposure, such as CCL3, CCL4, and CCL5 (Proietto et al., 2004). However, the in vivo relevance of these activities remains unclear.

Nonclassical DCs

Monocyte-Derived DCs

As a consequence of inflammation or infection, lymphoid and nonlymphoid organs can harbor DCs that originate from monocyte infiltrates (Serbina et al., 2003) and have been termed "monocyte-derived DCs" (moDCs) or "inflammatory DCs" (iDCs) (Mildner et al., 2013; Segura and Amigorena, 2013). moDCs are phenotypically difficult to discern from cDCs because they share similar expression patterns of MHC-II, CD11b, and CD11c; however, indicative of their monocytic past, moDCs express CD64, the Fc-gamma receptor 1 (FcγRI) (Plantinga et al., 2013; Tamoutounour et al., 2012). Monocytes have long been known to give rise to DC-like cells that can efficiently stimulate T cells when cultured in vitro in the presence of Csf2 (GM-CSF) and IL-4 (Sallusto and Lanzavecchia, 1994). In fact, it was the study of these BM culture-derived nonclassical DCs that yielded many of our insights into DC biology. Gene expression profiles of cDCs and BM culture-derived DCs, however, differ considerably (Xu et al., 2007). Earlier findings should probably be revisited using either ex vivo isolates or cDC equivalents generated in cultures driven by the growth factor Flt3L (Naik et al., 2005) to draw conclusions on cDC biology. A relation of moDCs and cDCs is supported by the fact that BM culture-derived moDCs express the transcription factor zbtb46 (Satpathy et al., 2012) that is restricted to cDCs in the immune system (Meredith et al., 2012b). It also remains to be determined how much BM culture-derived DCs reflect moDCs, i.e., their likely in vivo equivalent, which itself remains insufficiently defined. "TNF-α and iNOS-producing DCs" (or TIP DCs) that appear during pathogen-associated inflammation (Serbina et al., 2003) have been considered the prototypic moDCs. However, given the pronounced proinflammatory signature of these cells and their Csf2-independent development (Greter et al., 2012), they might represent activated effector monocytes rather than cDC-like cells (Mildner et al., 2013). Another population suggested to represent in vivo moDCs are DC-SIGN (CD209a)-positive DCs that appear in LNs after TLR ligand challenge (Cheong et al., 2010). Because these cells are Flt3L dependent and express zbtb46, they can also be interpreted as activated cDCs (Meredith et al., 2012a). Taken together, the definition of moDCs clearly requires further study. Moreover, because moDCs are most closely related to CD11b$^+$ DCs, these efforts will probably contribute to our understanding of the heterogeneous CD11b$^+$ cDC compartment.

Plasmacytoid DCs

pDCs are found circulating in the blood and in peripheral organs and display a characteristic surface phenotype and morphology, including a highly developed secretory compartment (Reizis et al., 2011). Identified first in humans (Siegal et al., 1999) and later in mice (Asselin-Paturel et al., 2001), pDCs harbor rearrangements of their IgH loci (Corcoran et al., 2003) and can, in transfer experiments, arise from common lymphoid progenitors (CLPs) (Manz et al., 2001). pDCs were therefore thought to be related to the lymphoid lineage. However, pDCs clearly share cDC characteristics, such as their strict Flt3L dependence for development (Kingston et al., 2009), a restricted potential to prime T cells, at least after activation (Sapoznikov et al., 2007), and their overlapping though distinct transcription profile (Robbins et al., 2008). pDCs are, however, best characterized by their unique functional property to rapidly produce large amounts of type I interferons upon viral infections (Nakano et al., 2001; Siegal et al., 1999), a direct consequence of their constitutive IRF7 expression (Honda et al., 2005). pDC development and homeostasis are regulated by the helix-loop-helix transcription factor E2-2 (also known as TCF4) (Cisse et al., 2008) that facilitates pDC development by directly suppressing expression of Id2 (Ghosh et al., 2010), a transcription factor critical for cDC development (Kusunoki et al., 2003). Conversely, early deletion of E2-2 leads to a complete absence of pDCs both in human and mouse (Cisse et al., 2008; Nagasawa et al., 2008), and excision of the E2-2 gene in mature pDCs initiates an alternative cDC transcription program including the expression of CD8α (Ghosh et al., 2010). These "converted" CD8α^+ pDCs bear D-J rearrangement indicative of their pDC past and can be discriminated from CD8α^+ cDCs by their expression of

the CX_3CR1 chemokine receptor and other markers (Bar-On et al., 2010). It remains to be shown whether and how these "ex-pDCs" contribute to immunity.

