Transcriptional Control of Dendritic Cell Development

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Abstract

The dendritic cells (DCs) of the immune system function in innate and adaptive responses by directing activity of various effector cells rather than serving as effectors themselves. DCs and closely related myeloid lineages share expression of many surface receptors, presenting a challenge in distinguishing their unique in vivo functions. Recent work has taken advantage of unique transcriptional programs to identify and manipulate murine DCs in vivo. This work has assigned several nonredundant in vivo functions to distinct DC lineages, consisting of plasmacytoid DCs and several subsets of classical DCs that promote different immune effector modules in response to pathogens. In parallel, a correspondence between human and murine DC subsets has emerged, underlying structural similarities for the DC lineages between these species. Recent work has begun to unravel the transcriptional circuitry that controls the development and diversification of DCs from common progenitors in the bone marrow.

INTRODUCTION

Classical dendritic cells (cDCs) are professional antigen-presenting cells that play a key role in shaping appropriate immune responses (1–3). Since their discovery by Steinman (4) in 1978, DCs have been recognized as potent activators of naive T cells, but they are now known to have nonredundant functions in innate immunity as well. Over time, the definition of the cells responsible for these activities has been refined, and several cell lineages are now appreciated. These include the initially identified cDCs, plasmacytoid DCs (pDCs), Langerhans cells (LCs), and monocyte-derived DCs (moDCs). Recent reviews have discussed the basis for recent changes to the classification of these cell types and their functions in the immune response (1, 2, 5). The maturation and activation of DCs in response to various stimuli such as Toll-like receptor (TLR) signaling have been reviewed recently (6). This review focuses on recent findings about the transcriptional basis for the development and function of the individual types of DCs.

Many relevant studies have addressed the heterogeneity of DC populations using different combinations of surface markers. Comparing transcriptional properties between studies can benefit from a simplified DC nomenclature, referring to a common lineage rather than citing the precise markers of each study. For example, Batf3-dependent CD8α^+ cDCs in the spleen and CD103$^+$ cDCs in peripheral tissues belong to the same lineage (7), which we refer to as Irf8$^+$ cDCs. And although Irf4$^+$ cDCs, the other major branch of cDCs, have been subdivided into Notch2-dependent and Klf4-dependent populations, the developmental basis has not been resolved; we will continue to refer to both of these as Irf4$^+$ cDCs.

We begin by reviewing the functional diversity of mature murine DC populations and the correspondence with human DCs, which suggest that DC subsets are organized around major immune effector modules promoting cytolysis, intracellular defense, extracellular defense and mucosal barrier immunity (**Figure 1**). We will then discuss recent progress in study of the progenitors giving rise to DCs and finish with the transcriptional basis of DC development and diversification into the recognized DC lineages.

HETEROGENEITY OF MATURE DENDRITIC CELLS

The in vivo function of some DC subsets has been demonstrated by transcriptional manipulations that selectively eliminate some but not all subsets. The Irf8$^+$ cDC subset expresses CD8α in spleen and CD24 or CD103 in the periphery (although CD24 expression and CD103 expression are not unique to this subset) (8). It requires the transcription factors Irf8 (9, 10), Batf3 (7, 11, 12), Nfil3 (13), Id2 (14, 15), and Bcl6 (16) for development.

The second major branch of cDCs comprises CD4$^+$ or CD8$^-$ cDCs, expresses Sirpα or CD11b, and is characterized by expression of the transcription factor Irf4 rather than Irf8. Antigen presentation to CD4$^+$ T cells appears to favor CD8$^-$ cDCs (17, 18), which are affected by loss of the transcription factors Traf6, Irf2, and Irf4 (3). Subsets of this branch are dependent on the transcription factors Notch2 and Klf4, indicating heterogeneity within Irf4$^+$ cDCs (19, 20). Production of IL-23 by Notch2-dependent Irf4$^+$ cDCs is required for effective type 3 immune responses (19, 21), whereas Klf4-dependent Irf4$^+$ cDCs appear to be required for type 2 responses by an unknown mechanism (20).

These two branches of cDCs can be distinguished by mutually exclusive expression of XCR1 and Sirpα. XCR1 is expressed by Irf8$^+$ cDCs, and Sirpα is expressed by Irf4$^+$ cDCs, independently of activation status and location (22). An alternative nomenclature has been proposed, whereby XCR1$^+$ Irf8$^+$ cDCs are referred to as DC1 and Sirpα^+ Irf4$^+$ cDCs as DC2 (23). However, because DC2 cells have already been functionally divided into Notch2-dependent

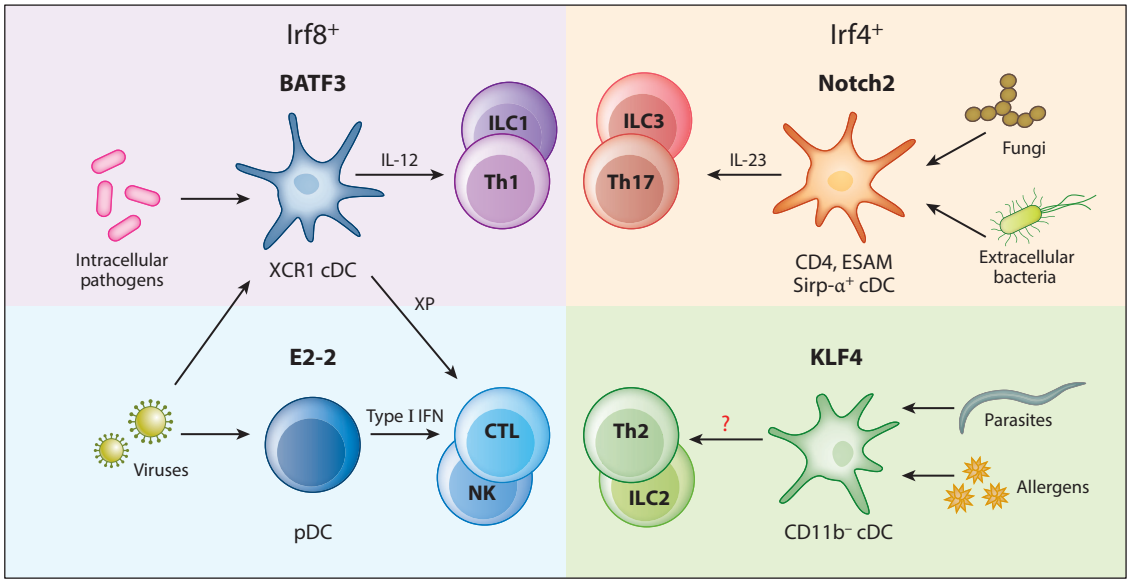


Figure 1

Dendritic cell (DC) subsets serve distinct immune effector modules. Transcription factor dependencies identify four subsets of DCs whose actions are directed primarily toward distinct immune effector modules. Irf8$^+$ DCs comprise plasmacytoid DCs (pDCs) and one branch of classical DCs (cDCs). Irf4$^+$ DCs are heterogeneous in their surface markers and display at least two transcriptional programs directed at different types of immune responses.

Irf4$^+$ cDCs and Klf4-dependent Irf4$^+$ cDCs, and so are already heterogeneous, we will not use the DC1/DC2 naming system here. In this review, we divide cDCs into Irf8$^+$ and Irf4$^+$ subsets, because these factors are differentially expressed and are developmentally involved in the two subsets. By contrast, Batf3, as we discuss below, is developmentally required only for Irf8$^+$ cDCs but is expressed by both Irf8$^+$ and Irf4$^+$ cDCs, such that its expression alone does not distinguish between these two cDC lineages.

pDCs are identified by expression of B220, Siglec-H, and Bst2, and their development requires the transcription factors Irf8 and E2-2 (24). pDCs have morphology and functions distinguishing them from cDCs. Various studies of in vivo ablation indicate that the function of pDCs is in producing abundant type I interferons in response to viral infections, leading to a general reduction in viral titers, but they do not appear to function directly in antigen presentation. Finally, LCs are capable of antigen presentation but are developmentally more related to the macrophage lineage. For example, LCs depend on IL-34 and not on the common DC cytokine Flt3-ligand (Flt3l) for their development. Also, LCs do not express key transcription factors characteristic of cDCs, such as Zbtb46, or pDCs, as we discuss below. We touch on some issues relating to LC development as they arise but do not focus on the transcriptional basis of their development.

