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Dendritic Cells Revisited

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Keywords

dendritic cells, Langerhans cells, monocytes, myelopoiesis, adaptive immunity, innate immunity

Abstract

Dendritic cells (DCs) possess the ability to integrate information about their environment and communicate it to other leukocytes, shaping adaptive and innate immunity. Over the years, a variety of cell types have been called DCs on the basis of phenotypic and functional attributes. Here, we refocus attention on conventional DCs (cDCs), a discrete cell lineage by ontogenetic and gene expression criteria that best corresponds to the cells originally described in the 1970s. We summarize current knowledge of mouse and human cDC subsets and describe their hematopoietic development and their phenotypic and functional attributes. We hope that our effort to review the basic features of cDC biology and distinguish cDCs from related cell types brings to the fore the remarkable properties of this cell type while shedding some light on the seemingly inordinate complexity of the DC field.

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INTRODUCTION

Found in all mammalian tissues, dendritic cells (DCs) are sentinel leukocytes that play a key role in the initiation and regulation of adaptive immune responses, as well as in innate immunity. Since their discovery by Ralph Steinman & Zanvil Cohn (1) almost 50 years ago, more than 90,000 publications referring to dendritic cells have been deposited in PubMed, underscoring the enormous progress in the field. At the same time, a myriad of cells have been grouped under the DC umbrella, generating confusion as to what constitutes bona fide DCs and how to describe their functional attributes and the changes they undergo during their life span (**Figure 1**). Here, we review some of the principles of DC biology, referring back to historical notions while incorporating recent advances in our understanding of cell origin, diversity, and function. We focus our discussion on the unique cell lineage that is nowadays called conventional or classical DCs (cDCs) (see the sidebar titled Terms and Definitions). As clarified throughout this review, these cells best correspond to the ones originally discovered by Steinman & Cohn and are distinct from plasmacytoid cells (PCs), Langerhans cells (LCs), and monocyte-derived cells (MCs), which have all been called DCs in some contexts. As the laboratory mouse has been the model of choice to study cDCs, mouse studies feature prominently in this article, but we also discuss human cDCs and draw

TERMS AND DEFINITIONS

Activated cDC: a cDC that has undergone activation and is capable of transmitting information to other immune and/or nonimmune cells. There are different types of activated cDCs with different functions that are dictated by external signals as well as by ontogenetically determined constraints in functional capacity. As such, activated cDC1s and cDC2s can influence different types of responses, from tolerance/regulation (e.g., induction of Tregs) to distinct types of immunity (e.g., innate immunity, Th1, Th2, Th17, Tfh, CTL)

cDC: a classical or conventional DC identified on the basis of ontogenetic and phenotypic criteria (**Figure 1**)

cDC activation: the process by which a resting cDC acquires the capacity to convey information to other immune and/or nonimmune cells

DC maturation: a term historically used to denote the acquisition of T cell immunostimulatory activity by cDCs in response to a given stimulus, such as engagement of pattern recognition receptors. The term maturation has come to equate specific phenotypic changes, specifically the upregulation of so-called maturation markers such as CD80, CD86, and CD40. However, upregulation of these markers is also found in tolerogenic cDCs, and not all immunogenic cDCs express them equally. Use of the term can be misleading to those not familiar with cDC biology, especially as it is sometimes also used to refer to cDC differentiation from progenitors

Differentiation: the developmental transition from a precursor cell to a differentiated state. In the final differentiation step of cDCpoiesis, pre-cDCs give rise to terminally differentiated cDCs expressing high levels of MHC-II and CD11c in both lymphoid and peripheral organs. Differentiated cDCs should not be confused with mature cells, a term that in the DC field was frequently used to denote a specific type of activation event

Monocyte-derived cell (MC): cell that can share phenotypic markers with cDCs, such as CD11c and MHC-II, especially under inflammatory conditions. MCs can act as APCs in tissues, but whether they have a major role in antigen transport to lymph nodes and in naive T cell priming remains a matter of debate

PC: plasmacytoid cell. PCs are capable of producing type I interferons upon infection. They mainly have a lymphoid origin and are ontogenetically distinct from cDCs

Resting cDC: a cDC in its sentinel environment-sampling mode and not actively transmitting information to other cells. However, it may still interact with its surroundings and receive tonic signals that maintain viability and competence

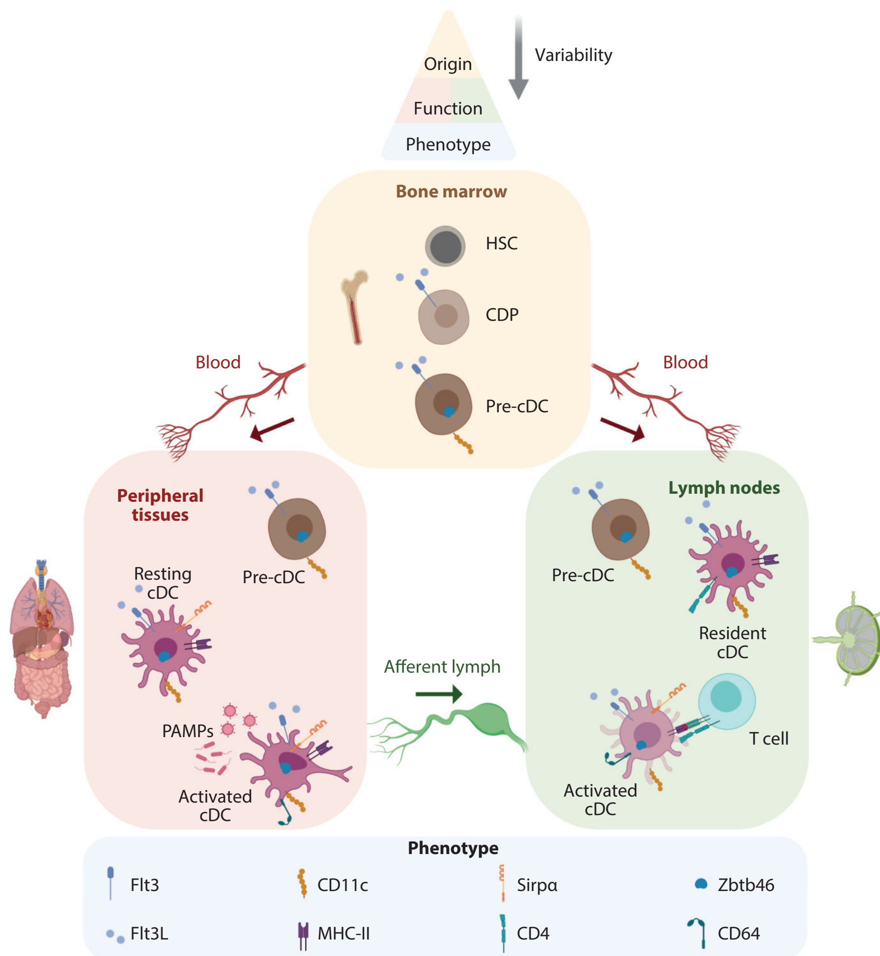


Figure 1

The life history of a cDC, from its origin in the bone marrow to its death in a lymph node. A cDC2 is depicted, but a similar path applies to cDC1s. cDCs develop from HSCs via intermediate stages with cDC-restricted potential, as CDPs and pre-cDCs. This is dependent on Flt3L acting on Flt3. Pre-cDCs egress from the bone marrow and travel through the blood to colonize lymphoid and nonlymphoid organs, where they differentiate into resting cDCs that function as sentinel cells. Changes to the local environment, including the presence of PAMPs (depicted), can be detected by cDCs, inducing activation. This involves multiple phenotypic changes that can include acquisition of markers typically restricted to macrophages and monocyte-derived cells (e.g., CD64, depicted), which complicates the experimental identification of cDCs. Activation is often accompanied by migration to dLNs through the afferent lymph. cDCs resident in dLNs can also be activated and can acquire antigens carried in afferent lymph. In the context of infection, activated cDCs in dLNs typically prime pathogen-specific T cells, as depicted. Abbreviations: cDC, conventional DC; cDC1, type 1 cDC; CDP, common DC progenitor; DC, dendritic cell; dLN, draining lymph node; HSC, hematopoietic stem cell; PAMP, pathogen-associated molecular pattern. Figure adapted from images created with BioRender.com.

parallels across the two species. We further include a list of terms used in this review with our own definitions, which may differ from those of others (see the sidebar titled Terms and Definitions). We hope that readers will find this article of value, whichever classification of DCs they choose to use.

Discovery and Early History of Dendritic Cells

The discovery of DCs[1], together with the later identification of pattern recognition receptors (PRRs), connected the innate and the adaptive immune systems proposed by Ilya Metchnikov and Paul Ehrlich, respectively (2). Before the discovery of DCs, lymphocytes were thought to be able to mount immune responses unaided (2). However, using an in vitro system developed by Robert Mishell & Richard Dutton (3), Donald E. Mosier (4) found that whole mouse splenocyte suspensions but not purified lymphocytes were capable of generating antibodies to heterologous erythrocytes and suggested that accessory cells in the spleen adherent cell fraction were required to help initiate adaptive immune responses. Accessory cells were initially thought to correspond to macrophages (5). When Steinman joined Cohn's laboratory, he found among mouse spleen cells a semi-adherent cell type with distinct morphology (1). These dendritic cells (from the Greek *dendron* for "tree," referring to their tree-like processes) had a stellate shape and, in contrast to typical macrophages, contained few lysosomes. Over the following years, a series of experiments by Steinman and his collaborators established that DCs are distinct from macrophages and the most potent splenic accessory cells for stimulating T cell responses, a function that became a defining property of the cells (reviewed in 6). Human blood counterparts of mouse splenic DCs were later identified (7), indicating that these cells exist across species.

In addition to T cell stimulation, a functional property ascribed to DCs was their ability to take up antigens and transport them to local lymphoid tissues. Early work suggested that LCs can leave the skin and move via the afferent lymph as veiled cells to draining lymphoid organs carrying antigenic material (8, 9). Skin LCs were postulated to represent a type of peripheral DC and were shown to undergo remarkable changes in culture that made them resemble the DCs traditionally isolated from mouse spleen (10). This led to the concept of DC maturation, the thought that cells in peripheral tissues are in an antigen-sampling state and then mature into an immunostimulatory state as they migrate to secondary lymphoid organs to present previously acquired antigens and prime T cell responses. This migration and maturation concept is a cornerstone of DC biology, as discussed in the section titled Functions of Conventional Dendritic Cell Subsets.