Langerhans Cells

LCs are a unique population of mononuclear phagocytes restricted to the epidermal skin layer. Identified in the 19th century, these cells gained special attention after the discovery of the splenic DC. LCs express MHC-II and could stimulate an MLR after in vitro culture with T cells. Moreover, it was the study of LCs that led to the realization of distinct functional DC "maturation" stages (Schuler and Steinman, 1985). LCs were long considered prototype sentinel tissue-resident DCs sampling their environment and migrating to skin-draining LNs to activate naive T cells (Romani et al., 1989). However, no firm evidence for a role of LCs in the priming of protective naive T cell immunity could be established (Romani et al., 2012). Additionally, the notion that LCs belong to the DC family has been confounded by their distinct gene expression that is similar to that of macrophages, rather than cDCs (Miller et al., 2012). Notably, LCs also share a prenatal origin with most tissue macrophages, which is in stark contrast to the short-lived cDC compartment relying on constant renewal by HSC-derived cells (see below). The LC compartment is established before birth from fetal liver-derived monocytes (Hoeffel et al., 2012), and LCs self-renew under physiological steady-state conditions without replacement by blood-borne precursors (Merad et al., 2002). In addition and in contrast to cDCs, LC development is independent of Flt3L (Ginhoux et al., 2009) but requires Csf1r receptor engagement (Ginhoux et al., 2006), though not by Csf1, but rather its alternative ligand IL-34 (Wang et al., 2012). Collectively, these data suggest that LCs are closer related to tissue-resident macrophages than to cDCs.

cDC Development

cDCs generally display a short half-life of approximately 3–6 days and are constantly replenished from BM precursors in a strictly Flt3L-dependent manner (McKenna et al., 2000). The transcription factor PU.1 (Anderson et al., 2000), Gfi1 (Rathinam et al., 2005), and Cbfβ (Satpathy et al., 2014) control the development of the common DC lineage and absence of either of these regulators leads to perturbed cDC development (Figure 1).

Myeloid and lymphoid branches of the immune system bifurcate early in hematopoiesis into common myeloid and lymphoid precursors (CMPs, CLPs) (Akashi et al., 2000; Kondo et al., 1997). Early adoptive CMP and CLP transfer experiments established that both cells have the potential to give rise to cDCs, but concluded that most steady-state cDCs, including CD8α-positive and -negative subsets, are of myeloid origin, because CMPs vastly outnumber CLPs (Manz et al., 2001). DCs thus initially share their origin with monocytes. Eventually, however, monocyte and DC committed precursors (MDPs) (Fogg et al., 2006) either give rise to "common monocyte progenitors" (cMoPs) restricted to monocytes and their descendants (Hettinger et al., 2013) or commit toward a pDC or cDC fate (Figure 1; Naik et al., 2006; Onai et al., 2007). A clonogenic CDP-derived pre-DC population was first identified in lymphoid tissues (Diao et al., 2006; Naik et al., 2006), subsequently found in the blood, and shown to populate lymphoid organs via the circulation to give rise to cDCs in lymphoid and nonlymphoid tissues (Liu et al., 2009; Varol et al., 2009; Bogunovic et al., 2009). Although these early studies suggested that pre-DCs can give rise to all cDCs (Liu et al., 2009), detailed studies on the potential of CDPs and pre-DCs in terms of their differential or preferential development into either $CD8\alpha^+$ and $CD103^+$ or into $CD11b^+$ cDC subsets in lymphoid and nonlymphoid tissues are missing. In the periphery, for instance, pre-DCs seem biased toward a $CD103^+$ cDC fate, whereas the origin of $CD11b^+$ cDCs is heterogeneous (Ginhoux et al., 2009). In support of this notion, IRF8-deficient mice lack $CD8\alpha^+$ and $CD103^+$ lymphoid and nonlymphoid tissue cDCs (Aliberti et al., 2003) in accordance with the strong reduction of CDPs (Schönheit et al., 2013) and pre-DCs (data not shown). These animals, however, do harbor $CD11b^+$ cDCs (Aliberti et al., 2003). Precursors of $CD11b^+$ cDCs might thus bypass an IRF8-dependent pre-DC stage or pre-DCs could themselves be heterogeneous. Pre-DCs of zbtb46-GFP reporter animals can indeed be subdivided according to expression of the lectin Siglec-H and zbtb46 (Satpathy et al., 2012). Of note, in these studies only zbtb46-GFP-expressing pre-DCs gave rise to $MHC\text{-}II^+$ cDCs in culture, and therefore truly represent committed pre-DCs (Figure 1). The exact potential of these different pre-DC subsets to develop in vivo into the main lymphoid and nonlymphoid tissue cDC populations needs further investigation. Additional insight might come from DC fate mapping studies, such as the one exploiting DNGR-1 expression (Schraml et al., 2013). It remains to be shown how well the short-lived DC compartment is amenable to the Cre-loxP-based approach. In addition, gene expression patterns of cDCs overlap considerably with that of macrophages and monocytes, in particular after activation of the latter, which can seriously confound such strategies.