Irf8$^+$ cDCs Function in Cross-Presentation and Defense Against Intracellular Pathogens

Cross-presentation of antigen to CD8$^+$ T cells is performed efficiently by the Irf8$^+$ CD8α^+ subset of cDCs (17), and IL-12 production by CD8α^+ cDCs is required for effective type 1 responses (**Figure 1**). The dependence of Irf8$^+$ cDCs on the transcription factor Batf3 for development helped to establish that IL-12 production by this branch of cDCs is important in defense against

intracellular pathogens (11, 25–28). Experiments using *Irf8*$^{-/-}$ mice had indicated that CD8α^+ DCs were a source of IL-12 during infection by *Toxoplasma gondii* (29); however, *Irf8*$^{-/-}$ mice harbor additional defects that could have contributed to altered responses to *T. gondii* infection (30, 31). In contrast, *Batf3*$^{-/-}$ mice exhibit a selective loss of CD8α^+ DCs and the CD103$^+$ peripheral counterpart (11). Studies with *Batf3*$^{-/-}$ mice showed that these DCs were the critical source of IL-12 for early defense against *T. gondii* infection (28). CD8α^+ cDCs express Tlr11 (32, 33) and highly express Irf8 (34), which may enable these responses. IL-12 produced by monocyte-derived DCs is also important at sites of infection (35), although their dependence on Irf8 and Batf3 is unknown.

Use of *Batf3*$^{-/-}$ mice lacking CD8α^+ cDCs also confirmed the unique cross-presentation capacity of this subset during tumor rejection (11, 36, 37). *Batf3*$^{-/-}$ mice containing DC-specific deletion of the type I interferon receptor were used to show that CD8α^+ DCs require signaling by type I interferon receptors to support antitumor responses (36, 37). Other studies indicate that cross-presentation by CD8α^+ DCs also requires maturational signals such as granulocyte-macrophage colony-stimulating factor (GM-CSF) or TLR ligands (38–40). Migratory Batf3-dependent CD103$^+$ DCs capture apoptotic-cell associated antigens in the lung and then travel to draining lymph nodes, where they present captured antigen to CD8$^+$ T cells (41). Batf3-dependent cDCs in islets of Langerhans are required for development of autoimmune diabetes, although it is not clear if this was due to a requirement for cross-presentation or for IL-12 production in priming CD4 T cells (42). Finally, Batf3-dependent cDCs were critical for promoting T cell responses in responses to viruses (2) and in the tumor myeloid compartment (43).

Targeting antigen to XCR1 uniquely expressed on Irf8$^+$ cDCs has been proposed for use in vaccines against influenza virus (44) and therapeutic cytotoxic vaccines for humans (45). Although Irf8$^+$ cDCs are the type of DCs best suited for cross-presentation, targeting other types of DCs using antigen coupled to anti-Dec205 or anti-DCIR2 antibodies allowed them to cross-present, suggesting that cross-presentation in vivo relies on common or redundant intracellular pathways (17, 46).

Notch Signaling Controls Terminal Maturation of Irf4$^+$ cDCs That Produce IL-23

Distinct functional specialization of Irf4$^+$ cDC subsets was revealed using mice with conditional deletion of Notch2 in DCs and *Citrobacter rodentium* infection, a mouse model for enteropathogenic *Escherichia coli*. IL-23 and IL-22 production by innate cells is required during early stages of *C. rodentium* infection (47) and CD11b$^+$ cDCs were identified as the nonredundant source of IL-23 after administration of bacterial flagellin (48). Deletion of Rbp-J, a mediator of Notch signaling, using a CD11c-cre deletor strain, led to a 50% reduction in the CD11b$^+$ subset of splenic DCs (49). Two populations of CD8$^-$CD11b$^+$ DCs can be distinguished based on CX3CR1 and ESAM expression (21). Notch2, but not other Notch receptors, controls the development of the CX3CR1^{lo} ESAM^{hi} subset in the spleen. Development of CD11c$^+$ CX3CR1$^+$ cells, which are likely monocyte derived and not a cDC subset, also requires Notch2 signaling in the small intestine, but their function in infections was not examined (50).

Mice with conditional deletion of Notch2 in DCs revealed that these CD11b$^+$ cDCs are the critical source of IL-23 required for clearance of *C. rodentium* (19, 51). *Irf4*$^{-/-}$ and *Ccr7*$^{-/-}$ mice, whose DCs exhibit migration defects (52), did not show the same susceptibility to *C. rodentium* infection as mice lacking Notch2 in DCs did (19). IL-23 is necessary for induction of IL-22 by ILC3 and Th17 cells (53, 54). Thus, it is the IL-23 production by small intestine lamina propria CD11b$^+$ DCs that induces IL-22 production by ILC3s, providing local control of the pathogen

(**Figure 1**). Notch2-dependent CD11b$^+$ DCs also modulate Th17 differentiation and function. Deletion of Notch2 in DCs leads to a decrease in Th17 numbers in the mesenteric lymph node (21). This shows that IL-23 from CD11b$^+$ DCs is necessary for Th17 differentiation in vivo and in vitro (55). A similar role for Notch2-dependent cDCs in supporting IL-17 production by CD8 T cells was shown for responses to skin-resident commensal organisms (56).

A Klf4-Dependent Subset of Irf4$^+$ cDCs Is Required for Optimal Th2 Responses

Circumstantial evidence had suggested a specific DC subset might regulate type 2 immunity. Depletion of CD11c$^+$ cells in *Itgax*-DTR mice significantly impairs Th2 responses against *Schistosoma mansoni* (57). A CD11b$^+$ DC subset expressing FcεR1 could induce in vivo type 2 immune responses to immunization with house dust mite (HDM) antigen (58). In addition, both CD11b$^+$ cDCs and moDCs were rapidly recruited to lung airways upon HDM challenge, but only CD11b$^+$ cDCs were able to migrate to the lymph node and trigger Th2 responses (59). However, in 2013, a study showed that moDCs are sufficient to induce Th2 responses in *Flt3l*$^{-/-}$ mice immunized with a high dose (100 μg) of HDM (60). In contrast, human CD141$^+$ DCs can induce CD4 T cells to produce Th2 cytokines, although their in vivo role is unverified (61) and other human DC subsets have not been excluded.

Further studies indicated that the Irf4$^+$ cDC branch may operate in Th2 responses in an HDM-induced allergy model (62). Two other studies examined *Irf4*$^{-/-}$ mice to argue that dermal cDCs expressing CD301b and PD-L2 mediate Th2 responses (63, 64), although these mice also exhibit a defect in DC migration from tissues to lymph nodes (52). The expression of major histocompatibility complex class II (MHC-II) and costimulatory molecules was reported to require Irf4 according to analysis of GM-CSF derived cDCs in vitro (18). However, in vivo, the expression of MHC-II was not dependent on Irf4 (52).

A subset of CD11b$^-$ Irf4$^+$ cDCs in the skin-draining lymph nodes was found to be dependent on the transcription factor Klf4 and to be required for Th2 responses (20). Klf4 conditionally deficient mice were known to have reduced CD11b$^+$ cDCs in their spleen; however, the nature of the defect had not been further analyzed with respect to cDC subsets or function (65). Conditional Klf4 deletion using CD11c-cre impaired Th2 cell responses during *S. mansoni* infection, *Schistosoma* egg antigen (SEA) immunization, and HDM challenge and promoted Irf4 expression in pre-cDCs (20). Involvement of a similar subset of CD11b^{lo} DCs in Th2 response had been suggested previously (66).

pDCs Are Required for Effective Antiviral Responses

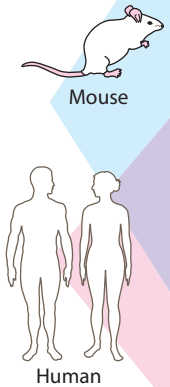
Three methods have been used to selectively delete pDCs and demonstrate their importance in antiviral responses. In the first model, diphtheria toxin receptor was expressed under the control of the human pDC specific BDCA-2 promoter (67). Analysis of these mice showed that pDC depletion reduced early IFN-I production during murine cytomegalovirus infection and increased viral burden, leading to expansion of natural killer cells. During vesicular stomatitis virus infection, survival and accumulation of virus-specific cytotoxic T lymphocytes was impaired.