Expanding the Dendritic Cell Family and Contracting It Again

Many subsequent studies implicated DCs in directing immune responses against allografts, pathogens, or cancer cells, as well as in maintaining self-tolerance (reviewed in 6). Researchers also began to appreciate that DCs, even the ones from mouse spleen, are heterogeneous. Ken Shortman's group first found a subgroup of mouse DCs expressing CD8αα in the spleen and thymus (11) and later described another subset expressing CD4 (12). Others also found subpopulations of murine DCs in the spleen, lymph nodes (LNs), Peyer patches, and skin (13). Gene expression analysis confirmed the segregation of splenic DCs into discrete subsets (14), a finding that was recapitulated across multiple mouse tissues (15). Eventually, two major subtypes of DCs, currently referred to as cDC type 1 (cDC1) and cDC type 2 (cDC2), were found to exist in lymphoid and nonlymphoid organs, in addition to the LCs found in skin and mucosa. Around the same time, cells in both humans and mice capable of producing large amounts of type I interferons in response to viruses and some Toll-like receptor (TLR) (see the section titled Sensing) agonists were argued to be able to differentiate into DC-like antigen-presenting cells able to stimulate T cell responses; they were renamed plasmacytoid DCs (16, 17). The DC family extended further with

[1]The term dendritic cell and abbreviation DC in this review are used in their historical context to refer to different cell types that have been argued to be part of the dendritic cell family. Only some of these constitute cDCs, as made clear herein.

the finding by Federica Sallusto & Antonio Lanzavecchia (18) that human monocytes cultured in vitro with granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-4 give rise to DC-like cells and with the observation that mouse monocytes can generate cells with DC-like features during inflammation in vivo (19). Collectively, the discoveries of subtypes of tissue DCs and cells that resemble DCs in certain scenarios led to a broadening of the concept of DCs to the point that the definition of DCs became nebulous.

A combination of ontogeny studies and gene expression profiling has provided some clarity. Ontogeny is immutable, and therefore, the relationship between two cell types can often be determined by referring back to their most recent common ancestor. A key development was the identification of clonogenic DC progenitors in mouse bone marrow (20–22). This indicated that plasmacytoid DCs and cDCs shared a recent common ancestor, justifying their joint inclusion in the DC family from an ontogenetic perspective. However, this has been challenged by more recent studies that now suggest that mouse plasmacytoid DCs predominantly originate from a progenitor distinct from that which gives rise to most cDCs (23–25). Furthermore, the gene expression profile of plasmacytoid DCs is different from that of cDCs (26–28). Therefore, it appears that plasmacytoid DCs are divergent from cDCs, and we henceforth refer to these cells as plasmacytoid cells (PCs) rather than DCs.

The adoption of ontogenetic and gene expression criteria also led to a reevaluation of the inclusion of LCs in the DC family. LCs derive primarily from fetal liver monocytes that colonize the skin during embryogenesis (29). Similar to many tissue macrophages, LCs maintain themselves by local proliferation in response to macrophage growth factors M-CSF (macrophage colony-stimulating factor) and IL-34 (30) and do not depend on the cDC growth factor Flt3L (31) (see section titled Defining Conventional Dendritic Cells in the Mouse). LCs have a distant gene expression relationship to cDCs (32) and are not labeled in a cDC-lineage-tracing mouse model (33). As such, LCs have recently been reclassified as macrophages rather than DCs (34). As for MCs, they are, by definition, derived from monocytes and therefore ontogenetically distinct from cDCs. MCs, including mouse and human monocyte-derived DCs and tumor necrosis factor/inducible nitric oxide synthase–producing (TiP) DCs, also have a distinct gene expression profile from cDCs (28, 35, 36). In sum, by ontogenetic and gene expression criteria, LCs, PCs, and MCs are clearly distinct from cDCs.

This leaves open the notion of function. Much of the impetus for broadening the DC family came from the historical definition of DCs as stimulators of T cells, a property that, over time, became synonymous with antigen-presenting cell (APC) function. However, almost all cell types can act as APCs in the appropriate milieu or experimental setup. LCs, PCs, and MCs likely do act as APCs in many instances and probably contribute to local restimulation of effector and memory T cells (36). The jury is still out as to how much of a role they play in naive T cell priming in secondary lymphoid tissues, which was the original attribute of Steinman's DCs. For example, the T cell–priming ability of PCs reported in earlier studies has been found in some instances to be likely due to contamination with cDC progenitors (23–25). Similarly, in the mouse, it has recently been shown that when MCs are rigorously separated from contaminating cDCs, they have minimal ability to prime T cell responses and do not migrate from the periphery to LNs (37). While we remain agnostic as to whether other cells can prime T cells, hereafter we focus our attention on cDCs.

DEFINING CONVENTIONAL DENDRITIC CELLS IN THE MOUSE

Many of the early and recent developments in the DC field have involved mouse studies. We first review some of the experimental approaches to studying cDCs in mice, continue with a

summary of the process of cDC differentiation from precursor cells (cDCpoiesis), and finish with a discussion of mouse cDC heterogeneity.

In Vitro Tools for Studying Mouse Conventional Dendritic Cells

In early days, isolating mouse DCs was a cumbersome process not easily reproduced across laboratories. In 1992, a protocol was published showing how to generate large numbers of mouse DC-like cells in vitro from whole blood or bone marrow cells using GM-CSF (38, 39). This culture system, with refinements, including the addition of IL-4 in some cases, was enthusiastically adopted and widely used to study mouse DC biology (40). However, it was recently found that GM-CSF cultures of bone marrow cells in fact yield a combination of MCs resembling macrophages and cDC-like cells that do not correspond to either cDC1s or cDC2s (41). As such, it is likely that some of the functions ascribed to cDCs from studies of GM-CSF cultures reflect the activities of macrophages as much as those of cDCs (40). In contrast, a different bone marrow culture system using Flt3L generates PCs and fully differentiated cDCs that more genuinely represent the cDC subsets found in vivo (20, 21). Addition of GM-CSF to Flt3L cultures increases the proportion of cDC1s, which are otherwise underrepresented (42). It is therefore possible that GM-CSF can help support the Flt3L-driven development and activity of cDCs, but Flt3L cultures provide a more relevant model for the study of cDCs than GM-CSF cultures.

Genetic Tools to Define Conventional Dendritic Cells in Mice

Supplemental Material >

Consistent with the above, mice lacking GM-CSF (*Csf2*$^{-/-}$) or its receptor (*Csf2rb*$^{-/-}$) have largely normal cDC populations (22, 43, 44), while mice deficient in Flt3L (*Flt3l*$^{-/-}$) or its receptor Flt3 (aka CD135, encoded by *Flt3*) display a dramatic reduction in cDC and PC numbers across all tissues (45, 46) (**Supplemental Table 1**). Further, Flt3L injection causes a dramatic increase in the numbers of cDCs in lymphoid tissues (47). As such, Flt3L dependence and responsiveness are often used as a means of defining mouse cDCs (**Supplemental Table 1**).

Monocytes were thought to be the immediate precursors of DCs until, as mentioned in the previous section, cDCs were shown to arise from distinct progenitors (21, 22, 48, 49). Those findings were complemented by fate-mapping strategies for cDC precursors in mice (**Supplemental Table 1**). One cDC-lineage-tracing mouse took advantage of the fact that DNGR-1 (encoded by *Clec9a*) is first expressed by cDC-committed precursors. In Clec9a^{Cre} × Rosa26-STOP$^{fl/fl}$-EYFP mice, these precursors express Cre recombinase, and consequently, their cDC daughters become YFP^{+} despite the fact that many of them, namely cDC2s, cease to express DNGR-1 (33). Monocytes and granulocytes are not labeled in those mice, but the converse is true in a recently developed fate-mapping mouse for *Ms4a3* (a gene expressed by granulocyte-monocyte progenitors), in which there is efficient labeling of monocytes and granulocytes, but not cDCs (50). Finally, Zbtb46 expression is upregulated in cDC-committed precursors, and mice in which Zbtb46 drives expression of a fluorescent protein or a diphtheria toxin receptor have proven broadly useful for marking cDCs or for selectively ablating them (51, 52). However, DNGR-1 and Zbtb46 can be expressed by cells other than cDCs, as can CD11c, which was the first genetic driver employed to induce gene expression in mouse DCs (53, 54). The advantages and limitations of these and other common models to study cDCs are described in **Supplemental Table 1**.

cDCpoiesis in Mice

Like all leukocytes, cDCs ultimately derive from hematopoietic stem cells (HSCs) (**Figure 1**). In the next sections, we describe the trajectory followed by HSCs to give rise to cDCs. We also discuss the process by which tissues become colonized with cDCs and the fate of those cells.

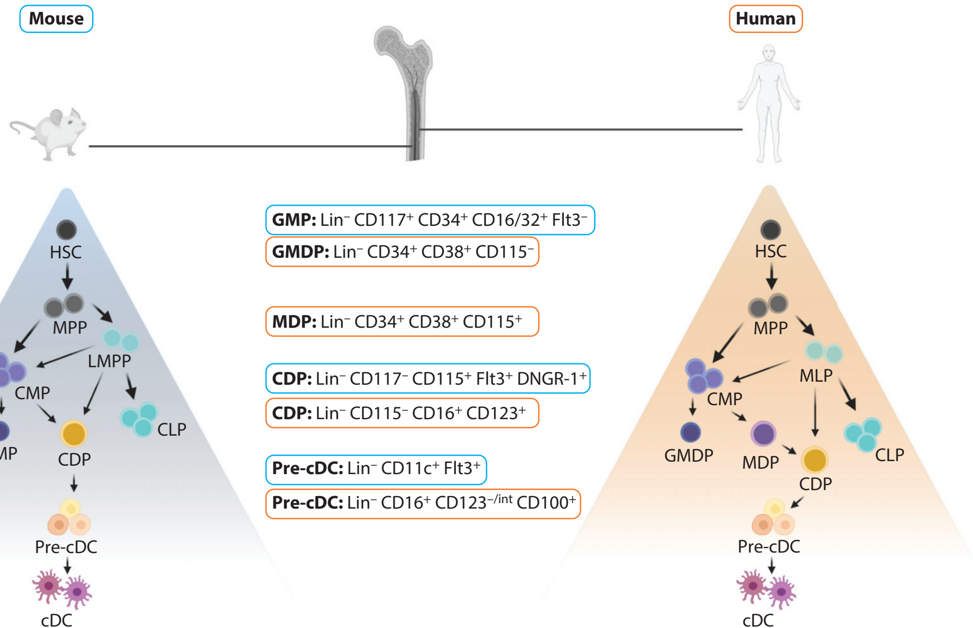


Figure 2

Mouse and human cDCpoiesis. Hematopoietic commitment pathway of cDCs in mice (*blue*) and humans (*orange*). Markers used to identify progenitor populations in mice or humans are listed in the center. Lineage corresponds to TCRβ, CD3, CD19, B220, NK1.1, and Ter119 for mice or CD20, CD14, and CD16 for humans. Abbreviations: cDC, conventional dendritic cell; CDP, common or conventional dendritic cell progenitor; CLP, common lymphoid progenitors; CMP, common myeloid progenitors; GMDP, granulocyte, monocyte and dendritic cell progenitor; GMP, granulocyte and monocyte progenitor; HSC, hematopoietic stem cell; LMPP, lymphomyeloid-primed progenitors; MDP, macrophage and dendritic cell progenitor; MLP, multipotent lymphoid progenitor; MPP, multipotent progenitors. Figure adapted from images created with BioRender.com.