Contributions of cDC Subsets in Lymphoid Organs

cDCs reside in tissues where they contribute functions that can, but must not necessarily, be related to their generic, well-accepted function as migratory APCs that mediate T cell cross-talk. Below we will discuss the current knowledge of cDCs that reside in lymphoid and selected nonlymphoid tissues.

Spleen

The spleen harbors at least three populations of resident cDCs: $CD8\alpha^+$ cDCs, $Esam^{hi}CD11b^+$ cDCs, and $Esam^{lo}CD11b^+$ cDCs. $CD8\alpha^+$ cDCs represent about 20%–30% of the total splenic DC compartment and are localized in the marginal zone where they sample lymph- and blood-borne Ags and pathogens (Idoyaga et al., 2009). Indeed, during infection, a fraction of systemic, Gram-positive bacteria can associate in the blood with platelets via the covalent opsonizing complement factor C3 and platelet receptor GPIb. These platelet-coated bacteria can thereby be specifically targeted to splenic $CD8\alpha^+$ DCs in order to allow protective immunity and avoid clearance by less immunogenic macrophages (Verschoor et al., 2011). However, this mechanism also renders $CD8\alpha^+$ cDCs a unique splenic entry portal for intracellular pathogens, such as *Listeria monocytogenes* (Neuenhahn et al., 2006). $CD8\alpha^+$ cDCs furthermore efficiently uptake apoptotic or necrotic cells from peripheral blood (Iyoda et al., 2002) and are thus able to present exogenous tumor- or virus-derived Ags. Depending on the context, Ag uptake can result in cross-tolerance or cross-priming that is required for the triggering of CTL responses to viral or cancer Ag in the

absence of cytosolic Ag generation by cDCs (Rock, 2003). Accordingly, BATF3-deficient mice, which specifically lack CD8α$^+$ cDCs, display for instance impaired West Nile Virus-specific CTL responses and ineffective tumor rejection (Hildner et al., 2008). Further functional properties of splenic CD8α$^+$ cDCs are their highly specific expression of the double-stranded RNA sensor TLR3 (Edelson et al., 2010) and the *T. gondii* sensor TLR11 (Koblansky et al., 2013) that combined with their predisposition to secrete IL-12 critically contributes to the early anti-parasite defense (Mashayekhi et al., 2011).

The splenic Esam^{hi}CD11b$^+$ cDC compartment shows a substantial overlap with CD4$^+$ cDCs and represents 50%–60% of the majority of splenic cDCs. Absence of this cell population, as a result of an impairment of the Notch-signaling pathway by CD11c-Cre-mediated DC-specific deletion of RBP/J (Caton et al., 2007) or NOTCH2 itself (Lewis et al., 2011; Satpathy et al., 2013), severely and specifically impairs CD4$^+$ T cell responses (Lewis et al., 2011). Like CD8α$^+$ cDCs, CD11b$^+$ cDCs are also located in the marginal zone and concentrate in the bridging channels (Dudziak et al., 2007). Recruitment of CD11b$^+$ cDCs to this microanatomical niche is mediated by the chemokine receptor EBI2 (Gatto et al., 2013) and might provide them with critical growth or differentiation factors, such as B cell-derived lymphotoxin (Kabashima et al., 2005). Esam^{hi} cDCs are poor producers of proinflammatory cytokines (Lewis et al., 2011). Of note, the identity of Esam^{hi} cDCs seems at least partially controlled by the transcription factor Runx3, because a DC-restricted Runx3 loss reduces their CD4$^+$ T cell priming capacity and shifts their gene expression signature toward Esam^{lo} cDCs (Dicken et al., 2013). Interestingly, Runx3-bound genes are cobound by zbtb46, suggesting cooperation of these transcription factors in the repression of the alternative Esam^{lo} cell fate (Dicken et al., 2013).

Esam^{lo}CD11b$^+$ cDCs, the third spleen-resident cDC subset, were most recently characterized (Lewis et al., 2011) and are probably overlapping with cells previously termed CD8α$^-$CD4$^-$ "double negative" (DN) cDCs (Vremec et al., 2000). These cells can be identified by their expression of DCAL2 (also known as Clec12a), which is expressed in 33D1$^-$CD8α$^+$ splenic cDCs but not in Esam^{hi}CD11b$^+$ cells (Lewis et al., 2011; Kasahara and Clark, 2012). In line with their "monocytic" gene expression signature, Esam^{lo} cDCs are superior producers of inflammatory cytokines upon TLR trigger (Kasahara and Clark, 2012; Lewis et al., 2011) and efficient Th1 cell inducers in response to in vivo CpG exposure (Kasahara and Clark, 2012). The distinct functions of Esam^{lo} and Esam^{hi} cDCs might suggest a labor division within the CD11b$^+$ cDC compartment with cytokine-producing Esam^{lo} cells representing "detectors," which may supply Esam^{hi} "presenter" cells with defined cytokine micro-milieus at the recognition site (Lewis and Reizis, 2012).