In a second model, conditional deletion of E2-2 using CD11c-cre led to lack of pDCs (68). This system was used to show that pDCs are important both for the innate response to acute MHV and in the adaptive response to chronic lymphocytic choriomeningitis virus Docile infections. In particular, pDCs allow optimal CD4$^+$ T cell activation, prevent their exhaustion, and allow sustained CD8 T cell responses during chronic viral infections.

A third model used a knock-in approach that targeted the 3′UTR of Siglec-H with IRES-DTR-EGFP (69). This study reported a greater impact of pDC deletion compared with the two other studies (67, 68), suggesting that pDCs were required for inflammation induced by TLR stimulation and bacterial and viral infections, as well as for controlling both $CD4^+$ T cell responses and $CD8^+$ T cell responses. The discrepancy between these findings and results of other approaches deleting pDCs may derive from the expression of Siglec-H on progenitors that affect other subsets of DCs (70), as discussed below. Thus, deletion of Siglec-H^+ progenitors would cause a reduction in more than pDCs, and would likely reduce cDC development as well, potentially explaining the relatively larger phenotype reported by these authors. On the other hand, the first system (67) relies on the fidelity of the human pDC specific BDCA-2 promoter used as a transgene in mice. Likewise, deletion of E2-2 using CD11c-cre could potentially lead to inactivation of the E2-2 gene in other lineages, such as macrophages, in which CD11c-cre is known to be expressed. However, this is unlikely to be a confounding factor, because E2-2 is not expressed in macrophages.

Correspondence Between Mouse and Human DC Subsets

Recent work supporting a correspondence between murine and human dendritic cell lineages has been reviewed elsewhere (71, 72) (**Figure 2**). XCR1 expression identifies the $Irf8^+$ cDC lineage in both humans and mice (73). In humans the $Irf8^+$ cDC lineage also expresses high levels of CD141 in dermis, liver, and lung tissues and is distinct from $CD1c^+$ and $CD14^+$ tissue DCs, which do not express CD141 (74). The homologs of murine peripheral $CD103^+$ DCs are the human tissue $CD141^+XCR1^+CLEC9A^+$ DCs. The homologs of murine $CD64^-CD11b^+$ DCs are the human



pDCs	Irf8⁺ cDCs	Irf4⁺ cDCs	LCs
Siglec-H⁺ Bst2⁺ Ly6C⁺	Langerin⁺ CD8a⁺ (LT) CD103⁺ (NLT) CD205⁺ CD24⁺	CD4⁺ (LT) CD11b⁺	F4/80⁺ CD205⁺ CD103⁻
CD45RA⁺ CD123⁺ CD14⁺ XCR1⁻ Sirpα⁺	XCR1+ Clec9A+ BTLA^{hi} Necl2⁺ CD14⁻ Sirpα⁻	Sirpα⁺ XCR1⁻	Langerin⁺ Epcam⁺ CD11b⁺ Sirpα⁺ E-cadherin⁺ XCR1⁻
CD303⁺ CD304⁺	CD141⁺	CD1c⁺ CD1a⁺ (skin) CD1b⁺	CD1c⁺ CD1^{hi}

Figure 2

Correspondence between murine and human DC subsets. Selected markers of the indicated cell types are shown for mouse (*blue*) and human (*pink*). Markers shared between mouse and human DCs are indicated by the overlap (*purple*). Abbreviations: cDC, classical DC; DC, dendritic cell; LC, Langerhans cell; LT, lymphoid tissue; NLT, nonlymphoid tissue; pDC, plasmacytoid DC.

CD1c$^+$ DCs. Like murine Irf8$^+$ cDCs, CD141$^+$ human cDCs are superior in cross-presenting cell-associated antigens.

Recent findings (2014) indicate that the similarity in gene expression patterns between human and mouse cDC subsets (74) extends to include intestinal DCs (16). Human intestinal CD103$^+$Sirpα^- DCs were related to human blood CD141$^+$ DCs and to mouse intestinal CD103$^+$CD11b$^-$ cDCs, which are Batf3-dependent Irf8$^+$ cDCs (19). The human CD103$^+$Sirpα^+ DCs were related to human blood CD1c$^+$ DCs and to mouse intestinal CD103$^+$CD11b$^+$ cDCs, which were Notch2-dependent Irf4$^+$ cDCs (19). The correspondence of these phenotypic subsets with functional specialization was less rigid, as both the human and the mouse CD103$^+$ DC subsets induced Th17 cells, but human CD103$^-$Sirpα^+ DCs induced the Th1 subset of helper T cells, reflecting similarity to inflammatory, monocyte-derived DCs. This and another study (75) suggested a role for the transcriptional repressors Prdm1 (Blimp-1) and Bcl6 in regulating DC activity in the intestine.

Some differences between human and murine Langerhans cells (LCs) have been suggested (76, 77). One difference may be due to their ability for cross-presentation (76). Human LCs, but not mouse LCs, expressed genes related to antigen processing and cross-presentation that were also expressed by murine Irf8-expressing dermal cDCs (77). Also, human LCs were more similar in gene expression to the human blood cross-presenting Irf8$^+$ lineage, the CD141$^+$BDCA-3$^+$ cDCs, whereas murine LCs are quite dissimilar from mouse Irf8$^+$ cDCs. This study suggests that human LCs may be more similar to the murine Irf8$^+$ cDCs than previously thought.

Expression of the C-type lectin langerin seems to differ between mouse and human DCs (78). In mice, Langerin is expressed by LCs and Irf8$^+$ cDCs. However, in humans, langerin is expressed by LCs, and at lower levels by the CD1c$^+$Irf4$^+$ cDCs, but not by Irf8$^+$ cDCs (78). This difference may complicate the ability of human CD1$^+$ cDCs to develop into LCs (79). CD1c$^+$ cDCs treated with GM-CSF, TGF-β, and BMP7 had high expression of langerin, EpCAM, and E-cadherin and numerous Birbeck granules, which was interpreted as an alternative pathway of LC differentiation. Conceivably, langerin does not represent a robust LC lineage marker in humans and these cells are simply activated CD1c$^+$ cDCs.

Human CD14$^+$ cells have been considered to be DCs with an unclear murine counterpart, but they have poor cross-presenting capacity when compared with other cells such as LCs (76). A recent study showed that human dermal CD14$^+$ cells have a transcriptional profile highly conserved with that of mouse macrophages and distinct from DCs. Thus, human CD14$^+$ cells are the counterpart to murine monocyte-derived CD11b$^+$CD64$^+$ macrophages. These are short-lived tissue macrophages rather than an authentic DC lineage (80).

ORIGIN OF DC PROGENITORS

DCs arise in vivo from the common myeloid progenitor (CMP) and the lymphoid-primed multipotent progenitor (LMPP) (81–84); however, observed clonal bias of fate-mapped LMPPs has challenged the presumed homogeneous multipotential nature of hematopoietic progenitors (84). Also, a type of mechanism of lineage divergence has been proposed based on a link between the actions of a transcription factor and the cell cycle (85). A proposed macrophage-DC precursor (MDP) (86, 87) retains DC and monocyte potential but excludes neutrophils or other myeloid lineages; however, its ontogeny—and even its existence—is under debate (88). MDPs give rise to committed monocyte progenitors (cMoPs) (89) and common DC progenitors (CDPs) (82, 90), but the basis for this divergence is unclear. Likewise, it is unclear how the CDP diversifies to generate the several mature DC subsets described above.