Early hematopoietic precursors of the cDC lineage. HSCs produce multipotent progenitors (MPPs) and lymphomyeloid-primed progenitors (LMPPs) that contain cells primed to give rise to specific lineages (**Figures 1** and **2**). In mice, cellular bar coding showed that LMPPs include cells destined to give rise to cDCs, suggesting that commitment to the cDC lineage can occur very early in hematopoiesis (55). LMPPs can also differentiate into common lymphoid progenitors (CLPs) to generate T, B, and natural killer (NK) cells and other innate lymphoid cells. Even though it has been shown that lymphoid progenitors can generate cDCs in some instances (56–59), fate-mapping studies indicate that CLPs and their progeny do not substantially contribute to the steady-state pool of cDCs in adult mice (60). A subset of LMPPs differentiate instead into common myeloid progenitors (CMPs), which can give rise to granulocyte-monocyte progenitors (GMPs) that in turn generate granulocytes (i.e., mast cells, basophils, eosinophils, and neutrophils) and mononuclear phagocytes including cDCs and monocytes (61) (**Figure 2**). Downstream of GMPs, a population loses granulocyte potential but retains the ability to generate mononuclear phagocytes. These cells were named macrophage–dendritic cell progenitors (MDPs), given their alleged ability to give rise to both cell types (62). However, a later study showed that the putative DCs descended from MDPs are not cDCs but MCs and put in question the existence of MDPs at the single-cell level (63). The notion that GMPs do not give rise to MDPs but, rather, are direct precursors of granulocytes, monocytes, and MCs is supported by a recent fate-mapping study (50). Therefore, the existence of the MDP is controversial, leaving unclear what routes are taken by LMPPs or CMPs to give rise to cDCs (**Figure 2**).

Committing to a cDC fate, from conventional DC progenitor to pre-cDC. The earliest precursors with DC-restricted potential were termed "common" DC progenitors (CDPs), as they were thought to be able to generate both cDCs and PCs (21, 22). As mentioned in the section titled Introduction, this was subsequently put in question by the demonstration that PC precursors are distinct from those that give rise to cDCs (23–25). Hence, CDP should more accurately stand for "conventional DC progenitor." CDPs give rise to pre-cDCs, a transition accompanied by the upregulation of CD11c (**Figures 1** and **2**). Pre-cDCs were first discovered in lymphoid tissues (48, 49). It was later shown that pre-cDCs are the cells that exit the bone marrow via the blood to seed all lymphoid and nonlymphoid tissues and generate cDCs (64) (**Figure 1**).

As discussed, Flt3L is a key factor for generating all cDC subsets (45, 46). Accordingly, CLPs, CMPs, and their immediate progeny are responsive to Flt3L, but their maintenance is not dependent on the cytokine (46). The dependence on Flt3L increases as precursors commit to the cDC lineage, as evidenced by severe reduction of CDPs and pre-DCs but not upstream progenitors in Flt3L-deficient mice (65, 66).

Pre-cDC homing into tissues and cDC life span. Which cues drive pre-cDC egress from the bone marrow, homing to specific organs, and localization within the tissue remains largely unexplored. Use of mixed bone marrow chimeras suggested that CXCR4 is required for the retention of pre-cDCs in the bone marrow. This approach also showed that CCR2 and CX_3CR1 are partially required to mediate pre-cDC homing into the lung but not the spleen, especially following local inflammation induced by LPS inhalation (67). A recent paper confirmed that the local increase in a subtype of inflammatory cDC2s during lung viral infection is CCR2 dependent (37). Whether CCR2 acts at the level of pre-cDCs coming from the bone marrow or in recruiting that particular cDC subtype from the circulation is not known. On the other hand, the presence of cDC1s in the thymus depends on the CCR7-CCL21 axis (68), and their increase in numbers in the brain after cerebral ischemia is dependent on CCR1 (69). This suggests that the homing of pre-cDCs to healthy and inflamed tissues is a complex process and might involve context- and/or tissue-specific pathways.

As for the life span in tissues, experiments with parabiotic mice have shown that cDCs are entirely replaced by bone marrow progenitors every 10–14 days, except for cDCs in the lung, which require 30 days or more depending on the cDC subset (31). These observations have led to the idea that most cDCs are postmitotic, short-lived cells constantly replenished by blood-circulating pre-cDCs. Nevertheless, fully differentiated cDCs proliferate in response to Flt3L (46), and a small percentage of tissue cDCs actively divide even at steady state (26, 31, 70, 71). In addition, pre-cDCs retain proliferative capacity even after seeding tissues, and multicolor fate mapping reveals that cDCs in lung and small intestine are organized as a network of miniclones of sister cells (72). The fate of cDCs is poorly understood, but those cells that migrate from peripheral tissues to the draining LNs (dLNs) are thought to die and to be scavenged locally (**Figure 1**).

Mouse Conventional Dendritic Cell Heterogeneity

As mentioned in the section titled Introduction, there are two basic subtypes of cDCs, and a recently adopted terminology refers to them as cDC1s and cDC2s (36) [not to be confused with archaic designations of DC1s and DC2s, which initially referred to cDCs and PCs, respectively (73)]. We review the phenotype and development of mouse cDC1s and cDC2s below, but first we focus on how to define mouse cDCs and pre-cDCs.

Defining cDCs and pre-cDCs. The flow cytometer is still the tool of choice to analyze mouse cDCs, even if single-cell RNA sequencing (scRNA-seq) and other technologies are increasingly

employed. Mouse cDCs can be identified by the expression of CD45, CD135, CD11c, and MHC-II and the lack of characteristic markers of PCs, T cells, NK cells, B cells, and erythrocytes (see **Supplemental Table 2**). cDC1 and cDC2 subsets in LNs can be resident cDCs that originated from pre-cDCs arriving via the blood from the bone marrow or migratory cDCs that emigrated from tissues via afferent lymph. Resident cDCs express lower levels of MHC-II and higher levels of CD11c than migratory cDCs. Similarly, in peripheral tissues, cDCs presumed to be poised to migrate or in the process of emigrating can be distinguished from their resting counterparts by increased levels of markers such as CD80, CD86, and CCR7 (35). Despite clear overall gene expression signature segregation between cDCs, macrophages, and MCs, few genes are uniquely expressed by cDCs. Among myeloid cells, genes preferentially expressed by cDCs include *Zbtb46*, *Flt3*, *Kit*, and *Ccr7*, while *Cd14*, *Fcgr1* (CD64), and *Mertk* are more restricted to macrophages, and a third group, including *Emr1* (F4/80), *CD68*, and *Csf1r* (CD115), is shared (15, 32).

Supplemental Material >

Pre-cDCs, the immediate precursors of cDCs, have low MHC-II expression but can be identified by the expression of other markers (**Figure 2**; **Supplemental Table 2**). Individual pre-cDCs were originally thought to be bipotential (21, 22). More recently, cDC1/cDC2 subset specification was shown to be able to occur during the CDP to pre-cDC transition to give rise to committed pre-cDC1s or pre-cDC2s (74–76). One study identified uncommitted pre-cDCs in mouse bone marrow as a population that lacks CD115 and CD117, while pre-cDC1s express CD117 and pre-cDC2s express CD115 (74) (**Supplemental Table 2**). Another study used Ly6C and SiglecH and found that pre-cDC1s lack both markers, while pre-cDC2s are Ly6C^{+} and SiglecH^{-} and uncommitted pre-DCs are Ly6C^{-} and SiglecH^{+} (75) (**Supplemental Table 2**). It is not known whether the two gating strategies identify exactly the same cells and whether they can be used to identify committed and uncommitted pre-cDCs in all tissues. When a Clec9a^{Cre}-based multicolor cDC fate-mapping mouse was analyzed, colonization of lung and intestinal tissues was suggested to predominantly involve committed pre-cDC1s and pre-cDC2s (72).

cDC1s. cDC1s in nonlymphoid organs do not express the CD8αα homodimer, which is typically found in cDC1s in the spleen, LNs, and thymus. Instead, peripheral cDC1s express the E-cadherin–binding integrin αE (CD103) or CD24 (although a subset of cDC2s in the lung express CD24, and the same is true for CD103 and cDC2s in the intestine) (77). All cDC1s express the chemokine receptor XCR1 and the C-type lectin receptors (CLRs) DNGR-1 (aka CLEC9A), CD205, and CD207, although the latter two are also expressed by other cells (e.g., LCs) and CD207 is not expressed by cDC1s in the intestine and pancreas (**Supplemental Table 2**). Compared to cDC2s, cDC1s express higher amounts of Flt3 and have low expression of CD11b, Sirpα, and other macrophage markers (35). cDC1s account for approximately 30% of total cDCs in the periphery and 40% in lymphoid organs. The exceptions are Peyer patches and the thymus, in which cDC1s are enriched. It was originally thought that the high abundance of cDC1s in the thymus was a consequence of their intrathymic origin from prothymocytes. Although the lymphoid versus myeloid origin of cDC1s in the thymus is still an area of debate, several studies indicate that thymic cDCs have a conventional origin from circulating pre-cDCs and are unrelated to T cells (77).