Lymph Node

Like splenic cDCs, LN cDCs can be subdivided into CD8α$^-$ and CD8α$^+$ subsets (Henri et al., 2001), but harbor in addition migratory DCs that entered via the afferent lymphatics from associated nonlymphoid tissues. The latter probably import insult-associated Ags for T cell stimulation (Ruedl et al., 2000), although their exact role versus LN-resident cDCs remains to be determined. LN-resident cDCs could serve to trigger T cell responses by cross-presentation of the immigrating monocytes alongside their cargo (Samstein et al., 2013). Cross-presentation by LN-resident CD8α$^+$ cDCs is, for instance, critical for responses to Ags imported by LCs that themselves seem incompetent to prime (Allan et al., 2003). However, it is less obvious in the case of bona fide cDC immigrants, which are themselves considered fully capable to prime T cells. Specific Ag transfer from moDCs to resident cDCs for naive CD4$^+$ T priming was also observed in yeast-infected animals (Ersland et al., 2010). Thus, cell-to-cell Ag transfer might be a more common theme to be explored in the future. Interestingly, LN-resident, but not migratory, cDCs have also been reported to sample follicular dendritic cell (FDC)-retained Ags (McCloskey et al., 2011). LN-resident cDCs might be important to maintain peripheral tolerance; however, recent experiments suggest that at least under certain conditions, migratory DCs are superior in this respect (Idoyaga et al., 2013). Surprisingly, LN-resident cDCs also seem to control LN entry of lymphocytes by modulating the maturation state of high endothelial venules (Moussion and Girard, 2011), which suggests cDC interactions with stromal components, an area that clearly needs further exploration.

Thymus

In contrast to spleen or LN, thymic DCs are composed of a main CD8α$^+$ and a minor CD8α$^-$ CD172a$^+$ (SIRPα) cDC subset (Wu and Shortman, 2005). Residing in the thymic medulla, these cells were originally thought to mediate negative T cell selection (Ardavín, 1997). However, the prime role in this process is played by the medullary thymic epithelial cells (MTECs) (Yano et al., 2008), which are in addition uniquely specialized to express a vast array of otherwise periphery-restricted tissue antigens (PTA), in part in an Aire-dependent manner (Anderson et al., 2002). Accordingly, constitutive cDC ablation does not result in an overt breakdown of central tolerance (Birnberg et al., 2008), although thymic DCs might still be required for efficient presentation of particular Ag, including the cross-presentation of MTEC-derived PTA. Interestingly, and in contrast to splenic CD8$^+$ cDCs, thymic CD8$^+$ cDCs harbor frequent D-J rearrangements, suggesting that most of these cells derive in steady state from intrathymic lymphoid precursors (Ardavín et al., 1993; Corcoran et al., 2003). The latter can be found within the DN1(c) thymocyte gate characterized as CD4 or CD8^{lo} CD117$^+$ CD44$^+$CD25$^+$ cells (Luche et al., 2011). However, adoptively transferred CMPs have also been shown to give rise to thymic CD8α$^+$ cDCs (Manz et al., 2001) and therefore these cells may be of mixed origin. Thymic CD8α$^+$ cDCs can cross-present Ags to T cells in the absence of licensing factors (Dresch et al., 2011) and these XCR1$^+$ cells seem efficiently attracted to the medulla by MTEC-derived XCL1 (Lei et al., 2011). Thymic CD8α$^-$ cDCs were suggested to immigrate at least in part from the blood (Li et al., 2009), localizing to the thymic cortex and perivascular regions, where they might sample blood-borne antigens (Baba et al., 2009). Thymic CD8α$^-$ cDCs cannot cross-present (Proietto et al., 2008). In experimental settings, adoptively transferred splenic cDCs were shown to contribute to negative selection by carrying peripheral Ags in a tolerogenic context (Bonasio et al., 2006). At least in the mouse, however, cDCs have limited access to the circulation and these cells do not leave the LN via efferent lymphatics. Interestingly, the migration of peripheral DCs to the thymus seems to depend on CCR2 (Baba et al., 2009), which suggests a contribution of moDCs. Moreover,

