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REVIEW

Classical DC2 subsets and monocyte-derived DC: Delineating the developmental and functional relationship

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To specifically tailor immune responses to a given pathogenic threat, dendritic cells (DC) are highly heterogeneous and comprise many specialized subtypes, including conventional DC (cDC) and monocyte-derived DC (MoDC), each with distinct developmental and functional characteristics. However, the functional relationship between cDC and MoDC is not fully understood, as the overlapping phenotypes of certain type 2 cDC (cDC2) subsets and MoDC do not allow satisfactory distinction of these cells in the tissue, particularly during inflammation. However, precise cDC2 and MoDC classification is required for studies addressing how these diverse cell types control immune responses and is therefore currently one of the major interests in the field of cDC research. This review will revise murine cDC2 and MoDC biology in the steady state and under inflammatory conditions and discusses the commonalities and differences between ESAM^{lo} cDC2, inflammatory cDC2, and MoDC and their relative contribution to the initiation, propagation, and regulation of immune responses.

Keywords: monocyte-derived DC (MoDC) · inflammatory cDC2 · ESAM^{lo} cDC2

Introduction

Dendritic cells (DC) are a heterogeneous family of cells that, despite their distinct ontogeny, share numerous functional features, including innate pathogen sensing and antigen (Ag) processing and presentation. Generally, DC are categorized into three main classes, namely, (1) conventional (or classical) DC (cDC), representing the most potent Ag-presenting cells (APCs) pivotal for the priming and differentiation of naïve Ag-specific T cells [1–7], (2) specialized type I interferon-producing plasmacytoid DC (pDC) [8, 9], and (3) monocyte-derived DC (MoDC), which under certain conditions may represent an emergency source of DC at the site of infection [10–13]. Additionally, epidermal Langerhans cells are commonly referred to as a fourth DC class, as they share a common origin with macrophages (Mφ) but functionally behave like typical cDC. Due to the heterogeneity within the DC network, several DC populations are phenotypically overlapping. For example, type 2 cDC (cDC2) comprise several tissue-specific populations that often display overlapping marker expression with MoDC and Mφs. This complicates the discrimination of these cells into clearly separated lineages and has resulted in some confusion about the specific immune-modulatory functions of individual DC subsets, particularly during inflammation [14–19]. Hence, a more accurate analysis of the interrelationship between cDC2 and MoDC will have significant implications for the interpretation

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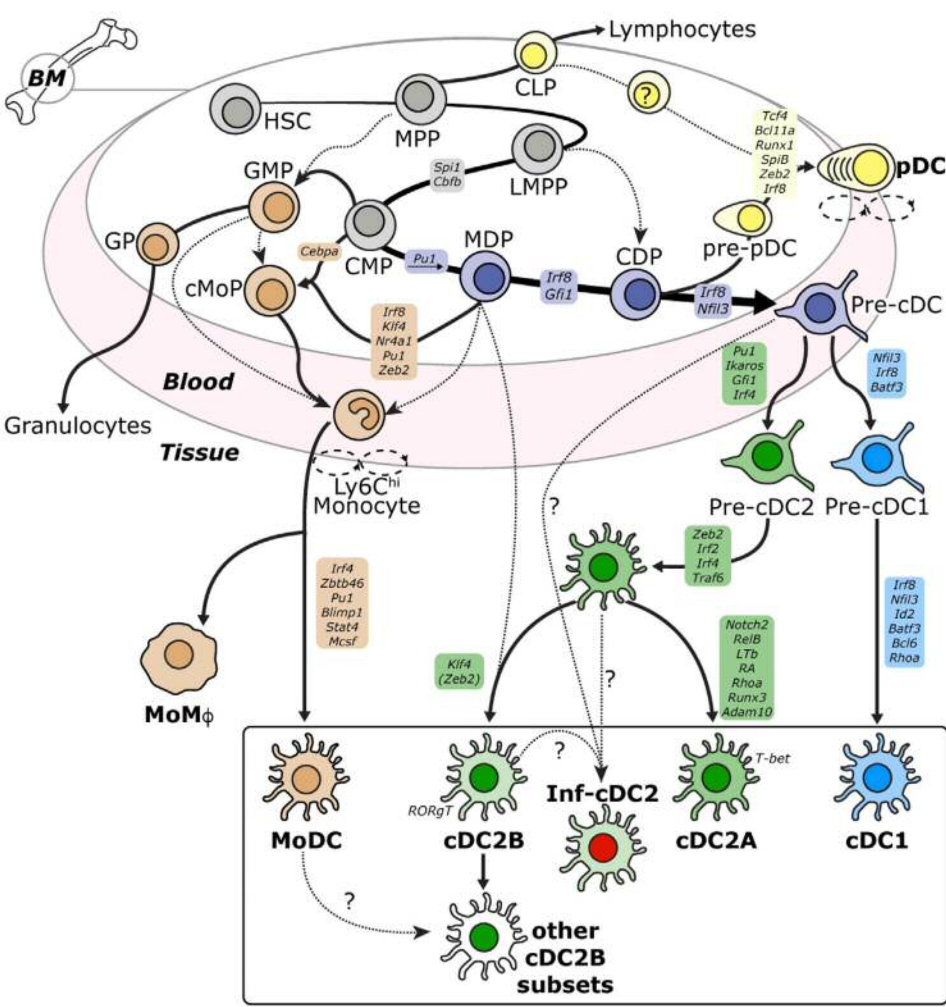