Differential transcription factor dependence for development is a useful means to distinguish cDC subsets (28, 78). cDC1 differentiation depends on *Irf8*, *Batf3*, *Nfil3*, and *Id2*. In fact, *Batf3* genetic deletion in mice results in selective loss or dysfunction of cDC1s (79) and has been used widely to study the necessity for cDC1s in different models (**Supplemental Table 1**). Deletion of *Nfil3* and *Id2* in mice also causes cDC1 deficiency (80, 81). However, cDC1s can reappear in *Batf3*-, *Nfil3*-, or *Id2*-deficient mice during inflammation or after irradiation (82, 83). In contrast, *Irf8* plays a nonredundant role, acting at different stages of cDC1 development (78). A recent paper

examined the function of three enhancers that regulate *Irf8* activity and showed that the +32-kb *Irf8* enhancer is necessary to maintain differentiated cDC1s in tissues but not for development of pre-cDC1s (84). In contrast, the +41-kb *Irf8* enhancer acts at the CDP level, allowing these cells to progress to the pre-cDC1 stage (**Supplemental Table 1**). Finally, the −50-kb *Irf8* enhancer regulates *Irf8* expression in monocytes and macrophages, and deficiency in that enhancer does not impact cDC1 development (84). The dynamic interplay between *Irf8* and other transcription factors during cDC1 development and specification was also recently clarified. Differentiation of CDPs into pre-cDC1s requires upregulation of *Id2* and Nfil3-driven downregulation of *Zeb2*, a repressor of cDC1 development. *Id2* expression in more differentiated cells blocks the activity of the +41-kb *Irf8* enhancer, thereby imposing a requirement on the +32-kb *Irf8* enhancer to maintain *Irf8* activity. A puzzling observation is that *Batf3* regulates *Irf8* expression in fully differentiated cDC1s, and yet pre-cDC1s develop normally in *Batf3*- but not in *Irf8*-deficient mice. A recent paper showed that *Batf3* induces the expression of *Irf8* only when activity is regulated by the +32-kb *Irf8* enhancer (85).

Supplemental Material >

cDC2s. The other major subset, the cDC2s, is more abundant and considerably more heterogeneous. Mouse cDC2s were first identified by their expression of CD4 (12). CD4 expression is restricted to a fraction of cDC2s in lymphoid tissues, but in general, most cDC2s express high levels of Sirpα and CD11b (**Supplemental Table 2**). In most tissues, cDC2s do not express typical cDC1 markers, such as CD8, XCR1, and DNGR-1. CD103 is also absent from most cDC2 populations, except in the intestine (35). Apart from the $CD11b^-$ $CD103^+$ population of cDC1s, the intestine harbors a $CD11b^+$ $CD103^+$ population of cDC2s and $CD11b^+$ $CD103^-$ cells, the identity of which is still debated. It has been suggested that intestinal $CD11b^+$ $CD103^-$ DCs migrate poorly to the dLNs; derive from circulating monocytes; develop in an M-CSF-dependent, Flt3L-independent manner; and display a gene expression profile more closely related to that of macrophages than that of cDCs (86). These observations suggest that these $CD11b^+$ $CD103^-$ DCs are MCs rather than cDC2s, although others have argued that they originate from pre-cDCs and are dependent on Flt3L (33, 87).

The development of cDC2s collectively is regulated by a group of transcription factors that includes those encoded by *Irf4* and *Irf2* and by the adaptor signaling protein encoded by *Traf6*. Initially, it was thought that Irf4 was required for the development of all cDC2s. However, a relatively small population of cDC2s persists in the intestine of *Irf4*$^{-/-}$ mice even though its function is impaired (88) (**Supplemental Table 1**). Therefore, it appears that Irf4 is essential to regulate cDC2 function, perhaps at the level of migration from the periphery to lymphoid tissues, even when not strictly required for cDC2 development (78).

Two subgroups of cDC2s have been defined based on differential requirement for either Notch2 receptor or Klf4 transcription factor for development (28, 78) (**Supplemental Table 1**). In the spleen, Notch2-dependent cDC2s can be identified by selective expression of ESAM (53, 88, 89). The Klf4-dependent subset is $CD11b^-$ (90), although a fraction of splenic $CD11b^+$ cDC2s also depends on this transcription factor (91). It was recently shown that splenic cDC2s can also be segregated by T-bet and RORγt expression (26). T-bet- and RORγt-expressing cDC2s have been called cDC2As and cDC2Bs, respectively (26) (**Supplemental Table 1**). The T-bet-labeled population corresponds, at least in part, to the previously described $ESAM^+$ population. The T-bet$^-$ fraction (presumably expressing RORγt) is heterogeneous and expresses CLEC10A and/or CLEC12A but also ESAM (26). Whether ROR-γt and T-bet are necessary for the development of these subsets, whether they are all cDC2s or include MCs, and how they overlap with the Notch2- or Klf4-dependent populations previously described are yet to be clarified.

Confusing Cells

In this section, we discuss mouse cells whose phenotype is not always distinct and that are difficult to categorize as either cDC1s or cDC2s.

Double-negative cells. Depending on the markers used to discriminate cDC1s from cDC2s (for example, CD4 versus CD8, CD103 versus CD11b, and XCR1 versus Sirpα), several laboratories have found by flow cytometry a population of double negative cells of varying size. Plausibly, the double negative population corresponds to either cDC1s or cDC2s lacking the markers in question rather than representing a third cDC subset. For example, a population of $CD103^{-}$ $CD11b^{-}$ splenic DCs that expresses Sirpα, CX3CR1, and CD8α and expands during infection likely corresponds to cDC2s (92–94). Our laboratory and others have found that XCR1 and Sirpα neatly subdivide most cells from the cDC gate ($CD11c^{+}$, $MHC\text{-}II^{+}$) into cDC1s or cDC2s, leaving an almost negligible double negative population in most tissues (90, 95). Therefore, XCR1 and Sirpα may be useful as initial flow cytometry markers to subdivide cDCs into cDC1/2s before additional antibodies are used to probe phenotype. However, we note that XCR1 can be downregulated in cDC1 in certain contexts, such as in some tumors (96).

CD64 and the macrophage headache. CD64, aka FcγRI, was originally proposed to distinguish macrophages and MCs from cDCs (97, 98). Although this might be the case in some tissues, there are bona fide DCs in mouse kidney that express CD64, are labeled in the $Clec9a^{Cre}$-based lineage-tracing mouse, and originate from pre-cDCs in a Flt3L-dependent manner in short-term adoptive transfer experiments (33, 95). More recent characterization of the $Clec9a^{Cre}$-based lineage-tracing mouse indicates that the lung also contains populations of $CD64^{+}$ Flt3L-dependent cDCs (72). In line with these observations, a recent paper showed that type I interferon induced after lung infection or inflammation leads to the appearance of lung cDCs expressing CD64 (37). Given the recent finding that the microbiota induces production of type I interferon from PCs, which acts on cDCs (99), it is conceivable that the proportion of CD64-expressing cDCs in tissues depends on the microbiota composition in any given animal facility. Although it is suggested that $CD64^{+}$ cDCs are a type of cDC2s (33, 37, 100), the possibility that they constitute a heterogeneous population of both cDC1s and cDC2s, or even a distinct cDC subset, has not been ruled out.

DEFINING CONVENTIONAL DENDRITIC CELLS IN HUMANS

cDCs are hardly present in mouse blood (101) but comprise approximately one-tenth of steady-state blood leukocytes in humans. Human blood DCs were first characterized by Steinman's group in the early 1980s, who showed that they are $HLA\text{-}DR^{+}$, lack T and B cell markers, are morphologically distinct from blood monocytes, and can efficiently stimulate a mixed-leukocyte reaction (7). It remains unclear today whether human blood DCs are a blood-resident cDC population, prematurely differentiated pre-cDCs en route to peripheral tissues, and/or cDCs that were resident in those tissues and are in the process of emigrating to the spleen via the blood. Later, the discovery of blood DC antigens (BDCAs) allowed further discrimination of human blood DC subsets (102) at around the same time as the study of human blood DCs was being largely superseded by culture systems. First, DC-like cells were found to develop when $CD34^{+}$ human stem and progenitor cells (HSPCs) from umbilical cord blood were stimulated with GM-CSF and tumor necrosis factor alpha (TNF-α) (103). But the key development came with a seminal study describing an in vitro culture method for developing DC-like cells from monocytes using

GM-CSF and IL-4 (18). This became the standard approach for studying human DCs and trying to harness their therapeutic potential (104). More recently, a renewed effort to characterize primary human cDCs has been undertaken, driven in part by a desire to establish whether the cDC1 and cDC2 subsets found in mice also exist in humans. This effort has since been boosted by technological advances, such as high-dimensional cytometry and scRNA-seq, which have allowed the cross correlation of a large number of surface markers between tissues and species, providing a better picture of human cDC heterogeneity and human cDCpoiesis while unearthing additional complexity.

Models for Human Conventional Dendritic Cell Differentiation

By definition, monocytes generate MCs, which differ by gene expression profiling from bona fide cDCs isolated from peripheral blood (105). It has been suggested that the MCs that develop in GM-CSF/IL-4 cultures (18) may in fact be an in vitro counterpart to CD14^{+} CD1c^{+} inflammatory cells mobilized during immune responses (106). Bona fide human cDCs can be generated in vitro from CD34^{+} cord blood HSPCs (107) containing CD115-expressing progenitors (108). As in mice, cDC development in humans is driven by Flt3L, and administration of the cytokine to humans or humanized mice triggers cDC expansion (47, 109). Similarly, Flt3L supplementation is key for driving development of human cDCs in culture. An effective system for growing human cDCs from HSPCs utilizes a two-step protocol of amplification and differentiation (110) and has been tweaked to improve efficiency and yield (107, 111). In such cultures, Flt3L, Notch signaling, and GM-CSF all act at the precursor level to cooperatively promote human cDC1 generation (112).