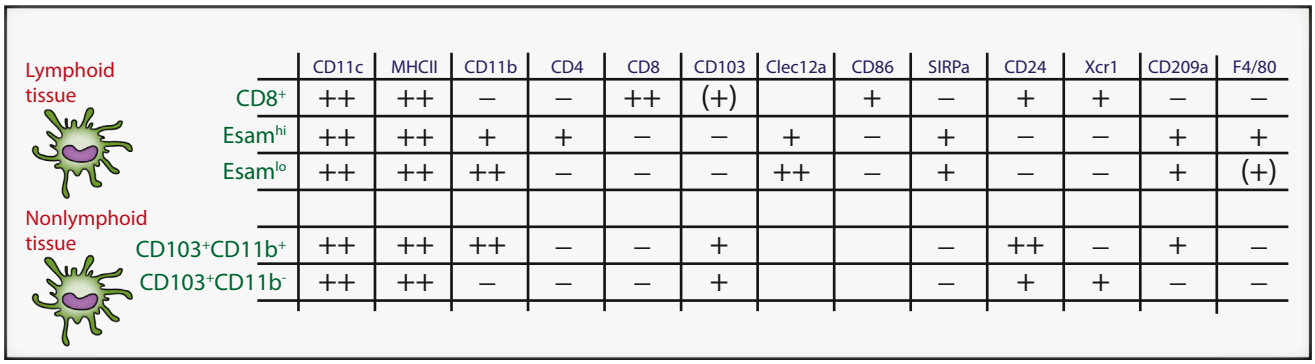

Figure 2. Surface Phenotype of DCs
cDCs in lymphoid and nonlymphoid tissue differentiate from a circulating pre-DC. Within the tissue, the different cDC subsets can be divided according to the indicated cell surface markers.

CCR9$^+$ pDCs have also been reported to carry peripheral Ags into the thymus (Hadeiba et al., 2012), although the physiological relevance of these pathways remains to be shown.

Bone Marrow

The BM hosts cDCs (Feuerer et al., 2003), which via intravital microscopy have been shown to be organized into discrete perivascular clusters (Sapoznikov et al., 2008). These cells are distinct from moDCs (Milo et al., 2013) and share activities and surface signatures with splenic cDCs (Sapoznikov et al., 2008) but have not been dissected into subsets. Moreover, attempts to assign to these cells a role as primary APCs for the stimulation of naive T cells in the BM niches have failed and instead revealed promiscuity—at least with respect to CTL responses (Milo et al., 2013). In an interesting twist, these BM-resident cDCs were found to constitute a critical survival niche for recirculating B cells by providing macrophage migration inhibitory factor (Mif) (Sapoznikov et al., 2008). This is reminiscent of the dependence of marginal zone B cells on moDCs for survival and differentiation, although the latter involves the TNF "superfamily" members BAFF and APRIL (Balázs et al., 2002). Finally, the BM recently emerged as a reservoir of long-lived CD4$^+$ and CD8$^+$ memory T cells (Tokoyoda et al., 2009). However, whether cDCs are involved in reactivation of the latter, or whether this is accomplished by the arguably more mobile, and after activation probably more potent, B cells (Lanzavecchia, 1987) remains to be shown.

Contributions of cDC Subsets in Nonlymphoid Organs

Like lymphoid organs, most nonlymphoid tissues contain at least two major subsets of cDCs that often share the αE integrin CD103 marker but can be distinguished according to CD11b expression (Figure 2). The study of nonlymphoid tissue DCs so far largely involved tissues that are in contact with body surfaces, such as the skin, lung, and intestine, and we will hence focus on these organs. Of note, cDCs in these tissues are found embedded in a network of tissue-resident macrophages and additional moDCs (Tamoutounour et al., 2013; Varol et al., 2009). Recruitment of the latter might be related to a constant exposure of these sites to bacterial products that create a low-grade inflammatory milieu and state of "primed homeostasis" (Zigmond et al., 2012). Steady-state presence of moDCs, which are barely distinguishable from CD11b$^+$ cDCs, probably complicates the interpretation of the available data. However, recently, additional markers were identified (such as CD64, CD24, and MAR-1) that should allow in the near future better separations of tissue-resident CD11b$^+$ cDCs from moDCs.