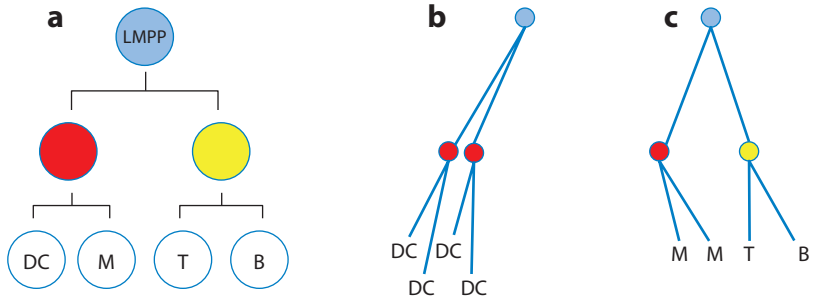


Figure 3

Bias in lineage potential of lymphoid-primed multipotent progenitors (LMPPs) revealed by cellular barcoding. (*a*) An abbreviated schematic for stages of differentiation of an LMPP (*blue*) through two progenitor stages (shown as *red* and *yellow*) into dendritic cell (DC), monocyte (M), or lymphoid lineages (T, B). (*b*) The fate history of an individual LMPP that exhibits clonal bias for generating DC lineages. (*c*) The fate history of an individual LMPP, which in this case exhibits combined monocyte and lymphoid potential, but not DC potential.

Clonal Bias in Early Bone Marrow Progenitors of DCs

Most models of hematopoiesis define various progenitors as homogenous populations that take on progressively restricted developmental potential (**Figure 3**). However, single cell in vivo fate mapping has uncovered unexpected variability in early bone marrow progenitors that give rise to DCs (84). As typically depicted, most models favor binary divergence and imply that a progenitor gives rise to progenitors of both downstream lineages. But these models do not guarantee, or even require, that a given individual progenitor cell gives rise to progeny cells that populate the entire spectrum of lineages. In cellular barcoding experiments, Naik et al. (84) used lentivirally inserted unique tags to trace the developmental fates of hundreds of individual LMPPs and hematopoietic stem cells (HSCs). Some individual LMPPs exhibited unexpected restrictions in potential. Although some LMPPs generated the expected broad diversity of lineages, most had some restriction and divided into combinations of lymphoid-, myeloid-, and DC-biased progenitors, and many showed a strong bias toward generation of progeny of only DC lineages.

The authors suggested a graded commitment model of hematopoiesis in which lineage imprinting can occur much earlier than previously suspected. This would mean that at single cell resolution, the steps of specification and commitment might be imprinted even earlier than typically reflected by the surface markers of the individual cells (**Figure 3**). This imprinting could involve, for example, epigenetic regulation of the transcription factors that are known to control DC development, although the details are still under investigation. Even without such information, these results imply that DCs are a distinct lineage based on separate ancestry.

Linkage Between Differentiation and Cell Cycle Length

Lineage divergence can arise from mutual antagonism between transcriptional factors that promote opposing fates. A new type of mechanism has been discovered in which lineage divergence results from regulation of cell cycle length by a transcription factor. Lineage divergence between lymphoid (B cell) and myeloid (macrophage) fates relies on the transcription factor PU.1 achieving either low or high levels, respectively. By examining the accumulation of PU.1 proteins in single progenitor cells during differentiation, Rothenberg and colleagues (85) uncovered a feed-forward

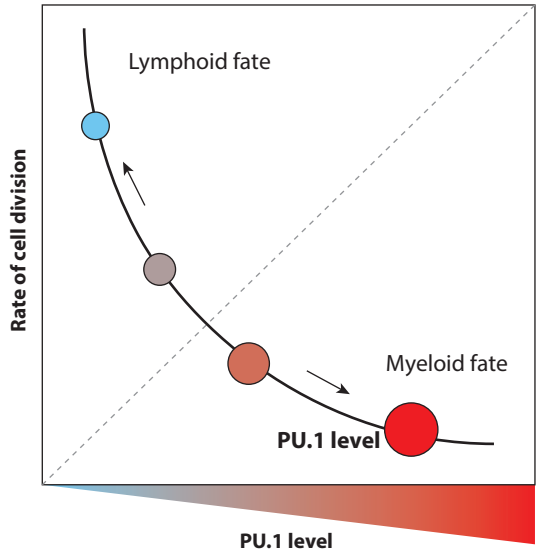


Figure 4

Cell cycle regulation by PU.1 promotes myeloid divergence. PU.1 can decrease the rate of cell division. Because PU.1 is relatively stable, a long interphase promoted the accumulation of PU.1 protein, which then reduces proliferation more efficiently. Rapid division of lymphoid progenitors prevents PU.1 levels from crossing a threshold (*dashed line*) sufficient for positive feedback by PU.1.

pathway that promotes a divergence in PU.1 protein levels. The essential feature of this mechanism is the action of PU.1 in blocking the cell cycle, coupled with the relatively long half-life of PU.1 mRNA and protein. By maintaining a rapid rate of cell division, B cell progenitors keep levels of PU.1 protein below a threshold required to block cell division (**Figure 4**). In contrast, myeloid progenitors divided more slowly, allowing PU.1 protein levels to increase and therefore further inhibit the cell cycle, thus promoting myeloid differentiation.

Experimentally, introduction of exogenous PU.1 into progenitors led to an increase in the expression of endogenous PU.1, and this was achieved by increasing the duration of the cell cycle (85). This result implies the existence of a positive feedback circuit between the regulatory factor PU.1 and the cell cycle. PU.1 is expressed at intermediate levels in uncommitted progenitors and must be downregulated for B and T cell development to occur, with higher PU.1 levels promoting macrophage development. The basis for heterogeneity in how progenitors diverge is still unresolved, but this new link between the cell cycle length and a transcription factor's level of expression may apply to other divergences. These results show that cell cycle duration functions as an integral part of a positive regulatory circuit to control cell fate.

Flt3 and Flt3L Requirements for DC Development

The development of pDCs and cDCs is dependent on Fms-like tyrosine kinase 3 (Flt3), or CD135; its ligand, Flt3L; and the downstream signaling factor STAT3. Flt3 is expressed on several early bone marrow progenitor populations (91, 92), including mature cDCs and progenitors of cDCs (93). Studies that analyzed *Flt3*$^{-/-}$ and *Flt3l*$^{-/-}$ mice each found deficiencies in DC development, but with varying severity (94, 95). One analysis of Flt3-deficient mice found an 85% reduction in all DC populations at 2 weeks of age compared to age-matched control mice, but the defect

improved in 9-week-old mice to a 43% cDC reduction and 65% pDC reduction (94). The initial characterization of Flt3l-deficient mice was based on analysis of mice between 5 and 14 weeks of age that had 4- to 10-fold reductions in splenic CD8α$^-$ DCs and 6- to 14-fold reductions in splenic CD8α$^+$ DCs (95). It is not clear whether the variable reductions were related to age differences among the mice examined. Conceivably, DC numbers are restored with increasing age in *Flt3l*$^{-/-}$ mice, as in *Flt3*$^{-/-}$ mice. However, at face value, these results suggest that DC development is more severely disrupted by *Flt3l* deficiency than by *Flt3* deficiency. Consistent with the latter conclusion, another study (96) comparing DCs from several nonlymphoid tissues of control, *Flt3*$^{-/-}$, and *Flt3l*$^{-/-}$ mice at 8 to 12 weeks of age reported a significant reduction in CD8α$^+$ DCs in both types of knockout mice but a reduction in CD11b$^+$ DCs in *Flt3l*$^{-/-}$ mice only. *Flt3l*$^{-/-}$ mice had more severe deficiencies in both types of cDCs compared with *Flt3*$^{-/-}$ mice. Although the basis for this discrepancy remains unresolved, conceivably Flt3l could signal through another receptor besides Flt3, making the loss of the ligand more severe than the loss of one receptor target. In any case, a direct comparison between *Flt3*$^{-/-}$ and *Flt3l*$^{-/-}$ mice across a wider range of ages is warranted.