Figure 1. Myeloid cell differentiation: from bone marrow hematopoietic stem cells to DC heterogeneity: cDC, pDC, and monocytes derive from quiescent long-term self-renewing hematopoietic stem cells (HSC) in the bone marrow (BM). These HSCs differentiate into short-term multipotent progenitors (MMP) and further into lymphoid-primed multipotent progenitors (LMMP). LMMP develop into common lymphoid progenitors (CLPs) and common myeloid progenitors (CMPs). While CLPs give rise to lymphoid cell lineages (e.g., T cells and B cells), CMPs progress to myeloid cell lineages by developing into granulocyte macrophage progenitors (GMPs) and macrophage and DC precursors (MDPs). MDPs segregate into common monocyte progenitors (cMoPs) and common DC precursors (CDPs). While cMoPs further differentiate into Ly6C^{hi} monocytes, CDPs differentiate into circulating precursors for cDC (precDC) and pDC (prepDC). Ly6C^{hi} monocytes and pDC circulate as mature cells throughout the body, whereas precDC leave the BM as precursor cells that seed both lymphoid and nonlymphoid tissues. In these tissues, precDC differentiate further into lineage-committed precDC1 s and precDC2, which mature into terminally differentiated cDC1 s and cDC2, respectively. In lymphoid tissues, cDC represent resident cDC subpopulations, while nonlymphoid tissue cDC can travel as migratory cDC to lymph nodes. The relationship of Inf-cDC2 with other DC is not known. Several key lineage-defining TFs that are involved in lineage commitment and function are indicated.

of studies addressing the functional specifications of these DC in inflammation and associated human diseases. Therefore, this review will revise some basic concepts of murine cDC2 and MoDC biology and discuss their parallels and differences under steady-state and inflammatory conditions. In particular, ESAM^{lo}CX3CR1^{+} cDC2B, which appear in the mouse spleen upon defective cDC2 homeostasis [16, 17], as well as inflammatory cDC2 (Inf-cDC2) [20], are often incorrectly referred to as being of monocytic origin. Here, we propose that these cells represent *bona fide* cDC2 populations with unique immune functions [18, 19].

Conventional DC

Across species, the cDC family can be classified as CD45^{+}CD11c^{+}MHCII^{+}CD26^{+}FLT3^{+} cells, which can be further divided into so-called XCR1^{+} cDC1 and SIRPα+ cDC2 based on their ontogeny and specific profile of cell-surface markers and transcription factors (TF), as well as functional characteristics [21] (Figure 1). With the advantage of high-dimensional flow cytometry and single-cell sequencing, it is now evident that both cDC1 s and cDC2 consist of several smaller and less

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	cDC1	ESAM^{hi} cDC2A	ESAM^{lo} cDC2B	Inf-cDC2	MoDC
General surface markers	CD11c, MHC-II, CD26, FLT3	CD11c, MHC-II, CD26, FLT3	CD11c, MHC-II, CD26, FLT3	CD11c, MHC-II, CD26, FLT3	CD11c, MHC-II
Specific surface markers	CD8*, CD24, CD36, CD103*, CD205, CLEC9A, Langerin*, NECL2, XCR1	CD11b, CD209A, SIRPα, DCIR2, CD4, CD103*, ESAM, F4/80	CD11b, CD209A, SIRPα, DCIR2, CD14, CCR2, CD64, CX$_3$CR1, Ly6C, CLEC10A, CLEC12A, Mgl2, (F4/80)	CD11b, SIRPα, CCR2, CD64, DC-SIGN, Ly6C, MAR-1	CD11b, CD209A, SIRPα CD14, CCR2, CD64, CX$_3$CR1, Ly6C CD16/32, CD88, CD115, CD206, CCR2, F4/80, Ly6C, MAR-1, MerTK
Transcription factors	BATF3, GFI1, ID2, Ikaros, IRF8, NFIL3, NOTCH2, PU.1, STAT3, ZBTB46	GFI1, Ikaros, IRF2, IRF4, NOTCH2, PU.1, STAT3, T-BET?, TRAF6, ZBTB46	GFI1, Ikaros, IRF4, KLF4, PU.1, (RORγT), ZBTB46, (ZEB2)	IRF4, IRF8, ZBTB46	GFI1, Ikaros, IRF8, KLF4, PU.1, ZBTB46
Differentiation/ survival factors	ADAM10*, FLT3, GM-CSF, RHOA	ADAM10, FLT3, GM-CSF, LTβR, RA, RELB, RHOA	FLT3, GM-CSF		GM-CSF, IL-4 (*in vitro*), M-CSF (*in vivo*)
Function	Ag cross-presentation, Type I immune responses	*Notch2-cDC2*: Bacterial + fungal infections, Type3 responses *T-bet-cDC2*: tissue repair	*KLF4-cDC2*: Type 2 responses, parasite infection, allergens *RORγT-cDC2*: pro-inflammatory	CD4$^+$ T cell priming, Type 2 responses Ag cross-presentation	Local T cell reactivation, T_H1 and T_H17 responses during infections
Cytokines	IFNγ, IL-12	IFN-III, IL-6, IL-23, TGFβ	IL-4, IL-12, IL-33, TNF	IFN-I, IL-12, TNF	IL-12, IL-23, iNOS, TNF
Human equivalent	CD141 (BDCA3)$^+$ cDC1: BATF3, BTLA, CD11c, CD26, CD117, CLEC9A, IRF8, MHC-II, Necl1, XCR1	CD1c (BDCA1)$^+$ cDC2: BCLA1, CD11b, CD11c, CD26, CLEC10A, MHC-II, IRF4, SIRPα	CD1c^{lo} cDC2: CD5, CD11b, CD11c, CD26, CLEC4A, CLEC10A, MHC-II, IRF4, SIRPα	Inf-DC (DC3?): CD1c, CD11b, CD11c, CD26, CD64, CD141, CD163	MoDC: CD11b, CD11c, CD14, CD16, CD64, CD88, CD115, CLEC4A, MHC-II, SIRPα