Skin

LCs residing in the skin epidermis were long considered the principal skin DC population and to be critical for the defense against external threats. Indeed, LCs efficiently phagocytose pathogens after epidermal cell injury and switch their chemokine expression pattern from CCR6 to CCR7, i.e., from a "sessile" to a "mobile" mode (Dieu et al., 1998), allowing them to emigrate toward cutaneous LNs (Stoitzner et al., 2003). However, whether LCs themselves can stimulate naive T cells or require cross-presenting LN-resident CD8α$^+$ cDCs is under debate (Mayerova et al., 2004; Allan et al., 2003). More recently, research has focused on the DC compartment of the skin connective tissue layer, the dermis, which was found to host multiple cDC subsets, as well as moDCs. Analysis of reporter mice for langerin (CD207), a C-type lectin required for characteristic LC Birbeck granules (Kissenpfennig et al., 2005), revealed the existence of langerin-expressing dermal DCs (Bursch et al., 2007; Ginhoux et al., 2007). In addition to LC "in transit," the dermis is currently believed to comprise four cDC populations: CD103$^+$CD207$^+$ and CD103$^-$CD207$^+$ cDCs, CD207$^-$CD11b$^+$ cDCs, and cDCs negative for CD207, CD11b, and CD103 (Henri et al., 2010). The exact relationship of these subsets remains unknown and mainly functions of CD103$^+$CD11b$^-$ cDCs and CD207$^-$CD11b$^+$ cDCs have been addressed. Of note, the latter can also include monocyte descendants, which show partial overlap in function and gene expression with skin CD11b$^+$ cDCs (Tamoutounour et al., 2013). All dermal DC populations display CCR7-dependent migration to cutaneous LNs (Henri et al., 2010; Ohl et al., 2004) and all dermal cDC subsets can efficiently present Ags in a MHC-II context (Bedoui et al., 2009).

Mirroring splenic cDC labor division, dermal cDCs display functional specification. Thus, only CD207$^+$CD103$^+$ dermal cDCs efficiently cross-present auto-Ag in a transgenic animal model in which keratinocytes express a membrane form of ovalbumin as a model antigen (Henri et al., 2010). Moreover, in HSV-1-infected mice, only CD103$^+$ dermal cells could present viral Ag to naive CD8$^+$ T cells (Bedoui et al., 2009). This establishes dermal CD103$^+$ cDCs as the functional equivalent of

splenic CD8α$^+$ cDCs (Edelson et al., 2010). Generation of CD103$^+$ cDCs accordingly depends on IRF8, ID2, and BATF3 (Ginhoux et al., 2009; Hildner et al., 2008).

Interestingly, migratory dermal cDCs rather than LN-resident cDCs seem critical for peripheral tolerance induction (Idoyaga et al., 2013; Tamoutounour et al., 2013). Induction of Treg cells needs retinoic acid (RA) (Coombes et al., 2007), produced by the enzyme retinaldehyde dehydrogenase (RALDH). Among dermal cDCs, RALDH activity is restricted to CD103$^-$CD11b$^+$ cells (Guilliams et al., 2010), suggesting that this population is specialized in raising Treg cell control to dampen immune responses. In contrast to splenic Esam^{hi}CD11b$^+$ cDCs and intestinal CD11b$^+$ DCs (see below), dermal CD11b$^+$ cDCs do not rely on the transcription factor NOTCH2 for their generation (Lewis et al., 2011). Additionally, a population of CD301b$^+$ dermal DCs, which are superior inducers of Th2 cell immune responses, express CD11b (Kumamoto et al., 2013). LN-directed migration of these cells, though not their generation, depends on IRF4 (Gao et al., 2013).

Intestine

The intestine harbors a complex system of organized lymphoid tissues as primary sites for the induction of adaptive mucosal immune responses, including ileal Peyer's patches, colonic isolated lymphoid follicles, and more remote mesenteric lymph nodes (MLNs). cDCs are in addition found in the lamina propria, the mucosal immune effector site, alongside a prominent population of monocyte-derived macrophages (Zigmond and Jung, 2013). Like other tissue cDCs, intestinal nonlymphoid organ cDCs are Flt3L dependent and pre-DC derived (Bogunovic et al., 2009). They are phenotypically defined by expression of the integrins CD11c, CD103, and more recently CD24 (Schlitzer et al., 2013) and can be further subdivided according to CD11b expression. All intestinal cDCs are migratory cells and found, for instance, in thoracic duct lymph after mesenteric lymphadenectomy (Cerovic et al., 2013). Of note, CD11b$^+$ and CD11b$^-$ CD103$^+$ DCs show distinct prevalence in proximal and distal sections of the gut, suggesting functional specialization (Denning et al., 2011). Most of the available data, however, are on the small intestine and unless stated otherwise, we refer henceforth to this tissue, in particular the ileum. Moreover, we restrict our discussion to the steady-state gut, which is probably in a constant state of "primed homeostasis," due to ongoing exposure to microbial stimuli. Finally, intestinal DCs were originally merely defined as CD11c$^+$ cells and with time subfractionated into intestinal macrophages (now best defined as CD11c$^+$CD64$^+$ cells) and DC subsets. This fact often prevents definitive conclusions concerning subset-specific functions in particular from earlier pioneering studies.

The generation of intestinal CD103$^+$CD11b$^-$ cDCs depends on BATF3, Id2, and IRF8 and these cells thus represent the gut equivalent of splenic CD8α$^+$ cDCs.