It has been known for some time that development of all DC lineages requires the transcription factors Ikaros (97, 98), PU.1 (99–101), and Gfi1 (102). One action of PU.1 in supporting DC development has to do with its role in driving expression of Flt3 (101), although this was demonstrated only in late stages of DC development. PU.1 is also involved in expression of Irf8 as early as the CMP or MDP (103, 104). More recently, the transcription factor Bcl11a was shown to be required for development of all DCs, owing to its role in controlling Flt3 expression (105, 106). Interestingly, IL-7 receptor (IL-7R) expression is also dependent on expression of Bcl11a (105). The in vivo development of both pDCs and cDCs from *Bcl11a*$^{-/-}$ progenitors was severely impaired (**Figure 5**). Likewise, in vitro development of pDCs from *Bcl11a*$^{-/-}$ bone marrow progenitors treated with Flt3L was severely impaired; however, development of DCs from *Bcl11a*$^{-/-}$ progenitors in response to GM-CSF was normal.

Regulation of Irf8 by Runx1 and Cbfβ

DC development is also dependent on the transcription factor Runx1 and the core-binding factor subunit β (Cbfβ) (107), which is required for the action of Runx family factors (108). These factors appear to support expression of Irf8 (107). The Runx cofactor Cbfβ is essential for the development of Flt3$^+$ MDPs in the bone marrow and all DC subsets in the periphery. Deletion of Cbfβ in hematopoietic lineages causes a loss of DC progenitors and erythroid progenitors, with increased granulocyte-macrophage progenitors (GMPs) and a myeloproliferative disorder (107). This defect might be secondary to severe reduction in Irf8 expression in Cbfβ-deficient progenitors. Forced expression of Irf8 into Cbfβ-deficient progenitors restored DC differentiation (107). This suggests that Runx proteins and Cbfβ control the expression of Irf8, placing them earlier in the hierarchy of transcription network of DC development.

Development of the MDP from the CMP and the Exclusion of Granulocyte Potential

The origin and potential of the MDP is an issue of some debate. Several studies document the DC potential of the CMP (defined as cKit^{hi} CD34$^+$ CD16/32$^+$ Sca1$^-$) (81, 109) and GMP (cKit^{hi} CD34$^+$ CD16/32^{low} Sca1$^-$) (109, 110). Subsequently, the MDP (macrophage and dendritic cell progenitor) was defined as the CX3CR1$^+$ fraction of the GMP, which was described as retaining developmental potential for macrophages and DCs, but not granulocytes (86).

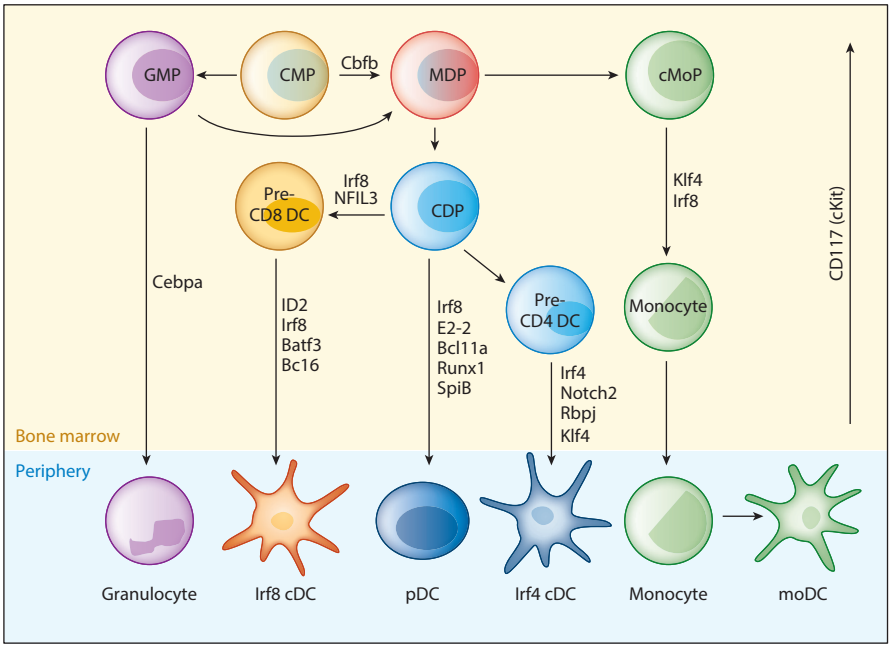


Figure 5

Stages and transcription factors for DC development. A scheme showing myeloid lineage development from the CMP, indicating transcription factors required for particular transitions between stages. Although unresolved, our scheme shows GMP and MDP divergence from the CMP. Commitment to Irf8 and Irf4 branches of cDCs can occur in the bone marrow. Relative level of cKit in progenitors is indicated by vertical position. Abbreviations: cDC, classical DC; CDP, common DC progenitor; cMoP, committed monocyte progenitor; CMP, common myeloid progenitor; DC, dendritic cell; GMP, granulocyte-macrophage progenitor; MDP, macrophage-DC precursor.

The close resemblance in the surface markers has led to suggestions that the MDP might develop from the GMP (87, 111) (**Table 1**). However, other groups have reported that the GMP has low DC developmental potential (112); specifically, the Flt3-negative fraction of the GMP has no potential for DC development (113). It is possible that the MDP develops directly from the CMP and the DC potential reported for the GMP earlier (109, 110) was conferred by cells that would now be defined as MDPs but could not be excluded from GMP at that time ($cKit^{hi}CD34^{+}CD16/32^{+}Sca\text{-}1^{-}$), given that CX3CR1 was not used. In essence, the GMP as initially defined included the MDP, and both may arise directly from the CMP. However, no studies have compared the transcriptional profiles of the MDP and CX3CR1-negative GMP fraction.

Initially, adoptive transfers indicated that MDPs generate DCs and monocytes but not granulocytes (86). In that study, in vitro single-cell analysis used GM-CSF to support differentiation and did not distinguish between DCs that could have come from monocytes instead of CDPs given that both are derived from MDPs. A follow-up study confirmed this indirectly. Adoptively transferred MDPs excluded granulocyte identity and instead developed into $CD11c^{+}$ DCs, pDCs, or $CD11c^{-}CD11b^{+}$ cells, presumed to be monocytes because they were $CX3CR1^{+}$ (87). Furthermore, the study in which the cMoP was identified was consistent with the understanding that the MDP, like the cMoP, lacks granulocyte potential in adoptive transfers (89). Exclusion of the granulocyte potential at the MDP stage was found to depend on Irf8, as MDPs from $Irf8^{-/-}$ mice retain potential to develop into neutrophils (114). This divergence between monocyte and

Table 1 Markers of lineage negative hematopoietic progenitors of mouse DCs[a]

	cKit CD117	CD34	FcgRII/III CD16/32	Flt3 CD135	MCSFR CD115	CX3CR1	Ly6C	Other markers	Reference
CMP	+	+	lo	n.u.	n.u.	n.u.	n.u.	n.u.	110, 181
GMP	+	+	+	n.u.	n.u.	n.u.	n.u.	n.u.	110, 181
MDP	+	(+)	(+)	n.u.	(+ PCR)	+	(–)Lin[b]	CD11b$^-$	86
MDP	+/–	(+)	(+/–)	(+) 75%	+	(+)	(–)Lin[b]	n.u.	94
MDP	+	n.u.	n.u.	+	+	+	n.u.	n.u.	115
MDP	+	n.u.	n.u.	+	+	n.u.	–	CD11b$^-$	89
MDP	+	n.u.	(+)	n.u.	(+/–)	+	n.u.	n.u.	88
cMoP	+	n.u.	n.u.	–	+	n.u.	+	CD11b$^-$	89
CDP	int/lo	(+)	n.u.	+	+	n.u.	(int/lo)	n.u.	82
CDP (pro-DC)	(int)	+	(+/–)	(+)	(+)	n.u.	–	CD43$^+$	90
Pre-DC	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	CD11c$^+$CD45RA^{lo} CD43^{int}CD172a^{int}	148
Pre-cDC	n.u.	n.u.	n.u.	+	n.u.	+	n.u.	CD11c$^+$sirpa^{int}	115
Pre-CD8 DC	int	n.u.	n.u.	+	(–)	n.u.	n.u.	CD11c$^+$MHCII^{int} Zbtb46$^+$CD24$^+$	104
Pre-CD8 DC	n.u.	n.u.	n.u.	+	n.u.	n.u.	–	CD11c$^+$MHCII$^-$ CD172a$^-$Siglec-H$^-$	150
Pre-CD4 DC	–	n.u.	n.u.	+	+	n.u.	n.u.	CD11c$^+$mMHCII$^-$	104
Pre-CD4 DC	n.u.	n.u.	n.u.	+	n.u.	n.u.	+	CD11c$^+$MHCII$^-$ CD172a$^-$Siglec-H$^-$	150