* tissue and/or subset-specific; () expression and/or function unknown

Figure 2. Phenotype and function of cDC subsets and MoDC. The different DC can be divided according to the indicated cell surface markers. They exhibit specific transcriptional regulation and display functional specialization. All DC have been characterized in both mice and humans.

well-characterized tissue-specific subpopulations with diverse immunomodulatory functions [2, 3, 15, 22-24].

cDC development and subset commitment

cDC are generally short-lived cells, with an average turnover of a few days [25, 26]. Therefore, they are constantly replenished by blood-borne progenitors of hematopoietic stem cell (HCS) origin, although a small fraction of mature cDC actively divide at steady state [26, 27]. HCS successively differentiate via the common myeloid progenitor (CMP), monocyte/DC precursor (MDP) [12, 28, 29], and common DC precursor (CDP) [29–31] into precommitted cDC (precDC) [30, 32] (Figure 1). These precDC are circulating cells that home to both lymphoid and nonlymphoid tissues. Here, driven by tissue-specific microenvironmental cues, precDC give rise to both terminally differentiated cDC1 and cDC2 subsets via precDC1 and precDC2 intermediates, respectively [32–36]. Notably, precDC committed to either cDC1 or cDC2 lineages can already be found in the BM, suggesting that lineage commitment might occur before entering the tissues [32, 37].

The development of both cDC1 and cDC2 *in vivo* critically depends on FLT3L [30, 31, 38, 39], whereas GM-CSF is essential for their survival [40]. While common transcription factors (TFs) regulate the terminal differentiation and/or functional maturation of cDC1 s, cDC2, or their subsets (e.g., ZBTB46/zDC, PU.1, IKAROS, GFI1, STAT3, and NOTCH2), other TFs and distinct metabolic pathways selectively regulate the commitment of either cDC1 or cDC2 (as recently reviewed by Murphy et al. [41], Bosteels et al. [36], Anderson et al. [23], and Cabeza-Cabrerizo et al. [4]). For example, cDC1 require the hierarchical expression of IRF8, NFIL3, ID2, and BATF3 for their development and survival, while cDC2 commitment mainly relies on ZEB2, RELB, IRF2, and TRAF6, as well as on IRF4 and KLF4 for their final differentiation and/or migration to draining LNs (as reviewed by Murphy et al. [41], Bosteels et al. [36], Anderson et al. [23], and Cabeza-Cabrerizo et al. [4]) (Figure 2). In addition to transcriptional programming, posttranslational protein modifications, e.g., by ADAM10, significantly contribute to cDC1 and cDC2 subset differentiation and homeostasis [17, 19, 42]. Notably, although much is known about the molecular signals that promote cDC fate under steady-state conditions, the molecular control of cDC development under inflammatory settings is largely unknown.

cDC2 heterogeneity

In contrast to the relatively homogenous cDC1 compartment, cDC2 constitute several different subpopulations, and this heterogeneity might be driven by the different microenvironmental cues in the residing tissues [14, 15]. cDC2 in the murine spleen can be defined either as NOTCH2- or KLF4-dependent cells [16, 43] or, more recently, as T-BET$^+$ cDC2A and RORγt$^+$ cDC2B cells [15] (Figure 2). Notably, the heterogeneity of cDC2 in the peripheral and mucosal tissues is even greater than that in the spleen.