BATF3-deficient mice display normal populations of Foxp3$^+$ Treg cells in lamina propria and MLNs. Moreover, their CD4$^+$ and CD8$^+$ T cells in the lamina propria and LNs show normal expression of the gut-homing receptors α4β7 integrin and CCR9 (Edelson et al., 2010). Thus, the ability to induce Treg cells (Coombes et al., 2007; Sun et al., 2007) and imprint a homing signature (Johansson-Lindbom et al., 2005) that has been reported for intestinal DCs seems to be redundant among these cells. So far, therefore, no unique functions have been assigned to CD103$^+$CD11b$^-$ cDCs, although it could be speculated that the latter might be associated with their cross-presentation potential. Although the uptake of infected apoptotic epithelial cells was shown to affect Th17-cell-mediated immunity (Torchinsky et al., 2009), so far no direct link to the CD103$^+$CD11b$^-$ cDC subset has been established for this process.

Paradoxically, in the gut it is the "CD103$^+$CD11b$^+$ cDC" population, which is functionally better defined, despite the fact that it is heterogeneous (Schlitzer et al., 2013; Schreiber et al., 2013). Intestinal CD103$^+$CD11b$^+$ cDCs were subfractionated via the surface markers CD24 and CD64, revealing their composition of bona fide Flt3-dependent CD24$^+$CD64$^-$ cells and contaminating CD24$^-$CD64$^+$ cells that represent intestinal macrophages. Development of CD24$^+$CD103$^+$CD11b$^+$ DCs depends on IRF4 (Schlitzer et al., 2013; Persson et al., 2013) and most likely also on NOTCH2 (Lewis et al., 2011). CD103$^+$CD11b$^+$ cDCs prevail in the small intestine over the colon, coinciding with a descending Th17 cell gradient between these tissues (Denning et al., 2011). Moreover, these cells have been shown to express IL6 and TGF-β mRNA, as well as RA-generating enzymes and efficiently induce the in vitro differentiation of Th17 cells, an activity that was dependent on the microbiota status of the donor animals (Denning et al., 2011). Indeed, CD103$^+$CD11b$^+$ DCs are required in vivo for the efficient generation of Th17 cells as evidenced in mice lacking these cells either due to a IRF4 and Notch deficiency (Lewis et al., 2011; Persson et al., 2013; Schlitzer et al., 2013) or as a result of their conditional ablation (Welty et al., 2013). In contrast, CD103$^+$CD11b$^+$ DC-derived RA seems dispensable for the in vivo generation of "gut tropic" T cells, as well as intestinal FoxP3$^+$ inducible Treg cell development (Persson et al., 2013; Welty et al., 2013), supporting the earlier notion that these activities are redundant among intestinal DCs. CD103$^+$CD11b$^+$ DCs seem to have unique direct access to luminal Ags, because they can translocate into the epithelium, phagocytose pathogens, and probably translocate to the MLNs (Farache et al., 2013). Moreover, ileal CD103$^+$CD11b$^+$ DCs were also reported to respond to a flagellin-mediated TLR5 trigger with production of IL-23, thus promoting an antibacterial defense cascade (Kinnebrew et al., 2010). Of note, in the colon CD103$^+$CD11b$^+$ cDCs are exceedingly rare, but might be functionally replaced by a population of CD103$^-$CD11b$^+$ cDCs. Interestingly, when retrieved from the lymph after lymphadenectomy, these cells spontaneously induced the differentiation of IFN-γ- and IL-17-producing effector T cells (Cerovic et al., 2013). However, CD103$^-$CD11b$^+$ cDCs remain poorly defined and are currently difficult to discern from monocyte-derived macrophages and moDCs.

Lung

The lung, like the intestine, is vulnerable to pathogenic insult and is constantly exposed to potentially harmful substances. Pulmonary immune responses pose the challenge to prevent immunopathology, such as asthma or allergy. Classical studies have highlighted the abundance of DCs in lung tissue and their dynamic accumulation upon insult (Holt et al., 1990). DC ablation from lungs by the CD11c-DTR approach (Jung et al., 2002) results in delayed T cell responses under various infectious models like intranasal influenza infection model

(GeurtsvanKessel et al., 2008). After phagocytosis of intranasally applied Ags, lung cDCs migrate to the draining LN in a CCR7-dependent manner and participate in the induction of T cell tolerance over innocuous Ag (Idoyaga et al., 2013).