Abbreviations: cDC, classical DC; CDP, common DC progenitor; CMP, common myeloid progenitor; cMoP, committed monocyte progenitor; DC, dendritic cell; GMP, granulocyte-macrophage progenitor; MDP, macrophage-DC precursor; n. u., marker not used.

[a]Parentheses indicate that the marker was not used to define the subset but its expression was analyzed in the study.

[b]Ly6C or Gr1 was included in the lineage gate.

neutrophil potential is thought to involve antagonism between Irf8 and the transcription factor C/EBPα (114). In summary, findings from these three studies were in agreement about the MDP having DC and monocyte potential, but no granulocyte potential.

Other studies examined the DC potential of MDPs but did not examine their granulocyte potential. One study focusing on Flt3 requirements in DC development used a definition of the MDP as cKit^{hi}CD115$^+$Lin$^-$ but noted that this population was predominantly CX3CR1$^+$ (94). This study showed that the MDP as initially defined (86) was heterogeneous for expression of CD115, with the CD115$^+$ fraction of the MDP also being predominantly Flt3$^+$. Another study examining CD103$^+$ DC development showed that the MDP can give rise to DCs in the liver, spleen, and kidney (12). Finally, a study (115) defining the MDP as Lin$^-$Sca-1$^-$CD115$^+$Flt3$^+$CX3CR1$^+$ concluded that the MDP is not found in the bloodstream and that it develops from Lin$^-$Sca-1$^-$Flt3$^+$cKit^{hi}CX3CR1$^-$ myeloid progenitors, a population that likely represents the CMP. However, these three studies did not examine the granulocyte potential of MDPs (12, 94, 115).

Sathe et al. (88) challenged the existence of a progenitor with DC and monocyte but not granulocyte potential. Using MDPs as defined by Fogg et al. (86) and Waskow et al. (94), they

reported granulocyte potential upon adoptive transfer into lethally irradiated recipients, and in in vitro colony formation assays (88). The frequency of colonies with granulocyte morphology developing from the MDP increased in response to GM-CSFS to SCF, IL-3, and erythropoietin, compared to M-CSF. This suggests that previous in vitro studies may have underrepresented the granulocyte potential of MDPs. However, this would not explain the discrepancy with previous studies that used in vivo adoptive transfers. This study did not use the 2009 Liu et al. (115) MDP definition, which includes CD115 expression ($CX3CR1^+CD115^+Flt3^+$) (115)—a potentially important omission, given that $CD115^+$ progenitors within the $cKit^{hi}$ population were not identical to $Flt3^+$ populations in bone marrow (94). Such discrepancies warrant further detailed analysis of the MDP with optimal surface marker definitions as well as detailed analysis of granulocyte development in the studied populations.

Another difference is that MDPs were heavily skewed to monocyte production in previous in vitro studies, discordant with in vivo findings of Sathe et al. (88). Limiting dilution analysis found DC precursor frequency was 0.02%, compared to 6.4% for macrophages, whereas in vivo, 2.4% of cells developing from the MDP were macrophages and 14% were DCs. By contrast, the population with the greatest DC potential was the $Sca1^-cKit^{hi}CD16/32^{lo}CD115^+Flt3^+$ fraction, a $CD115^+$ fraction of CMP, consistent with potential for granulocytes, DCs, and macrophages.

Divergence of Monocytes and the Common DC Progenitor from the Macrophage-DC Precursor

The MDP diverges into the CDP (82, 90) and the monocyte lineage, which can also generate DCs under some conditions (116). In 2013, Hettinger et al. (89) identified the cMoP, which is clonogenic and arises from the MDP. The cMoP is undergoing cell division and can be identified as $Ly6C^+Flt3^-CD115^+CD117^{hi}$. It can be found in bone marrow and the spleen. The cMoP gives rise to the major monocyte subsets and to macrophages, but not to DCs or neutrophils. The transcriptional basis for this commitment process has not been reported, and the basis for this divergence has not been defined.

In response to signaling by GM-CSF and IL-4, monocytes can generate monocyte-derived DCs (moDCs), which have characteristics of cDCs (116). DC-SIGN was reported as a marker for moDCs both in vitro and in vivo (117). These $DC\text{-}SIGN^+$ moDCs were able to cross-present cell-associated antigens, as previously shown (118) for moDCs. However, moDCs identified by DC-SIGN expression were also dependent on Flt3L (119), suggesting a potential in vivo origin from CDPs. Further, it was recently reported (in 2015) that moDCs derived in vitro by a "widely used protocol" that uses only GM-CSF are heterogeneous, consisting of at least two populations that resemble macrophages or DCs (120). At present, it is not possible to distinguish clearly which DCs are derived from monocytes rather than CDPs in vivo. This finding (120) calls into question a large amount of literature in the area of vaccine design and immune therapy that was based on GM-CSF–derived DCs. A systematic reevaluation of cDCs in place of GM-CSF–derived DCs may be in order in this area.

The Role of GM-CSF in DC Development and Homeostasis

One study has reported that GM-CSF signaling is required for development of Batf3-dependent DCs (121), whereas other studies have suggested that GM-CSF simply regulates CD103 expression on these DCs and not their development (122, 123). CD103 may be induced by GM-CSF, IL-3, and TGF-β (38, 40, 123, 124). Batf3-dependent cDCs with a $CD24^+CD11b^-EPCAM^{lo}$ phenotype are present in all organs of *Csf2rb*$^{-/-}$ mice, but they have lower than wild-type

expression of CD103 (123). GM-CSF treatment induced CD103 expression on CD24$^+$Sirpα^- cDCs derived from Flt3l bone marrow cultures. Although CD103 expression is often used to identify Batf3-dependent Irf8^{hi} cDC lineage in peripheral organs (42), it is not a universal marker of this lineage; Batf3-independent Irf4$^+$ intestinal cDCs also express CD103 (96). Nonetheless, combined GM-CSF and Flt3l treatment strongly enhances the in vitro outgrowth of Batf3-dependent CD103$^+$ cDCs (125), although the molecular basis of this effect was not determined.

TRANSCRIPTIONAL CONTROL OF DC DIVERSIFICATION FROM THE COMMON DC PROGENITOR

The CDP is thought to generate at least four distinct subsets of DCs: pDCs, Irf8$^+$ Batf3-dependent cDCs, and two types of Irf4$^+$ cDCs. The divergence of these various lineages involves a number of transcription factors, including Id2, Nfil3, E2.2, Irf8, Irf4, Zbtb46, Klf4, and Notch2 (**Figure 5**).