To date, it is not precisely known how NOTCH2- and KLF4-dependent subpopulations overlap with cDC2A and cDC2B, respectively. While NOTCH2-dependent cDC2 form a uniform population of cells expressing CD4 and high levels of the adhesion molecule ESAM, T-BET $^+$ cDC2A also express ESAM and CD4, but here, T-BET does not completely correlate with ESAM expression, as ESAM expression was also found on some T-BET-negative cDC2 [15, 16, 44]. On the other hand, both KLF4-dependent cDC2 and RORγt$^+$ cDC2Bs form a heterogeneous population of ESAM^{lo} cells that display variable expression of CD4, CLEC10A, and/or CLEC12A [15, 16, 43]. While both NOTCH2 and the GTPase RHOA control cDC1 and ESAM^{hi} cDC2 homeostasis, they do not control ESAM^{lo} cDC2 homeostasis [45]. Moreover, the maintenance of splenic ESAM^{hi} cDC2 is dependent on retinoic acid as well as LTβR signaling. In contrast, ESAM^{lo} cDC2 express high amounts of LTβR, and their homeostasis is independent of LTβ signaling [46]. Importantly, this phenotypical heterogeneity also translates into the functional specialization of the different cDC2 subsets, and cDC2A and cDC2B differ in their ability to induce and control immune responses [15].

Functional specialization of cDC subsets

Both cDC1 and cDC2 are present in most lymphoid and nonlymphoid tissues [21]. While lymphoid-resident cDC subpopulations

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reside their whole lifespan within secondary lymphoid organs, cDC in peripheral tissues travel in a CCR7-dependent manner to tissue-draining LNs. Here, they cooperate with lymphoid-resident cDC to control both immunogenic and tolerogenic immune responses. Although all cDC have a superior capacity to prime naïve T cells, cDC1, and cDC2 exhibit different MHC-I or MHC-II Ag-presentation capacities. Generally, cDC1 excel in cross-presenting soluble and particulate Ag on MHC-I to $CD8^+$ T cells [4-7, 47-49]. cDC1 also initiate the early activation of type 1 innate lymphoid cells (ILC1) and NK cells [6, 47-50], indicating that cDC1 are particularly important for type 1 immune responses during viral infections and tumor surveillance [51].

In contrast, the exact function of cDC2 is less clear, and especially in peripheral organs, diverse environmental cues may determine tissue-specific cDC2 functions [46, 52-55]. The general consensus is that cDC2 preferentially prime type 2 $CD4^+$ T-cell responses via MHC-II presentation [6, 43, 48] and initiate type 3 immune responses by activating ILC3 and T helper type-17 (TH17) cells [6, 16, 44]. These TH17 cells are typically induced by splenic $ESAM^{hi}$ cDC2 and dermal $CD11b^+$ cDC2 in response to extracellular pathogens, while they also produce IL-23, scavenge soluble Ag from the circulation and are required for germinal center B-cell responses [44, 52, 56]. Moreover, splenic $ESAM^{hi}$ cDC2 are also associated with tissue repair [15]. In contrast, splenic $ESAM^{lo}$ cDC2 are poor in MHC-II presentation, although they may robustly present low-dose Ag to $CD4^+$ T cells *in vitro* [56] and rather excel in the production of proinflammatory cytokines, such as TNF and IL-12. Therefore, they regulate type 2 responses to parasites and allergens by activating ILC2 and TH2 cells [16, 43, 44], a comparable function to dermal $CD11b^{lo}$ cDC2 [52]. As discussed, the tissue-specific variation in cDC2 development and function remains a key challenge in cDC2 research. For example, dermal $CD11b^{lo}$ cDC2

Notably, this functional separation into cDC1 and cDC2 is not absolute, as under certain conditions, both cDC subsets can fulfill redundant tasks. In particular, cDC1 can activate $CD4^+$ T cells through MHC-II presentation and initiate TH1 responses [57], while cDC2 can, depending on the type of Ag and activation stimulus, efficiently cross-present Ag both *in vitro* and *in vivo* [58–62]. This highlights that cDC are heterogeneous in terms of development and function, and a better understanding of their biology will benefit the development of novel treatments for a wide range of diseases.

Monocyte-derived DC

Monocytes are circulating leukocytes that migrate into tissues in the steady state and at an increased rate during inflammation, where they exhibit both proinflammatory and anti-inflammatory properties. As such, monocytes play an essential role in innate defense against many pathogens. They can be separated into $Ly6C^{hi}CX3CR1^-$ classical (inflammatory) and $Ly6C^{lo}CX3CR1^+$ nonclassical (patrolling) monocytes, each with their specific developmental and functional characteristics [63, 64]. Historically, monocytes have been considered precursors of tissue-Mφ [64, 65]; however, it has been demonstrated that under (pathogenic) inflammation, monocytes have the potential to differentiate *in situ* into cells with DC-like functions (MoDC) [10, 40, 66-69] (Figure 1). However, the developmental origin of MoDC and their relative contribution to maintaining the steady-state cDC network *in vivo* remain elusive (*see below*).