Human Conventional Dendritic Cell Ontogeny

As in mice, human HSCs differentiate into MPPs that give rise to CMPs and CLPs (113) (**Figure 2**). CMPs were argued to give rise to a granulocyte, monocyte, and DC progenitor (GMDP) that developed through the MDP and CDP stage before generating cDCs (114). However, only 10% of putative MDPs are able to generate both monocytes and cDCs at the single-cell level (114), reminiscent of data from mouse studies (63). Previously, it was shown that early multipotent lymphoid progenitors (equivalent to the mouse LMPPs) can give rise to monocytes and DC-like cells (115). Although the latter were likely MCs, another study subsequently reported that the same multipotent lympho-myeloid progenitors can give rise to cDCs, particularly cDC1s, and can do so even more efficiently than CMPs (116). Therefore, as in mice, it appears that human cDC specification may occur late, downstream of a CMP, or early in hematopoiesis, at the level of the multipotent lymphomyeloid progenitor (**Figure 2**).

Supplemental Material >

Human pre-cDCs were identified in bone marrow, peripheral blood, and peripheral lymphoid organs (111), and similar to their murine counterparts, they can be subdivided into uncommitted pre-cDCs, pre-cDC1s, and pre-cDC2s (27, 76, 117) (**Supplemental Table 2**). Notably, pre-cDCs have the ability to prime naive T cells and secrete cytokines in response to innate immune stimuli (76), properties normally associated with a more differentiated cDC state (see the section titled Functions of Conventional Dendritic Cell Subsets). These pre-cDCs express markers such as CD123, CD303, and CD304, which had historically been considered to identify PCs, and as aforesaid, pre-cDC contamination may therefore explain some reports of human PCs acting as potent stimulators of T cells (76). A summary of the phenotypic markers currently used to identify the different human DC precursor populations is presented in **Figure 2**.

Human Conventional Dendritic Cell Subsets

Like mouse cDCs, human cDCs are heterogeneous and can be subcategorized into subsets. In the next sections, we review human cDC subsets and the factors influencing their differentiation.

Human cDC1s and cDC2s. Since the first gene expression analysis of total cDCs from the peripheral blood and tonsils (118), gene expression profiling has become the go-to system to study human cDC heterogeneity. For example, at a time when the existence of cDC1s in humans was questioned, a comparative analysis showed that human BDCA-3$^+$ (CD141$^+$) DCs from peripheral blood display a gene expression signature resembling that of mouse splenic CD8α$^+$ DCs (119), paving the way for a series of studies showing that BDCA-3$^+$ DCs are indeed cDC1s (110, 120–122). A summary of the markers currently used to identify human cDC populations can be found in **Supplemental Table 2** and is discussed below.

Supplemental Material >

Human cDC2s and cDC1s are found in blood and in lymphoid and nonlymphoid tissues (123). XCR1 and DNGR-1 act as canonical markers for human cDC1s across tissues and species, but the cells can be further identified by expression of CD141 and CADM1 (124) (**Supplemental Table 2**). As in mice, cDC1s are rarer than cDC2s. The latter can generally be identified by expression of CD1c and SIRPα and absence of cDC1 markers XCR1 and DNGR-1 (124). However, as with their murine counterparts, human cDC2s are more heterogeneous than cDC1s (123) and show a different gene expression profile across tissues (26, 27, 125, 126). A uniform nomenclature for these subsets is still lacking, and human cDC2 classification and characterization remain an active area of investigation.

A number of transcription factors have been implicated in the development of human cDCs, either through in vitro manipulation of human cells or by the discovery of mutations that lead to specific defects in hematopoietic differentiation (28, 127). As in mice, development of cDC1s depends on BATF3 and IRF8 in humans (127, 128). An autosomal dominant mutation in *GATA2* leads to absence of blood cDCs, PCs, tissue cDCs, monocytes, and lymphoid cells, which is associated with a lack of LMPPs and reduced levels of GMPs (129). The patients variably display susceptibility to mycobacterial, human papillomavirus, and opportunistic fungal infections; alveolar proteinosis; and monocytopenia and an increased incidence of myelodysplastic syndrome and leukemia (130). Patients with *IRF8* mutations have also been identified with susceptibility to infections, including *Mycobacterium* infection, and with myeloproliferative disease, likely secondary to increased Flt3L availability (130). These patients can present with a near complete absence of monocytes and cDCs, together with a reduction in CD4$^+$ and CD8$^+$ cells and deficient T cell responses (131).

Additional human cDCs? Several groups have used transcript analysis of single human myeloid cells to suggest further subdivision of DCs into additional subsets named DC3s, DC4s, and DC5s (27). DC3 refers to a subpopulation of inflammatory cDC2-resembling myeloid cells marked by low expression of *IRF8* (132, 133), whereas DC4s, which lack expression of CD141 and CD1c and are identified by the surface marker CD16 (27), are possibly a subset of monocytes based on expression of CD16, CD14, and SLAN (126, 134). DC5s, or AS-DCs, express AXL and SIGLEC6 and share markers and functions akin to those of pre-cDCs (76). AS-DCs are additionally identified by high expression of *IRF8* and have recently been confirmed to act as a progenitor population for cDC2s (132). In sum, DC3s, DC4s, and DC5s are tentative populations found as a result of scRNA-seq clustering that may correspond to new subsets of cDCs or may turn out to be developmental states of cDC1s and cDC2s, plus some macrophage variants and

Supplemental Material >

inflammatory cDCs. An overview of phenotypic commonalities and differences between mouse and human cDC subsets can be found in **Supplemental Table 2**.

Translational Application of Human Conventional Dendritic Cells

Many clinical trials have tried to adoptively transfer human DCs for antitumor immunity, but results have been underwhelming, perhaps because MCs were often used instead of cDCs (104). New methods for generating human cDCs in vitro might help in this regard. Similarly, the use of Flt3L to expand cDCs in vivo has shown encouraging results in mouse cancer models (135), and it is now being tested in clinical trials. In addition, studying the signals that are involved in the egress of cDC progenitors from the bone marrow and that attract them to tissues during steady state and upon challenge might reveal new strategies to mobilize cDCs in therapy. Directly targeting human cDC subsets in vivo has emerged as another potential immunotherapy strategy. This is based on delivery of antigens and adjuvants to cDC1s and/or cDC2s using antibodies to receptors such as DEC205, DNGR-1, and CLEC12A and has shown promising results in mice and nonhuman primates (104).

Translational application requires understanding of the basic principles of cDC biology. These appear to hold across mice and humans. It is clear that cDC1s and cDC2s exist in both organisms, and many of their functional properties are conserved (see the section titled Functions of Conventional Dendritic Cell Subsets). Nevertheless, questions are often raised as to the degree to which mouse studies translate to humans. And this reminds us that when we talk about mice, we are referring in most cases to C57BL mice raised in specific-pathogen-free facilities, which lack the genetic and microbiota diversity that shapes the immune system (136). The use of diverse mice or of animals evolutionarily closer to humans, despite limitations in feasibility, may offer additional opportunities to cement basic cDC biology and facilitate its translation to human immunotherapy.

FUNCTIONS OF CONVENTIONAL DENDRITIC CELL SUBSETS

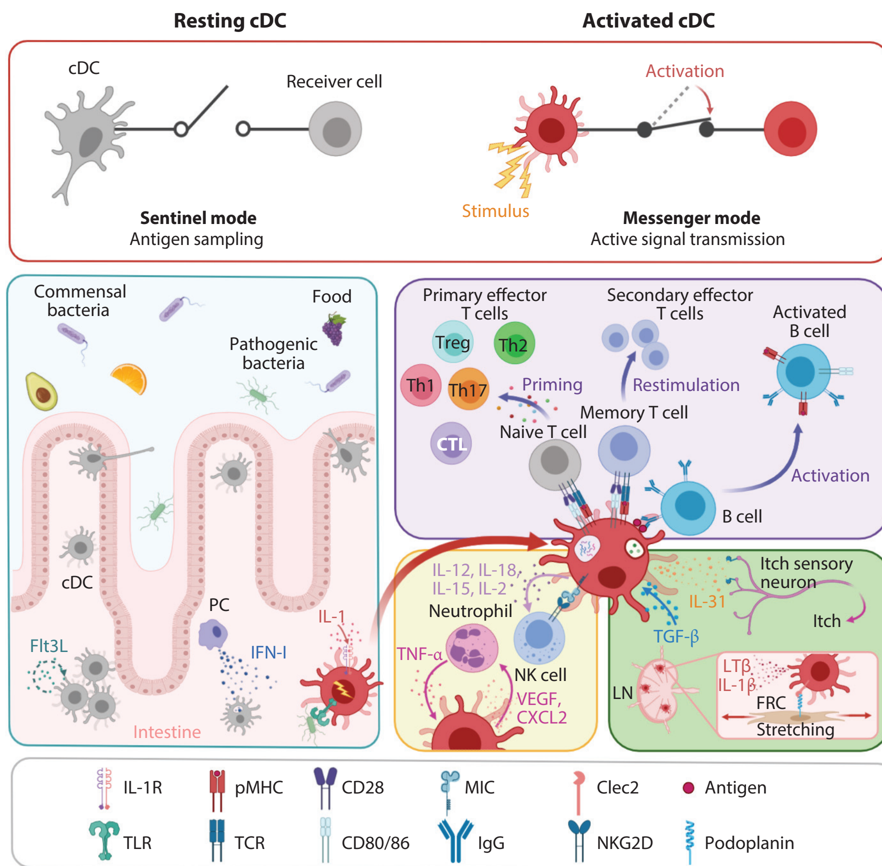
In the preceding two sections, we deliberately avoid referring to cDC properties because we feel that it is important to be able to define cells independently of function in order to be able to ask about the latter. We now review the functions of cDCs and how they vary or not by subset. We follow the logic of the immune response, starting by describing cDCs in peripheral tissues and how they sense infection and injury and become activated (**Figure 3**). We move on to cDC migration and antigen trafficking to dLNs and the anatomical superimposition of cDCs and T cells in the latter tissue and how it contributes to immunity. We end with a discussion of the canonical functions attributed to cDC1 and cDC2 subsets and the extent to which they may have been overstated. Our discussion is heavily biased toward mouse studies, but many of the principles herein apply equally to humans. Notably, although a primary function of cDCs is to prime and direct different classes of T cell responses, we do not cover the molecular details of that process and refer the reader to a companion review in this volume (137).