Plasmacytoid DC Divergence from Classical DCs at the Common DC Progenitor

Most models of DC development depict pDCs as diverging from cDCs at the CDP stage of development (126), with E2-2 expression favoring pDC development and Id2 expression favoring cDC development. The basic helix-loop-helix (bHLH) transcription factor E2-2 is required for pDC development in mice and humans (127). E2-2 and other bHLH factors, including E12, E47, and HEB, can form homodimers or heterodimers and bind the E-box DNA motif (128). E2-2 controls expression of *Bcl11a* and *Irf8* (129, 130), which support pDC development, and other pDC-specific genes, such as *Tlr7*, *Tlr9*, and *Bdca2* (127, 130). Id2 antagonizes E2-2 activity by forming inactive heterodimers with E2-2 (130), such that competition between E2-2 and Id2 at the CDP stage has been proposed as the basis for pDC/cDC divergence.

However, the basis for lineage divergence between pDCs and cDCs is not fully explained at present. Competition between E2-2 and Id2 can account only for a divergence between pDCs and Irf8$^+$ cDCs; it does not account for the divergence of pDCs from Irf4$^+$ cDCs. Id2 deficiency eliminates development of LCs and Irf8$^+$CD8α^+ cDCs but leaves Irf4$^+$ cDC lineages intact (14), even though they eventually express Id2. Further, E2-2 is first expressed in CDPs, whereas Id2 does not increase until the pre-cDC stage (131). The basis for E2-2 expression in DC progenitors, and loss of E2-2 in developing cDCs, is not understood.

Although a clonogenic pDC progenitor has not been identified, progress has been made (132, 133). A population of CCR9$^-$ pDCs in bone marrow that express E2-2 are the immediate precursors of CCR9$^+$ fully differentiated pDCs, but this precursor retains plasticity to develop into the Irf4$^+$ cDC—for example, in response to GM-CSF (132). This diversion to Irf4$^+$ cDCs is accompanied by decreased E2-2 expression and increased Id2, PU.1, and Batf3. And although the MDP and CDP are defined as CD115$^+$, a progenitor that expresses E2-2 and has abundant pDC potential is contained in the CD115$^-$ fraction of a cKit$^{int/lo}$ Flt3$^+$ population of bone marrow cells (133).

Expression of Irf8 and Irf4 Distinguishes Major cDC Branches

The distinct requirements for Irf8 and Irf4 in cDC development were initially unclear. These branches of cDCs have mutually exclusive expression of Irf8 and Irf4. Originally, the absence of CD4$^+$ cDCs in *Irf4*$^{-/-}$ and *Irf4*$^{-/-}$*Irf8*$^{-/-}$ double-deficient mice (134, 135) was interpreted as a requirement for Irf4 for the development of this entire branch of cDCs (136). However, these initial studies did not use CD24 and Sirpα, which now reliably distinguish between Irf8$^+$

and Irf4$^+$ cDCs. *Irf4*$^{-/-}$ and *Irf4*$^{-/-}$*Irf8*$^{-/-}$ double-deficient mice did retain CD4$^-$CD8α^- cDCs (134, 135), subsequently identified as CD8α^-CD11b$^+$ cDCs, but these exhibited defective migration due to impaired induction of CCR7 (52). Also, *Irf4*$^{-/-}$ mice retain peripheral lamina propria CD103$^+$CD11b$^+$ cDCs, which express Irf4 (19). These cells produce IL-23 in response to *Citrobacter rodentium* infection, in contrast to *Notch2*$^{-/-}$ CD11b$^+$ cDCs, which do not (19). In summary, whereas Irf8 appears to be required for the development of pDCs and CD8α^+ cDCs, it now seems that Irf4 is not strictly required for development of the Irf4$^+$ cDC branch; rather, it seems to be required for some functions of these cDCs.

Zbtb46 Identifies Progenitors Committed to cDC Lineages

The transcription factor Zbtb46 (BTBD4), a Broad Complex, Tramtrack, Bric-a-Brac, and Zinc Finger (BTB-ZF) family member, is selectively expressed by cDC lineages (119, 137). It is also expressed by some vascular endothelium, and at low levels in the megakaryocyte-erythroid progenitor (MEP) (137). Zbtb46 is expressed in both Irf8$^+$ and Irf4$^+$ branches of cDCs in lymphoid and peripheral tissues. During DC development, Zbtb46 is first expressed in a fraction of cells defined as pre-cDCs (138), which could be fractionated by Siglec-H and Zbtb46-GFP expression (137). Zbtb46-GFP$^+$ cells in the pre-cDC fraction, including those expressing Siglec-H, were strongly restricted to cDC potential, and largely excluded pDCs (137). This suggested that Zbtb46-GFP expression is a marker for cDC commitment. Further, it showed that Siglec-H is not a strict marker of pDC commitment. This helps to explain the previously discussed discrepancies that resulted from using Siglec-H to express DTR in an attempt to deplete pDCs (70).

Zbtb46 is not necessary for cDC development (137, 139). Enforced *Zbtb46* expression into bone marrow progenitors caused some increase in cDC development away from neutrophils (137), and loss of *Zbtb46* increased expression of MHC-II proteins (139). A minor increase in *Csf3r* and *Lifr* was also seen in *Zbtb46*$^{-/-}$ cDCs, although the impact of these changes was unclear.

Development of Irf8$^+$ cDCs Selectively Requires Nfil3, Id2, and Batf3

A reporter line expressing GFP from an *Irf8* locus transgene identified a role for PU.1 in initiating Irf8 expression at an early bone marrow progenitor similar to the MDP (103). PU.1 appeared to initiate *Irf8* expression from an enhancer located approximately 50 kb upstream of *Irf8*. A separate analysis of a knock-in Irf8-EGFP fusion protein showed a progressive increase in development of HSCs into GMPs and CLPs but not MEPs (140). Loss of Irf8 diverted development at the GMP toward neutrophils, indicating that Irf8 acts to exclude granulocyte potential (103), consistent with a previous report that *Irf8*$^{-/-}$ progenitors produced more neutrophils and fewer CDPs and mature DCs than wild-type progenitors (113).

Nfil3 (E4BP4) is a transcriptional repressor required for CD8α^+ DC development (13). Batf3 expression was reduced in *Nfil3*$^{-/-}$ DC progenitors, suggesting that Nfil3 acts upstream of Batf3 during DC development. Consistently, retroviral expression of *Batf3* into *Nfil3*$^{-/-}$ progenitors rescued development of Sirpα^- CD24$^+$DEC205$^+$ DCs. Nfil3 also acts in the development of all innate lymphoid cell subsets (141–143), where it has been thought to regulate eomesodermin and Id2. The mechanism of Nfil3 regulation of Id2 expression in developing DCs is unclear.

Id2 is also required selectively for Irf8$^+$ cDCs (14). The lack of a DNA-binding domain in Id proteins causes heterodimers formed with E proteins to be inactive (14, 15, 124, 144, 145). E2-2 is required for pDC development (127), consistent with Id2 overexpression blocking in vitro pDC development (15) and *Id2*$^{-/-}$ mice having increased pDCs (14). Examination of an Id2-GFP reporter line suggested an ordered action of Irf8, Id2, and Batf3 in CD8α^+ DC development

(124). Id2 expression in a pre-cDC population (138) could prevent E2-2 activity to stabilize cDC commitment, but there is no explanation for its selective requirement in development of Irf8$^+$ cDCs but not Irf4$^+$ cDCs.

Batf3 Maintains Irf8 Autoactivation of a Clonogenic Pre-CD8 DC Progenitor

Like Nfil3 and Id2, Batf3 is selectively required for the development of the Irf8$^+$ branch, but not the Irf4$^+$ branch, of cDCs (7, 11). The basis for this action was recently (in 2015) reported (104). Batf, Batf2, and Batf3 all have a leucine zipper domain that is able to heterodimerize with Jun factors and also interact with Irf factors through leucine zipper residues that face away from the Jun partner (146). However, both Batf and Batf3 can interact with both Irf8 and Irf4 (147), so it seems unlikely that the selective requirement for Batf3 in Irf8$^+$ cDC development relies on selective interactions with Irf8 rather than Irf4. However, Batf3 must interact with Irf8 to support Irf8$^+$ cDC development (147).