MoDC are highly heterogeneous, and multiple subsets of *in vitro* and *in vivo* MoDC have been characterized in both mice and humans depending on the context of infection, cancer, and inflammation. More recent studies using single-cell RNA sequencing identified multiple MoDC subtypes in GM-CSF-derived cell cultures based on gene expression profiles aligned with human peripheral cDC2 [71]. Moreover, depending on the type and location of the infection and the inflammatory setting, MoDC are termed monocyte-derived cells, TNF/iNOS-producing DC (Tip-DC), or inflammatory DC [21, 68, 72]. However, whether these different terms refer to the same cells or indicate specific (activation) stages of (Mo)DC is still under debate and has caused some confusion [40, 72-74]. These data suggest that monocytes give rise to functionally distinct DC during inflammation; however, whether these different MoDC fates are driven by environmental inflammatory cues or whether specialized monocyte precursors exist is still largely unknown.

MoDC differentiation under inflammatory conditions

Upon infection, $Ly6C^{hi}$ monocytes are recruited from the BM into the tissue in a CCR2- and CD62L-dependent manner, where the inflammatory milieu directs their differentiation into MoDC [60, 63, 66]. Consistently, reduced levels of circulating monocytes in CCR2-knockout mice correspond to the abrogated presence of MoDC in tissues [75–79]. Although the exact molecular pathways remain unclear, during their differentiation into MoDC, monocytes rapidly acquire the expression of MHC-II, CD11c, and other DC-related genes, while Ly6C expression is downregulated. These differentiated MoDC can still be distinguished from $Ly6C^{lo}$ nonclassical monocytes by the lack of CD43 expression [73, 78, 80].

In contrast to cDC, normal MoDC development was observed in FLT3L-knockout mice, indicating that MoDC are not derived from precDC. Although GM-CSF (eventually in combination with IL-4) is routinely used to generate MoDC *in vitro* [81–84], MoDC *in vivo* develop normally in the absence of GM-CSF and instead depend on M-CSF [40, 85-88]. Acquisition of DC fate is driven by PU.1, which acts as a suppressor of Mφ fate by restraining MAFB [69, 89]. Additionally, BLIMP-1 and IL-4/STAT4 signaling are essential for the induction of MoDC fate [90–93]. Although MoDC share a common gene signature and overlapping marker expression (e.g., CD64, Ly6C, and MerTK) with Mφs [94–96], MoDC express ZBTB46, underlining that MoDC are part of the DC family as opposed to activated Mφs. However, the significance of ZBTB46 for MoDC development is unknown. Of note, much of our knowledge about MoDC differentiation results from the gut, i.e., a tissue with high inflammatory signaling during the steady

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state, and MoDC development in other tissues or inflammatory settings might be different.

Steady-state MoDC subsets

Although MoDC were initially identified as a cell type that was absent from steady-state tissues [10], it is now well accepted that certain cDC populations in mucosal tissues (and possibly most peripheral tissues) depend on renewal by monocytes. For example, cells of the poorly defined CD11b$^+$CD103$^-$ cDC population in the lamina propria (LP) exhibit a gene expression profile closely resembling that of Mφ [73, 97] and express CCR2 [98], suggesting that they are derived from circulating monocytes [70, 85]. Similarly, these CD11b$^+$CD103$^-$ intestinal cDC hardly migrate to mesenteric LNs, although under inflammatory conditions, CD11b$^+$CD103$^-$ cDC can migrate in a CCR7-dependent manner and induce IL-17 and IFN-γ production by T cells [98, 99]. While some reports claim that these cells develop in a FLT3L-independent but M-CSF-dependent manner, others argue that CD11b$^+$CD103$^-$ cDC do originate from precDC and are FLT3L-dependent [98, 100]. In addition, transfer experiments have demonstrated that monocytes give rise to interstitial Mφs, while precDC develop into intestinal cDC [80, 85, 98]. Ms4a3 fate-mapping experiments established that granulocyte-macrophage-progenitor (GMP)-derived monocytes give rise to tissue-specific resident Mφs but not to steady-state cDC [101]. Nevertheless, low but stable Ms4a3 labeling of cDC2 could be observed in organs such as the lung, liver, and intestine [101]. In line with this, several reports have demonstrated that skin DC comprise a heterogeneous group of cells of mainly cDC but potentially also of MoDC origin [6, 102-104]. MoDC may also be recruited to the skin in a CCR2-dependent manner to replenish emigrated epidermal Langerhans cells during inflammation [105] and differentiate into CD11b$^+$CX3CR1$^+$ cDC in steady-state and inflamed lungs [70, 106, 107]. Additionally, some MoDC are found in skeletal muscle, although they do not migrate to the LN in the steady state [94]. Together, these findings suggest that monocytes can contribute to the steady-state cDC network in peripheral tissues, particularly in mucosal tissues, although the contribution of monocytes in maintaining the DC network might be lower than local cDC self-renewal *in situ*.

MoDC function

In vitro-generated GM-CSF DC have been used extensively in studies examining prototypic cDC function [81-84, 108, 109]. Although these types of studies demonstrate that MoDC are excellent in Ag presentation and T-cell priming, the exact function of MoDC is still unclear, especially as these observed DC-like functions of MoDC might be due to the substantial proportion of cDC2 present in these heterogeneous CD11c$^+$ DC cultures [110].