Sensing

cDCs play a key role in detecting infection, facilitated by their strategic localization at all possible sites of pathogen entry. In the lungs, for example, cDC1s extrude long protrusions across the epithelial layer into the conducting airways to sample airborne antigens (138), while in the intestine, they are able to interdigitate in the epithelial cell layer to capture luminal bacteria (139–141). In contrast, cDC2s have more limited access to antigens in the intestinal lumen and access them

when pathogens cross the epithelial layer or when other cells translocate them to the lamina propria (142). This differential positioning might influence the types of antigen that can be acquired by different cDC subsets and the timing of antigen sampling, although the relevance of any such differences to immunity remains unclear.

The ability of cDCs to initiate immunity to pathogens is largely a result of microbial sensing via innate immune receptors. As predicted by Charles Janeway (143), the latter correspond to PRRs, which recognize ubiquitous microbial signatures, referred to as pathogen-associated molecular patterns (PAMPs). PRRs include TLRs and CLRs that sense extracellular or internalized ligands. They further include cytoplasmic sensors such as RIG-I-like receptors, NOD-like receptors (NLRs), and cytosolic DNA sensors [e.g., cyclic GMP-AMP synthase (cGAS)], which detect cytoplasmic invasion by viruses and some bacteria. Signaling via PRRs dramatically changes the properties of cDCs and brings to the fore their ability to transmit signals to other immune cells and instruct antigen-specific adaptive immune responses. PRRs and other innate immune receptors[2] may also respond to ligands other than PAMPs, including, as independently proposed by



(Caption appears on following page)

[2]The term PRR is sometimes used generically to refer to any innate immune receptor that detects PAMPs or DAMPs. Here, we reserve the use of the term PRR for receptors that can signal in response to PAMP

Figure 3 (*Figure appears on preceding page*)

Activation of cDCs. cDC activation is the process by which a resting cDC becomes competent to convey information to other cells in response to extrinsic or intrinsic stimuli (*red box*). Resting cDCs sample material from their environment (the gut, in this example), including by interdigitating between epithelial cells. They also receive tonic signals such as Flt3L and type I interferon (derived in part from PCs responding to commensal organisms), which regulate proliferation and survival or maintain responsiveness (*blue box*). Activation is triggered in this example by pathogen-derived stimuli such as TLR agonists or cytokines such as IL-1. Activated cDCs convey messages to cells of the adaptive and innate immune systems, as well as nonhematopoietic cells. In adaptive immunity (*purple box*), cDCs in secondary lymphoid tissues prime naive T cells and restimulate memory T cells by presenting processed antigens and providing costimulatory and other signals. Activated cDCs can additionally secrete mediators that can help instruct naive T cell–activated differentiation into CTLs; Th1, Th2, or Th17 cells; or Tregs. Finally, activated cDCs can also present antigens to B cells, leading to antibody responses. In innate immunity (*yellow box*), activated cDCs secreting VEGF and CXCL2 can recruit neutrophils that reciprocally provide TNF-α. NK cells can respond directly to NKG2D ligands presented by cDCs, which secrete IL-12, IL-18, IL-15, and IL-2, providing NK cells with positive signals for proliferation and cytotoxicity. Activated cDCs can also provide signals to nonimmune cells (*green box*). For example, CLEC-2 upregulated on activated cDCs binds podoplanin on LN FRCs, allowing for FRC stretching and LN expansion during immune responses. cDCs also provide LTβ and IL-1β to FRCs and endothelial cells in LNs. As a final example, TGF-β produced during cutaneous wound healing responses induces IL-31 release by activated dermal cDC2s, which increases the sensitivity of itch sensory neurons and induces an itching response in wounded mice. There are many types of activated cDCs, whose functional properties depend on ontogenetic constraints, the nature of the activation signal, exposure to local immune modulators, spatial organization, and temporal factors. Note that activation of cDCs can also result in cDCs that maintain tolerance and suppress immune responses (not depicted). Abbreviations: cDC, conventional DC; CTL, cytotoxic T lymphocyte; DC, dendritic cell; FRC, fibroblastic reticular cell; IFN-I, type I interferon; LN, lymph node; LTβ, lymphotoxin beta; MIC, MHC-I chain–related sequence; NK, natural killer; NKG2D, natural killer group 2, member D; PC, plasmacytoid cell; pMHC, peptide-MHC complex; TCR, T cell receptor; Th1, T helper 1 cell; TLR, Toll-like receptor; TNF-α; tumor necrosis factor alpha; Treg, regulatory T cell; VEGF, vascular endothelial growth factor. Figure adapted from images created with BioRender.com.

Polly Matzinger (144) and Walter Land et al. (145), self-ligands exposed by damaged cells. The latter have been termed danger signals or damage-associated molecular patterns (DAMPs) (144, 146).

Gene expression and proteomics studies established that cDC subsets vary in their expression of innate immune receptors for PAMPs and/or DAMPs (147–149). cDC1s, unlike cDC2s, express high levels of TLR3, an endosomal PRR that detects viral double-stranded RNA within dead cell debris internalized by cDC1s (150). High expression of TLR3 is conserved across mouse and human cDC1s (147, 151, 152) and facilitates cDC1-mediated cross priming of $CD8^+$ T cells against virus-infected cells (150, 152). Mouse cDC1s also express TLR11 and TLR12, both of which recognize a profilin-like molecule from *Toxoplasma gondii*. They respond by producing vast amounts of IL-12, orchestrating innate and adaptive immunity to this pathogen (153–155). Finally, cDC1s across species are commonly identified by their high expression of DNGR-1 (156–158), a CLR that binds filamentous actin, a DAMP exposed by dead cells (159, 160). Signaling via DNGR-1 favors rupture of phagosomes to promote cross presentation of dead cell–associated antigens and is important for cytotoxic T lymphocyte (CTL) effector and memory responses against viruses and allografts (161–165).

In contrast to cDC1s, $CD103^+$ $CD11b^+$ cDC2s in the mouse intestinal lamina propria express TLR5, produce IL-23, and induce T helper (Th) 17 cell responses in response to flagellin from

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triggering, as per the original definition (143). Confusingly, some PRRs can additionally respond to DAMPs, although not all DAMP receptors respond to PAMPs.

pathogenic bacteria (166–168). Moreover, unlike cDC1s, mouse and human cDC2s uniquely express TLR7, which senses single-stranded RNA, and high levels of viral sensors RIG-I and MDA-5 (147, 149). Human AS-DCs (putative pre-cDCs) express SIGLEC6 and AXL, receptors involved in efferocytosis, suggesting they may respond to DAMPs. They also express TLR4, pointing to their capacity to produce an inflammatory response to bacterial infection (27). In fact, TLR4 is generally expressed across cDC subsets (149), and its canonical agonist, LPS, is commonly used to stimulate cDCs in experimental settings.

In sum, cDCs express a wide repertoire of innate immune receptors, endowing them with the ability to respond to a broad range of pathogens and tissue stresses. Differential receptor expression across subsets is broadly conserved between mice and humans and may impact the contribution of individual cDC subsets to immune responses to different pathogens. Whether this reflects a true division of labor remains unclear, although it has been argued that, for example, because cDC2s preferentially express NLRs and cytosolic virus sensors, they are specialized for responding to intracellular pathogens (147).

Conventional Dendritic Cell Activation

The concept of a mature DC, as originally proposed by Steinman (169), referred to acquisition of the ability to stimulate T cells and prime adaptive immune responses. DC maturation was accompanied by phenotypic changes, namely, the increased surface expression of MHC molecules and so-called maturation markers such as costimulatory molecules CD80, CD86, and CD40, as well as upregulation of the chemokine receptor CCR7, which allowed maturing cDCs from the periphery to migrate to lymphoid tissues. DC maturation was also potently induced by PRR triggering, consistent with a role in promoting immunity to infection. However, phenotypic signs of maturation can take place in conditions that do not lead to immunity and may even instruct tolerance (170). Furthermore, depending on the trigger and subset, cDCs can mature to instruct differentiation of different classes of effector T cells, including Th1, Th2, and Th17 cells and regulatory T cells (Tregs) (170). Therefore, the original concept of immature (off) versus mature (on) cDCs requires refinement, not least because mature has confusingly also been used in the context of cDCpoiesis to refer to differentiated cDCs. The term activated cDC is preferentially adopted here, as expounded below. The transcriptional control of cDC activation is reviewed in Reference 171.

Revisiting cDC activation. It is perhaps easiest to think of cDCs as existing in two fundamental states (**Figure 3**). The first is a resting or quiescent state in which cDCs are in antigen-sampling mode and carry out their role as sentinel cells, detecting changes to their environment. In this resting state, cDCs receive tonic signals in the form of soluble mediators, such as Flt3L or type I interferons, which maintain their survival and contribute to a poised state that facilitates responsiveness to immune challenge (46, 99). The second state is an activated state that can lead to several outcomes, including immunity and tolerance. What defines a cDC in its activated state is the ability to interact (or prepare to interact) with other cells and thereby communicate information about its environment (**Figure 3**). Although the essence of cDC biology is largely focused on the ability to convey signals to T cells, cDCs also present antigens to B cells (172) and interact with other immune and nonimmune cells (173–177) (**Figure 3**). Therefore, the capacity to transmit messages to other cells is the key difference between a cDC in an activated state and a cDC in its resting state, and the term activation denotes the process by which a resting cDC becomes competent to convey this information. At this stage, we refer to these cells as activated cDCs (**Figure 3**). In this terminology, we accept that activated cDCs need not be immunostimulatory and can

display a multitude of information-transmitting functions depending on the nature of the activating stimulus, environmental modulators, and cell-intrinsic constraints imposed by ontogeny and differentiation state. Notably, we also accept that transition into an activated state may not always involve an external stimulus such as PAMP recognition. As such, so-called homeostatically matured cDCs in the steady state (178) should be considered activated cDCs whose functions might be predominantly concerned with maintaining tolerance or, at least, with not inducing immunity. These themes are further explored below.

cDC activation leading to immunity. Activation of cDCs has often been studied from the perspective of generating APCs capable of priming T cells, which is important for vaccination and cancer immunotherapy. Proinflammatory cytokines such as TNF-α and type I interferons can induce phenotypic maturation of cDCs and have been used in maturation cocktails to stimulate MCs for immunotherapy in cancer patients (179). However, such cells do not produce cytokines such as IL-12 that direct the differentiation of naive $CD4^+$ and $CD8^+$ T cells. Production of these cytokines requires PRR triggering (99, 180). For example, cDCs activated indirectly by exposure to inflammatory mediators are able to induce $CD4^+$ and $CD8^+$ T cell proliferation, but those T cells lack effector function, including the ability to produce IFN-γ (181–184). Nevertheless, in some instances of viral infection or inhalation of house dust mite allergen, priming of CTL or Th2 responses, respectively, can occur independently of direct PAMP recognition by cDCs (185, 186). In the case of infection with influenza A virus, signaling via IL-1R, an inflammatory cytokine receptor that signals much like TLRs, was necessary and sufficient to elicit antigen-specific CTL priming by cDCs (186). Finally, even in the context of triggering via PRRs, cDC activation leading to initiation of adaptive immune responses can be potently amplified by inflammatory mediators, particularly type I interferons (187, 188). Similarly, including engagement of CD40 by T cell–derived CD40L, contact with T cells greatly potentiates cDC activation initiated by PRR triggers and increases their production of immunomodulatory cytokines such as IL-12 and IL-10 (180, 189).