Two main cDC populations have been identified in the lung: intraepithelial $CD103^+CD11b^-$ DCs and $CD103^-CD11b^+$ DCs in the submucosa of the conducting airways (GeurtsvanKessel et al., 2008). Development of lung $CD103^+CD11b^-$ cDCs depends on Id2 and IRF8 (Ginhoux et al., 2009) aligning these cells with the classical CD8 and CD103 DC lineage. Accordingly, lung $CD103^+CD11b^-$ cDCs are specialized in cross-presentation of antigens to $CD8^+$ T cells (del Rio et al., 2007). After influenza virus infection, migratory $CD103^+$ DCs can efficiently induce $CD8^+$ T effector cells in a CD24-dependent manner (Kim et al., 2014). Furthermore, $CD103^+$ cDCs express receptors for apoptotic bodies along with the machinery to cross-present ingested dead cells (Desch et al., 2011). Interestingly, lung $CD103^+CD11b^-$ cDCs can penetrate the epithelial cell layer to gain direct access to the airway lumen without disrupting the barrier function (Guilliams et al., 2013). These structures, which are reminiscent of trans-epithelial dendrites reported for intestinal CX_3CR1^{hi} macrophages (Niess et al., 2005), may provide access to pulmonary Ags. Notably, and in contrast to the skin, lung $CD103^+CD11b^-$ cDCs and not $CD11b^+$ cDCs prominently express RALDH after Ag inhalation and seem thereby to facilitate de novo Treg cell induction (Khare et al., 2013). Of note, it was also reported that lung $CD103^+CD11b^-$ cDCs might have a uniquely extended life span as compared to their $CD103^+$ cDCs equivalent in other tissues (Ginhoux et al., 2009).

Lung $CD103^-CD11b^+$ cDCs remain more enigmatic. As opposed to skin, LN, or spleen, these cells are in the lung outnumbered by the cross-presenting $CD103^+$ cDCs (Ginhoux et al., 2009). Earlier studies suggested that lung $CD11b^+$ cDCs are potent producers of chemo- and cytokines under physiological as well as under allergic inflammation (Beaty et al., 2007), although in this study contaminating moDCs had not been excluded. More recently, $CD11b^+$ cDCs, further defined as being $CD24^+$, were shown to be dependent on IRF4 (Schlitzer et al., 2013). $CD11b^+CD24^+$ cDCs were found to direct a Th17 cell response to *Aspergillus fumigatus* challenge, possibly due to their production of IL-23 (Schlitzer et al., 2013). $CD11b^+CD64^-$ cDCs were also reported to efficiently induce Th2 cell immunity in house dust mite-specific T cells (Plantinga et al., 2013). In contrast to migratory $CD103^+$ DCs, pulmonary $CD11b^+$ DCs were shown to trigger after influenza infection the activation of CTLs that remain in the draining LN and display a phenotype reminiscent of T memory rather than T effector cells (Kim et al., 2014). In summary, these data suggest a labor division among lung cDCs, in that $CD103^+$ cDCs might be specialized in triggering CTL immunity after viral infections and the induction of immunological tolerance, whereas $CD11b^+$ cDCs might efficiently initiate $CD4^+$ T cell responses and T cell memory.

Concluding Remarks

The recent years have seen major advances in our understanding of the classical DC compartment, starting with the definition of cDC ontogeny and thereby the firm establishment of these cells as a separate lineage distinct from monocytes and tissue-resident macrophages. Moreover, in the mouse there is now solid evidence for the existence of defined DC subsets, the $XCR1^+$ ($CD8\alpha^+$ and $CD103^+$) cDCs and $CD11b^+$ cDCs, that display functional specialization. However, critical gaps remain. Of note, progress is somewhat impeded by certain "ghosts in the DC closet." The popular distinction between "immature" and "mature" DCs, a scheme that originally emerged from in vitro cultures, implies that the former cells are functionally incompetent; however, DCs can clearly contribute distinct critical functions as they pass through their different activation states. Another issue is that the DC field is dominated by surface marker-based definitions. CD103, for instance, is used as a direct marker for the cDC subset but its expression is regulated by Csf2 (Zhan et al., 2011). Lack of $CD8\alpha^+CD103^+$ cDCs in Csf2-deficient mice has hence been interpreted as evidence for a critical role of this factor in $CD8\alpha^+$ and $CD103^+$ cDC development (Bogunovic et al., 2009; King et al., 2010; Greter et al., 2012) or simple loss of CD103 expression (Edelson et al., 2011). Emerging unbiased approaches to the study of cDCs, including massive parallel single-cell RNA-seq (MARS-seq) (Jaitin et al., 2014), might help resolve these issue and provide a new definition of functional entities within the cDC compartment. Another important outstanding issue is whether moDCs contribute merely quantitatively or also qualitatively to the functions provided by the cDC compartment and immunity. Finally, to get us closer to fulfill Ralph Steinman's vision of the rational exploitation of the powerful DC activities for vaccination and anticancer therapy, we require a better alignment of the murine cDC subpopulations with the less-well-characterized human DCs. Taken together, the future of DCs is bright but there's much left to do and to cite Ralph Steinman's enthusiastic concluding remark at the annual DC meetings, "Let's get back to work."

ACKNOWLEDGMENTS

We thank members of the S.J. lab for discussion and in particular E. David for help with bioinformatics.

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