MoDC have been described *in vivo* during pathogenic inflammation (e.g., *Leishmania major* infection, *Listeria monocytogenes* infection, fungal infections, and viral infection), as well as during inflammatory diseases such as psoriasis, asthma, and colitis [103, 111]. However, MoDC are very dynamic cells whose function and activation may vary during different immune responses, which complicates the determination of their precise functions *in vivo*. Moreover, the different MoDC subsets do not necessarily exhibit similar functions. For example, it has been indicated that MoDC favor TH1 cell immunity via IL-12 production [10, 67, 112] but can also induce TH17 responses [103]. Several reports have demonstrated that MoDC are as competent as cDC in Ag presentation in both *in vivo* and *ex vivo* models [10, 67, 94, 112, 113] and that MoDC can migrate from the tissue to the draining LN to contribute as APCs to adaptive immune responses [11, 114]. However, other studies indicate that MoDC are poor activators of naïve T cells [115–117], and a rigorous separation of MoDC from contaminating cDC revealed that MoDC have only a minimal ability to prime naïve T cells [18] (see below). Therefore, it seems that MoDC act as local APCs in tissues reactivating Ag-experienced effector and memory T cells [21]. Other reports propose that MoDC might act as shipping vessels transporting tissue Ag to LNs [118–120], which is, however, in contrast to more recent studies indicating that MoDC cannot migrate to LNs [121].

Additionally, efficient *in vitro* cross-presentation by MoDC has been documented [13, 113], although MoDC may use distinct mechanisms to prime CD8$^+$ T cells than cDC [60]. Although MoDC might be able to cross-present Ags, their contribution to *in vivo* CD8$^+$ T-cell priming is less clear, as MoDC might not migrate to the T-cell areas in secondary lymphoid organs. Moreover, although IRF4 is required for cross-presentation by *in vitro*-generated MoDC, *in vivo* cross-priming does not depend on IRF4-expressing cells but rather on BATF3$^+$ cDC1 s [60, 122], and it has been suggested that MoDC function as bystander cells transferring MHC-I/Ag complexes to cDC for the induction of efficient T-cell responses [123]. In addition to mediating immunity, MoDC can also regulate tolerogenic immune responses, either by the production of IL-10 and regulatory T-cell induction or by directly killing effector T cells or Ag-loaded cDC [124, 125]. Taken together, the exact function of MoDC *in vivo* remains largely unknown, although MoDC might be less efficient in LN migration and function at the site of infection. MoDC might have distinct functions restricted to tissues other than lymphoid tissues, and while MoDC are poor in Ag presentation but produce higher levels of proinflammatory cytokines than cDC, some MoDC might augment cDC-mediated T-cell priming [126].

Defining cDC and MoDC populations

Both cDC2 and MoDC can be found in any tissue upon inflammation, but proper phenotypical discrimination remains challenging, as both cDC2 and MoDC express overlapping surface markers (including CD11b, CD11c, SIRPα, and MHC-II) [10, 20, 34, 69] (Figure 2). Although these markers in combination with CD14, CD64, and cDC-specific CD26 allow the distinction between cDC2 and MoDC in the lung and small intestine [95, 97, 127], this

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staining might be less useful for the identification of these cells in other organs, as, for example, cDC in skin-draining LNs express CD14 at relatively high levels [74]. Moreover, certain FLT3L-dependent cDC2 in the steady-state kidney and lung express the initially proposed Mφ-specific marker CD64 [128, 129], and this receptor is also upregulated on activated cDC [18, 100]. Therefore, it is advised to include additional staining for the complement C5a receptor (CD88) as well as the MAR-1 antibody to distinguish cDC2 from MoDC and Mφs [20, 95, 96, 104, 127, 130]. Note that although MAR-1 was originally described to specifically recognize FcεRI, a recent publication indicates that this receptor is absent on MoDC and that MAR-1 staining on these cells might be due to cross-reactivity with the FcγRI alpha chain (CD64) [131].

As MoDC are by definition derived from monocytes, they are ontogenetically distinct from cDC. As discussed, the dependence on either FLT3L or CSF-1 of cDC and MoDC, respectively, may form an important basis to distinguish these two DC populations throughout the different tissues [21]. Moreover, several TFs have been described to distinguish cDC from MoDC. Since CDPs express CLEC9A, all their cDC progeny can be traced by CLEC9A fate mapping [100]. Monocytes, however, are marked by the expression of MS4A3, allowing unique tracking of MoDC in MS4A3 lineage tracing models [101]. In addition to phenotypic classification, the identification of specific TFs and growth factors that control the development and function of cDC and MoDC is of additional value for the clear discrimination between these DC lineages in multiple organs and across species [21]. Initially, ZBTB46 and L-MYC were described as cDC-restricted TFs, but although they are absent from pDC and Mφs, *in vitro*-produced MoDC express both ZBTB46 and L-MYC [73, 74, 132]. Therefore, the identification of MAFB as a monocyte-/Mφ-restricted TF further aided the discrimination between cDC and MoDC [133].