In the conditions described above, cDC activation is elicited by positive signals originating from microbial or viral invaders. It has been argued that DAMPs can also function as positive signals, substituting for PAMPs in induction of immunity (144–146). Some DAMPs, such as F-actin acting via DNGR-1 (see preceding section titled Sensing), do not trigger the features of cDC1 activation induced by PAMPs but primarily affect antigen handling (163, 165). Other DAMPs turn out to be contaminated with PAMPs such as LPS (190). Thus, while DAMPs can synergize with PAMPs in cDC activation, it remains important to establish to what degree they are sufficient to convert the resting state of cDCs into a T cell–priming state in the absence of other stimuli (191). In this regard, it is interesting to note that the ability of cDCs to enhance B cell responses does not always correlate with the features of activation that have been associated with T cell priming function (156). Thus, there may yet be forms of cDC activation that can promote immunity and about which we know little.

cDC activation and tolerance. In addition to their prominent role as the initiators of adaptive immunity, cDCs act in the induction and preservation of immune tolerance. In the thymus, central tolerance is maintained by resident cDCs that present self-antigens expressed by themselves or acquired from thymic epithelial cells, resulting in the negative selection of autoreactive T cells (192, 193). In the periphery, cDCs are also thought to delete or induce anergy in self-reactive T cells that escaped thymic negative selection, i.e., to induce recessive peripheral tolerance (194, 195). Several mouse studies indicate that this depends on cognate interactions between cDCs and Tregs (99, 196). Indeed, targeted deletion of MHC-II in cDC1s, preventing cDC1-Treg interactions, leads

to priming of self-reactive CTLs (197, 198). These observations suggest that Tregs may raise the cDC activation threshold necessary for productive immunity to above that which is induced by basal levels of cytokines such as type I interferons induced in response to PAMPs from microbiota (99). Alternatively, cDCs in steady-state conditions might activate self-reactive T cells but do so in a way that maintains their susceptibility to inhibition by Tregs in a three-cell cluster (e.g., through growth factor deprivation). Of course, in addition to recessive tolerance, cDCs can actively contribute to dominant tolerance by inducing the conversion of naive $CD4^+$ T cells into Tregs. In the intestinal mucosa, tolerance against the commensal microbiota is promoted by $CD103^+$ cDC1 induction of Tregs via the synergistic action of cDCs providing TGF-β and retinoic acid derived from dietary vitamin A (199). In turn, Treg-derived signals enhance cDC secretion of IL-10 and other factors, which can further induce Treg conversion in a positive-feedback loop (200).

It has been suggested that activated cDCs that induce tolerance are in a semimature state, while the induction of T cell responses requires full DC maturation (201). It is unlikely that differences in cDC tolerogenicity versus immunogenicity are quantitative. Transcriptomic comparison of mouse steady-state $CCR7^-$ resting cDCs versus $CCR7^+$ cDCs (the latter thought to correspond to homeostatically activated cDCs that induce tolerance) reveals changes in gene expression that are just as, if not more, dramatic than those undergone by resting cDCs upon stimulation with PRR agonists (178). The signals that elicit this homeostatic activation of cDCs are unclear. The original concept of DC maturation emerged from the in vitro culture of LCs (10). Their spontaneous maturation in culture may have been triggered by the loss of signals that retain LCs in a quiescent state in the epidermis, as in vivo ablation of TGF-β signaling led to upregulation of MHC, CD80, and CD86 and increased migratory activity (202). Similarly, homeostatic activation of cDCs in vivo might involve spontaneous (or programmed) loss of negative signals. This could synergize with weak positive signals emanating from microbiota (99), from encounter with dead cells (203), or even from the movement of cDCs themselves. For example, rupture of the nuclear envelope in cDCs migrating through tight constrictions can lead to cytoplasmic exposure of chromatin and activation of the cGAS cytosolic DNA sensor (204).

A recent study highlighted a cDC activation program present in naive and tumor-bearing mice that includes expression of genes encoding both T cell regulatory and stimulatory molecules, reminiscent of the transcriptional profile of cDCs undergoing homeostatic activation (205). The regulatory component of this program, namely, upregulation of programmed death ligand 1 (PD-L1), was induced upon engagement of AXL, presumably by apoptotic cells, while stimulatory activity, including IL-12 production, was positively regulated by IFN-γ and inhibited by IL-4 (205). However, IFN-γ can also drive upregulation of PD-L1 on cDCs, which contributes to tumor immune escape (206, 207). On this note, the field of cancer immunology has contributed greatly to our understanding of the signals that promote a poorly immunogenic or tolerogenic cDC fate. In the tumor microenvironment, IL-10, TGF-β, IL-6 and vascular endothelial growth factor (VEGF) can all dampen the ability of cDCs to prime and stimulate T cells (104), an area of intensive research because of its translational potential.

cDC activation and innate immunity. Although the essence of cDC biology is focused on their ability to interact with T cells, cDCs have emerged also as regulators of innate immune responses. Activated cDCs can induce NK cell cytotoxicity and proliferation by a combination of both direct cell-cell interactions (e.g., via engagement of NKG2D) and secretion of soluble mediators including IL-12, IL-18, IL-15, and IL-2 (208, 209). Additionally, IL-12 made by cDC1s in response to *T. gondii* (153) is required for clearance of infection even in T cell–deficient mice, firmly establishing that these cells can play a nonredundant role in innate immunity (155, 210). Cross talk between cDCs and neutrophils also plays a role in shaping early responses to bacterial infection

or cell death: Activated cDCs secrete VEGF or CXCL2, respectively, which recruits neutrophils that can reciprocally provide TNF-α, enhancing cDC activation (175, 176).

cDC activation and nonimmune cells. In addition to communicating with innate and adaptive immune cells, cDCs also convey information to nonimmune cell types. For example, cDCs provide lymphotoxin β and IL-1β to sustain the network of LN high endothelial vessels and stromal cells in the steady state and during immune responses (211–213). In mice lacking cDCs, LN cellularity is strikingly reduced as a result of loss of high endothelial venules, which limits egress of lymphocytes from blood (212, 214). IL-1β production by LN cDCs induces VEGF expression by fibroblastic reticular cells (FRCs), which contributes to cell growth during initial LN expansion (211). Similarly, CLEC-2 upregulated on activated cDCs during immune challenge binds to podoplanin on LN FRCs, inhibiting contractility signaling and permitting FRC stretching and LN expansion (215). A recent paper indicates that cDCs can also communicate with sensory neurons: IL-31 released by activated dermal cDC2s in response to TGF-β produced during cutaneous wound healing acts on itch sensory neurons to potentiate the itching response of wounded mice (177).

Antigen Transport to Tissue-Draining Lymph Nodes

Antigenic material from the periphery must be delivered to dLNs for presentation to T cells. This can be achieved either by active transport by migratory cDCs or by passive draining via the lymphatic system, as discussed below.

Migratory cDCs. One of the key features of tissue cDCs is their ability to migrate to dLNs. CCR7 upregulation allows activated cDCs to sense a haptotactic gradient of CCL21 generated by lymphatic endothelial cells that attracts them into afferent lymphatics and subsequently into dLNs via the subcapsular sinus (216). Interestingly, another G protein–coupled receptor, MRGPRA1, was recently shown to also mediate cDC2 chemotaxis to skin dLNs in response to substance P secreted by neurons activated by dermal allergens (217). Once in the dLN, cDCs move to specific compartments by interactions with LN stromal cells (218). The timing of cDC migration can vary, but in mice, arrival of antigen-bearing migratory cDCs at dLNs can occur as early as 8–12 h after virus infection (219, 220).

Mouse studies have contributed to the idea that migratory cDCs are indispensable for delivering antigens to dLNs and for productive T cell responses (221–224). Both migratory cDC1s and migratory cDC2s are involved in the transport of antigen to lymphoid tissues in the context of cell-associated (225) and soluble antigen (226) and during viral infection (219, 220, 227). This seems to be the case even in scenarios where antigen presentation to T cells is carried out exclusively by nonmigratory dLN-resident cDC1s, to which antigen is transferred from migratory cDCs, perhaps as a means of antigen dispersal (219, 228). However, it is now clear that the nature of the antigen itself, including its molecular size and route of immunization and dosage, can be an important determinant of its mode of delivery to dLNs, and in some cases, it can bypass the need for active transport by migratory cDCs.

Free-draining antigen in lymph is sampled by resident cDCs. Passive drainage of soluble and particulate antigen to dLNs has recently been proposed as a mechanism for the acquisition of antigen by LN-resident cDCs (229–231). Experimentally, virus-sized antigens ($<$200 nm) can freely enter peripheral lymphatics, reaching the dLN within minutes of administration to mice (222). There, molecules smaller than 70 kDa are able to penetrate deeper via a semipermeable conduit system onto which resident cDCs are anchored and ideally positioned to acquire soluble

antigen (232). However, when injected in the footpad, influenza A virus can also be captured in a SIGNR1-dependent manner by cDC2s found in the LN medulla and elicit virus-specific T cell proliferation as early as 2 h after administration (230). Similarly, a population of LN-resident cDC2s has been identified that extends processes into lymphatic sinuses and is able to directly sample afferent lymph independently of the conduit system and elicit robust $CD8^+$ and $CD4^+$ T cell responses against heat-killed *Pseudomonas aeruginosa* (231). Finally, resident cDC1s lining LN medullary sinuses were also shown to phagocytose live *Plasmodium* sporozoites that actively migrate to skin dLNs and to cross present sporozoite antigen to $CD8^+$ T cells 8 h after immunization, independently of skin-derived migratory cDCs (233).