Although there might be discussion in the literature about the origin of certain cDC populations, for example, splenic ESAM^{lo} cDC2 and cDC that appear during inflammation [17, 20], recent studies have demonstrated their cDC2 origin [18, 19]. These novel insights and their earlier misidentification as monocyte-derived cells will have consequences for the interpretation of original studies addressing the migration and T-cell priming capacities of MoDC.

Splenic ESAM^{lo} cDC2

As discussed, in the steady-state spleen, a fraction of cDC2 can be characterized by the lack of ESAM expression and is supposed to comprise a mixture of cDC precursors, cDC2B, and MoDC. The precise origin of ESAM^{lo} cDC is unknown, but they show a strong similarity with MoDC due to the expression of monocyte lineage-associated markers such as CD14, CD36, CCR2, CSF-1R, CX3CR1, Ly6C, and lysozyme, although at lower levels than monocytes and Mφs [16, 17, 19]. As their functional characteristics are not typically associated with cDC2, the current literature suggests that splenic ESAM^{lo} cDC may comprise MoDC or even activated Mφs that have acquired a cDC phenotype and function [17, 32]. However, steady-state renewal of splenic cDC does not rely on monocytes [34, 70]. Similarly, CLEC9A-Cre-mediated fate mapping [100] and the similar expression levels of ZBTB46 between ESAM^{hi} and ESAM^{lo} cDC2 [73] indicate that ESAM^{lo} cDC are *bona fide* cDC. Although ESAM^{lo} cDC2 might not be of monocytic origin, their distinct transcriptional requirements suggest that the ESAM^{hi} and ESAM^{lo} cDC populations may develop from different myeloid precursors. While adoptively transferred MDP gives rise to both ESAM^{hi} and ESAM^{lo} DC, transferred CDP predominantly develops into ESAM^{hi} cDC2, supporting the hypothesis that splenic cDC2 populations arise from different progenitors [16] (Figure 1). ESAM^{lo} cDC development is KLF4 dependent, and this TF also regulates monocyte development and Mφ polarization [43, 134]. Furthermore, the development of ESAM^{hi} and ESAM^{lo} cDC2 is regulated by RUNX3, and the lack of RUNX3 in cDC shifts the gene expression of ESAM^{hi} cDC2 toward that of ESAM^{lo} cDC2 [135]. Whether the ESAM^{hi} or ESAM^{lo} cellular fates are already appearing in the BM or might be driven by the local spleen environment is unclear.

Defective ESAM^{hi} cDC2 homeostasis, e.g., in CD11c-specific NOTCH2/RBPj-KO and ADAM10-KO mice, results in a strong expansion of the ESAM^{lo} cDC population that compensates for the loss of ESAM^{hi} cDC2, whereby the homeostatically defined cDC2 numbers in the spleen are kept constant [16, 17, 19]. These emerging ESAM^{lo} cells express PU.1 and ZBTB46, and ESAM^{lo} cDC2 in CD11c-specific ADAM10-KO mice strongly expand upon FLT3 treatment but further appear different from steady-state ESAM^{lo} cDC2 [19]. They do not represent an emergency MoDC population but most likely a diverging branch of splenic cDC2 that share a prominent gene expression signature with LN-resident cDC2 [19]. Together, ESAM^{lo} cDC2 can be easily confused with MoDC, but they are distinct from monocyte-derived cells and do represent a *bona fide* cDC2 population. Because the origin and function of ESAM^{lo} cDC2 seem to differ from those of other cDC2, these cells might be further classified into a separate cDC3 subtype.

Inflammatory cDC2

During inflammation, a population of activated inflammatory CD64^{+}Ly6C^{+}MAR-1^{+} DC appear in the tissues, the exact ontogeny and function of which remain to be identified. As monocytes are rapidly recruited to the site of inflammation, inflammatory DC are thought to be either MoDC [20] or Mφs [136], which is further supported by the observation that these cells do not express *Zbtb46*-GPF in lineage-tracing models [133]. However, *bona fide* cDC2 may also be recruited in a CCR2-dependent manner into inflamed tissues and acquire CD64 expression. These inflammatory cDC2 (Inf-cDC2) represent an activated cDC population that is induced in a type I IFN-dependent manner during respiratory viral infections and allergy [18, 137, 138]. Although Inf-cDC2 are ZBTB46^{+}, precDC-derived, and FLT3-dependent cells, it is unknown whether they are derived from the cDC2B population or whether they represent a distinct cDC2 subset.

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Inf-cDC2 express, in addition to cDC2-specific cell markers such as CD26, SIRPα, and IRF4, the monocyte markers CD64 and MAR-1 but not MerTK [18, 137]. Uniquely, Inf-cDC2 also acquire transcriptional and functional cDC1 characteristics, such as the expression of IRF8 [18]. These unique hybrid features allow Inf-cDC2 to efficiently prime $CD4^+$ T cells but also execute established cDC1 functions, including IL-12 production and cross-presentation of extracellular Ag to $CD8^+$ T cells [18, 137]. Moreover, Inf-cDC2 express CCR7 and have considerable potential to migrate to LNs.