Collectively, these studies highlight a potentially underappreciated role for LN-resident cDCs as early initiators of adaptive immunity in the context of soluble antigens and pathogens that freely drain to dLNs. In contrast, migratory cDCs may predominate in the setting of highly tropic or slowly replicating pathogens, where the antigen remains localized to the periphery and does not freely enter afferent lymph. Indeed, an exclusive reliance on antigen delivery by migratory cDCs could potentially be detrimental, given the delay that cDC migration imposes on the generation of T cell responses as compared to those induced by lymph-borne antigen (231). Instead, a temporal model might apply whereby initial T cell priming is induced by LN-resident cDCs and enhanced function is conferred by late-arriving migratory cDCs with higher expression of antigen:MHC complexes and with such immigrant cDCs providing additional signals needed to drive strong effector responses, especially of the Th1 type (234). These considerations are important for the rational design of vaccination strategies and optimization of vaccine delivery.

Conventional Dendritic Cells in Lymphoid Tissues: Location Matters

The distinct localization of $MHC\text{-}II^+$, $CD206^-$, and $CD206^+$ migratory cDCs (corresponding to cDC2s and a mixture of cDC1s and LCs) to specific LN regions after skin immunization was first shown with a fluorescent dye (235). The development of modern imaging techniques such as histocytometry allowed for subsequent high-resolution mapping of cDC subset territories in LNs (231). At steady state, cDC1s, regardless of their migratory or resident origin, localize predominantly deep in the T cell zone (paracortex). In contrast, resident cDC2s are found primarily in the outer cortex and migratory cDC2s in the interfollicular zone, close to the T cell–B cell border (236). cDC1 and cDC2 positioning mirrors the distribution of $CD8^+$ and $CD4^+$ T cells, respectively (226), and has been attributed to spatially distinct chemokine gradients. For example, stromal cells in the T cell zone secrete CCL21 and CCL19, preferentially attracting cDC1s, which express higher levels of CCR7 than cDC2s (226, 237). $CD8^+$ T cells also secrete high levels of the chemokine XCL1, which is the ligand for XCR1, one of the canonical markers of cDC1s (238). Nevertheless, we know that segregation is not static, especially during inflammation (223, 239).

In the spleen, which lacks an afferent lymph supply, cDCs are often thought to be resident and constitutively localized to the T cell zone of the white pulp. However, cDC1s have also been found within the red pulp and marginal zone and shown to migrate to the T cell zone of the white pulp upon activation by PAMPs (153, 240, 241). CD103, which is expressed by migratory cDC1s in LNs, is expressed by migrating spleen cDC1s (241). Splenic cDC2s have been found in the marginal zone, close to B cell follicles (242, 243), but also in the red pulp and the T cell zone. Thus, the spleen may also have a migratory cDC population that moves centripetally into the T cell zone in response to certain stimuli.

The spatial organization of cDC subsets in the dLNs has ramifications for the induction of immunity. Immunization can generate steep antigen gradients within the dLNs (244). As a result of their peripheral positioning, resident cDC2s acquire antigen faster and more easily than the

centrally located cDC1s (244). This arrangement enables rapid cDC2-induced $CD4^+$ T cell activation, perhaps so that help is available when B cells and $CD8^+$ T cells are stimulated by antigen. Finally, although the discussion in the last two sections has been T cell–centric, there is increasing evidence that both resident cDCs and migratory cDCs also present antigens to B cells and that this also involves their specific (re)localization within the LN (172). Anatomical regulation of antigen presentation to B cells by cDC subsets requires further exploration.

Revisiting Dogmas About Conventional Dendritic Cell Subset Function in Antigen Presentation

The notion that cDC subsets are functionally specialized to induce distinct responses has been inextricably linked to their identification as discrete cell types. It is certainly the case that ontogeny determines some cDC properties, such as the ability of activated cDC1s to produce high levels of IL-12, which is conserved between mice and humans (245) and may relate to their high expression of IRF8 (246). However, we should remember that cDC subset identity and function can be altered in inflammatory settings. For example, a recent study demonstrated that some cDC2s can start to upregulate IRF8 and produce IL-12 during viral infection, behaving much like cDC1s yet at the same time upregulating CD64 and resembling MCs (37). The observation of functional plasticity has not stopped some of the properties ascribed to cDC subsets from becoming entrenched dogmas. One such notion is that cDC1s are specialized to cross present antigens to $CD8^+$ T cells while cDC2s prime $CD4^+$ T cells.

Thanks largely to the *Batf3*$^{-/-}$ mouse model, a nonredundant role for cDC1s in inducing CTL responses against tumor and viral antigens has been established (35). However, there is a tendency to interpret results from such loss-of-function experiments as reflecting a functional specialization of cDC1s, forgetting that these experiments do not tell us so much what cDC1s do as what cDC2s (and other cells) cannot do. In other words, the fact that *Batf3*$^{-/-}$ mice cannot reject transplantable tumors means that cDC2s or MCs lack the capacity to compensate for cDC1s in priming CTL responses to those tumors. It does not mean that cDC1s are specialized to prime CTLs. This can only be assessed when we have a loss-of-function mouse for cDC2s. In fact, there is much evidence that cDC1s are also able to prime $CD4^+$ T cells (247–250), in which case the fact that $CD4^+$ T cell responses are largely unaffected in *Batf3*$^{-/-}$ mice may simply mean that cDC2s can compensate, i.e., that both cDC subsets can do it. Further, any differential ability of cDC2s and cDC1s to prime $CD4^+$ or $CD8^+$ T cells in vivo may be dictated not so much by intrinsic antigen-processing and -presenting specializations but by strategic positioning of the cells within dLNs, leading to differential acquisition of antigen, as described in the preceding section.

When a defect in $CD8^+$ T cell priming or tolerance is observed in *Batf3*$^{-/-}$ mice, there is also a tendency to interpret it as a consequence of loss of cross presentation by cDC1s. While this may be true in some settings [e.g., antitumor CTL responses absolutely require cross priming (251)], it could also reflect another nonredundant activity of cDC1s, such as the aforementioned IL-12 production, which protects from infection by *T. gondii* (252). In some cases, it could even reflect a cDC1-independent effect of Batf3 loss on the T cells themselves (253, 254). It should be remembered that, in the context of viral infection, even if cross presentation dominates in many instances (220), infected cDC1s can present viral antigens directly (255). In addition, MHC-I:peptide complexes can be acquired by cDCs from other cells in a process called cross-dressing (256). Finally, cDC1s are the platform for $CD4^+$ T cell help for $CD8^+$ T cell responses (255, 257), and many of the functional experiments looking at the necessity for cDC1s in $CD8^+$ T cell responses do not distinguish defects in antigen (cross) presentation from lack of help that diminishes CTL activity and $CD8^+$ T cell memory.

To specifically demonstrate that loss of cross presentation by cDC1s underlies a given CTL response defect in *Batf3*$^{-/-}$ mice, we need mice in which that process is selectively ablated in cDC1s rather than mice in which cDC1s are missing. The finding that DNGR-1-deficient mice have a defect in cross presentation of dead cell–associated antigens was the first step in this direction (158). It revealed that cross presentation by cDC1s can be necessary for robust CTL responses, and in particular, for the generation of tissue-resident memory CD8^{+} T cells, against some but not all viruses (161–163, 258), as well as for chronic allograft rejection by CD8^{+} T cells (164). More recently, the discovery that *Wdfy4* expressed by cDC1s is required for cross presentation allowed for generation of another mouse strain bearing cDC1s that are selectively unable to cross present and mount effective responses against tumors and viruses (210).

What WDFY4 does and, more generally, the mechanisms underlying cDC1 cross presentation remain elusive. Like other cDC functions, cross presentation may, in part, be a consequence of the innate immune receptors expressed by the cells and how these receptors contribute to uptake and handling of antigen-bearing cargo. For example, DNGR-1 induces cross presentation of dead cell–associated antigens by signaling to induce phagosomal rupture (165). However, this property is intrinsic to the receptor and can function in heterologous cells (165). In that context, it is worth remembering that many of the cases in which cross presentation by cDC1s is supposedly essential (e.g., experiments with tumor implantation into *Batf3*-deficient mice) likely involve dead cells as an antigen source. cDC1s have long been known to excel among cDC subsets at internalizing dead cell material, and some but not all of their superiority at cross presentation is attributable to that property (259, 260). It is possible that in other contexts, such as cross presentation of immune complexes or soluble antigens, cDC2s or even other cell types compensate (37, 261, 262). It is also important to note that mouse studies, however elegant, are not always predictive of the situation in humans. Human cDC1s share with their mouse counterparts the ability to efficiently take up cell corpses and cross present exogenous antigens (120, 121), but cross presenting capacity is reportedly not as restricted to cDC1s in humans as it is in mice (263–266). This is not to say that we cannot exploit the cross presenting and IL-12-producing abilities of cDC1s for therapy. In fact, cDC1 presence in tumors is positively associated with cancer patient survival and the success of checkpoint blockade immunotherapy (104), making these cells ideal candidates for the development of immunotherapies that mobilize CD8^{+} T cells against infectious diseases or cancer.

CONCLUDING REMARKS

Recent advances in immunology have shown the limitations of tackling the diversity of immune cells by grouping them into functional categories. For example, it is now appreciated that T cells come in many different types (γδ, αβ, mucosal-associated invariant T, natural killer T, Treg, CTL, Th, etc.) that can perform a multitude of tasks, from immunity to tissue repair, such that defining a T cell solely by function is next to impossible. Instead, immune cells can be classified by origin. This is easiest when ontogeny leaves an indelible and unambiguous mark, such as TCR or BCR locus rearrangement. But for myeloid cells, this is not the case even if in-depth phenotyping has begun to reveal constellations of markers that serve as good proxies for ancestry. Ontogenetic analyses combined with deep phenotyping give us an opportunity to revisit the DC family independently from functional definitions, thereby allowing us to ask whether our traditional views of how DCs function remain applicable today. Here we have presented the evidence indicating that cDCs constitute a distinct lineage of cells with the capacity to seed tissues and maintain immune homeostasis in the steady state while rapidly responding to local insults and initiating and directing innate and adaptive immunity. The focus on cDCs, while it excludes the extended DC family,

remains true to the essence of the cell that was defined by Ralph Steinman almost 50 years ago and expands our understanding of its specialized roles in immunobiology.

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