Taken together, the $CD64^+Ly6C^+MAR\text{-}1^+$ DC population that appears during inflammation is a mixture of MoDC and a novel cDC subset [18]. Although MoDC were initially associated with TH2 immune responses during allergic reactions [20], after careful separation from Inf-cDC2, these MoDC failed to migrate to LN and prime naïve T cells, although they locally presented Ag on MHC-II [18].

Concluding remarks

To date, there is no optimal strategy for separating cDC and MoDC across different tissues and species, which results in uncertainties about their individual immune functions. Nevertheless, recent technical advances, especially high-dimensional flow cytometry and single-cell sequencing approaches, have facilitated their more precise and refined definition through the identification of an increasing number of lineage-specific markers. The use of these markers greatly enhanced our understanding of the distinct cDC, MoDC, and Mφ lineages. This resulted in the consensus that cDC are essential for the initiation of immune responses by priming naïve T cells in LNs, while the main function of MoDC might be the *in situ* propagation of immune responses. Nevertheless, the lack of cDC2- and MoDC-specific ablation models complicates the further functional delineation of these DC subsets.

As the transcriptional requirements for both cDC2 and MoDC are still largely unclear, this limits the development of future genetic targeting approaches. For example, although several transcriptional regulators of cDC2 development and function have been identified in recent years, no single TF is known that selectively ablates all cDC2 subsets. Moreover, the widely accepted overarching TF in cDC2 biology, IRF4, might not be involved in cDC2 development but rather control the migration capacity of these cells [139]. Targeting of CLEC4A4 (DCIR2) or MGL2 will affect not only specific cDC2 subsets but also certain Mφ and LC populations, respectively [140, 141]. Although mutations in the *Zeb2* enhancer region resulted in the complete loss of cDC2, this mutation also abrogated monocyte development [142], while ZEB2 also marks pDC [143, 144]. *Conversely*, the classical monocytic fate mapping model using CX3CR1-cre also targets cDC2 [145].

The DC network is highly conserved between mice and humans (Figure 2). Human $CD141^+$ DC express XCR1 and CLEC9A and are equivalent to mouse cDC1 s, while human $CD1c^+$ cDC represent mouse cDC2, and human cDC2 can also be divided into at least two functionally distinct subsets [15, 146, 147]. However, in contrast to mice, human DC research has mostly focused, due to valid experimental limitations, on MoDC generated from peripheral blood monocyte cultures. Human $CD14^+CD16^-$ (the major monocyte population) and $CD14^+CD16^+$ monocytes are closely related to $Ly6^{hi}CX3CR1^{lo}$ classical monocytes in mice, and both can differentiate into MoDC and acquire DC characteristics, at least during *in vitro* GM-CSF culture. Among these human MoDC, $CD16^+$ cells express higher levels of CD11c and CD86 and produce TGFβ, while $CD16^-$ MoDC produce high levels of proinflammatory cytokines, including IL12p70 [81, 84, 148, 149]. Although the major classes of DC are present in both species, there are certain differences in the transcriptional programming driving DC development and function, and caution must be used by directly comparing the murine and human systems. Additionally, another challenge ahead is to better characterize all DC types during inflammation, as their versatility is even greater.

MoDC are extensively used in experimental studies and for vaccination purposes, i.e., in cancer immunotherapy approaches, and a large number of clinical trials using MoDC have established their safety and efficacy against various tumors. Given this interest in MoDC as the main target for the development of novel immunotherapies, elucidation of their biology in relation to cDC is of considerable importance, especially because MoDC-based vaccines only yield modest clinical responses in certain anticancer treatment regimens [150]. This is likely because MoDC, in strong contrast to cDC, orchestrate local immune responses and lack LN migration and naïve T-cell activation capacities. Given this division of labor and the lessons learned from MoDC-based clinical studies, focusing on specifically targeting cDC subsets might improve the development of novel (c)DC-based vaccines.

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Abbreviations: **Ag**: antigen · **DC**: dendritic cell · **cDC**: conventional (or classical) dendritic cell · **cDC1**: type 1 conventional dendritic cell · **cDC2**: type 2 conventional dendritic cell · **APC**: Antigen-presenting cells · **pDC**: plasmacytoid dendritic cell · **MoDC**: monocyte-derived dendritic cell · **Mφ**: macrophage · **Inf-cDC2**: inflammatory type 2 conventional dendritic cell · **TF**: transcription factor · **HCS**: hematopoietic stem cell · **CMP**: common myeloid progenitor · **MDP**: monocyte/dendritic cell precursor · **CDP**: common dendritic cell precursor · **GM**: granulocyte-macrophage-progenitor · **preDC**: precommitted dendritic cell · **LNs**: lymph nodes · **ILC1**: type 1 innate lymphoid cell · **TH1**: T helper cells type-1 · **TH2**: T helper cells type-2 · **TH17**: T helper cells type-17 · **MHC**: major histocompatibility complex · **TIP-DC**: TNF/iNOS-producing dendritic cells

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