Late progenitors of cDCs include the pre-cDCs (115, 148) and CCR9$^-$ immature pDCs (132, 149). Recently (in 2015), clonogenic progenitors committed to either Irf8$^+$ cDCs or Irf4$^+$ cDCs were identified and called pre-CD8 DCs (or pre-DC1s) and pre-CD4 DCs (or pre-DC2s), respectively (104, 150). Both of these progenitors could be identified by expression of Zbtb46-GFP but were distinguishable on the basis of other markers, including cKit expression levels (104). Notably, the pre-CD8 and pre-CD4 DCs could be identified in the bone marrow, suggesting that developmental divergence can occur earlier than previously thought.

Direct examination of the pre-CD8 DC revealed how Batf3 functions in Irf8$^+$ cDC development (104). Specification of the pre-CD8 DC could occur in *Batf3*$^{-/-}$ mice, but not *Irf8*$^{-/-}$ mice. Gene profiling of the committed pre-CD8 DC progenitor cell showed increased expression of Id2, Batf3, and CIITA and loss of Zeb2 compared with the CDP. By contrast, expression of these factors in the pre-CD4 DC progenitor did not increase compared with the CDP. Notably, both progenitors continued to express Irf8, already expressed by the CDP through a pathway of transcriptional autoactivation involving Irf8 and PU.1 (104). In contrast to the pre-CD8 DC progenitor, pre-CD4 DC progenitors initially expressed Irf8, but Irf8 expression was progressively reduced while Irf4, Id2, and Batf3 expression increased. The *Batf3*$^{-/-}$ pre-CD8 DC progenitor also began its course with abundant expression of Irf8, but this gradually ended. Thus, it appears that a major action of Batf3 is to maintain Irf8 autoactivation following specification of Irf8$^+$ cDC fate. But why the requirements of Irf8 autoactivation change between the CDP stage and the specified cDC stage is still unclear.

ChIP-Seq and functional analysis identified an enhancer region that binds Irf8 and Batf3 that was active in Irf8$^+$ cDCs but not pDCs or Irf4$^+$ cDCs (104). This region contained potential AP1-IRF composite elements that may cooperate for Irf8-Batf3 binding. However, the event that triggers specification is uncertain. Also, how the pre-CD4 DC progenitor is specified and committed, and how pDCs and the pre-CD4 DC progenitor diverge, is unclear. Perhaps the delay in Batf3 and Id2 expression, coupled with low E2-2 expression, allows for Irf8 transcriptional decay.

Compensation for Batf3 by Batf and Batf2 in Irf8$^+$ cDC Development

Normally it is Batf3 that supports Irf8$^+$ DC development, but compensation by Batf and Batf2 can occur in Batf3$^{-/-}$ mice that are infected with intracellular pathogens such as *Mycobacterium tuberculosis* or that are treated with IL-12 (147). The IL-12-driven compensation requires generation of IFN-γ in vivo and appears to be due to induction of Batf and/or Batf2, which may act in place of Batf3 in stabilizing Irf8 expression during cDC development. The action of these factors relies on the shared ability of the leucine zipper regions to mediate interactions with Irf8 (147), and so it

presumably represents compensation in the autoactivation of Irf8, described above. Conceivably, this pathway is of use in expanding the numbers of Irf8$^+$ cDCs during infections. It is not known at what progenitor stage Batf and Batf2 might be induced, but it is possible either one would be able to rescue Irf8 expression in the pre-cDC stage.

Compensation for Batf3 may be at work in settings other than infection (151, 152). In adoptive transfer of bone marrow into lethally irradiated mice, CD8α^+ DC development was seen as independent of Nfil3, Id2, and Batf3 (152). Likewise, development of Clec9a$^+$ cDCs was Batf3-dependent in vitro, as expected, but not when transferred into sublethally irradiated humanized mice (151). Batf3 expression can compensate for the loss of Nfil3 in CD8α^+ DC development (13). Perhaps irradiation acts in the same way as infection and IL-12 to induce Batf or Batf2 and compensate for the lack of Batf3 and Nfil3 in double-deficient cells to promote CD8α^+ DC development.

Irf4$^+$ cDCs Are Heterogeneous

Extensive heterogeneity of CD8α^- DCs is reported in the literature by studies using surface markers (19–21, 49) or transcriptional profiling of single cells (153). However, functional studies based on lineage ablation approaches have so far identified only two functional subsets of Irf4$^+$ cDCs, defined as sensitive to loss of Notch2 and Rbp-J or to loss of Klf4. In both cases, only a subset of Irf4$^+$ cDCs is affected, suggesting these factors may act later in development than the Irf8/Irf4 divergence. Immature Irf4$^+$ cDCs appear to encounter Notch ligand Delta-like (DL) 1 expressed by stromal cells, including fibroblasts, in the spleen or lymph nodes (154), which appears required for induction of CD4 and ESAM. This signaling event may enable Irf4$^+$ cDC to experience lymphotoxin, which supports Irf4$^+$ cDC homeostasis (155). But Irf4$^+$ cDCs that remain in *Notch2*$^{-/-}$ mice were also affected, although no functional deficits were identified. The basis for selective expression of IL-23 by this subset in response to certain pathogens remains unexplained. Irf4 reportedly inhibits IL-12 production by DCs (156), and Stat3 activation may also block CDK9/P-TEFb recruitment to the p35 promoter (157). So conceivably, such differences between Irf8$^+$ and Irf4$^+$ cells may change the relative balance of p19 and p35 production, favoring IL-23 in Irf4$^+$ DCs.

Klf4 can act as a repressor or activator of transcription and regulates development in several epithelial tissues, including skin, lung, and intestine (158–165). Among hematopoietic cells, Klf4 is expressed in myeloid cells and is required for monocyte development (162, 163, 166) as well as for in vitro M2 macrophage polarization (65, 162, 166, 167).

In mice, Klf4 is required for the emergence of particular fractions of Irf4$^+$ cDCs (20) that appear necessary for Th2 response to several stimuli. Klf4 is highly expressed in pre-cDCs and is expressed at lower levels in mature splenic cDCs. Early hematopoietic deletion of Klf4 reduced the expression of Irf4 in pre-cDCs but still allowed for divergence of CDPs into Irf8$^+$ and Irf4$^+$ branches (20). Deletion of Klf4 affected DC development by largely eliminating the Siglec-H$^-$ fraction of pre-cDCs, which is predominantly a CD115$^+$Flt3$^+$ committed pre–CD4 DC (104, 150). This seems to imply that the Irf4$^+$ cDCs that develop in the absence of Klf4 arise from the pDC-skewed population of Siglec-H$^+$ CD115$^-$ progenitors (133). Despite the requirement for Klf4 on a fraction of Irf4$^+$ cDCs, the transcriptional mechanism of action remains to be defined.

Development of Human DC Progenitors

Although analysis of human DC development has been more difficult compared to analysis of mouse DC development, recent studies (2015) have made significant progress toward this goal

(168, 169). A culture system for human DCs was developed based on the use of a stromal cell line combined with cytokines Flt3L, GM-CSF, and stem cell factor (SCF). These studies identified human equivalents of MDPs, CDPs, and a pre-cDC equivalent. The human GMP previously identified (170) was found to be heterogeneous and divisible into five major populations based on differential expression of GM-CSR, CD115, and IL-3R. An IL-3R^{hi} fraction of the GMP was identified as the human CDP equivalent. The other four GMP fractions consist of all combinations of GM-CSFR and CD115 expression within the IL-3R^{int} GMPs. A developmental potential equivalent to the MDP was found in the CD115$^-$GM-CSFR$^+$ fraction of the GMP (169). A pre-cDC equivalent was found in the CD115$^-$Flt3$^+$GM-CSFR$^+$CD45RA$^+$ fraction of Lin$^-$cKit^{hi} progenitors. The transcription factors associated with these progenitors were not defined (168).

cDC Homeostasis Is Regulated In Vivo

A 2014 report identified a unique transcriptional basis by which cDCs appear to control their proliferation and homeostasis (171). Mature DCs exhibit substantial proliferative activity (138, 155). The transcription factors c-Myc and N-Myc regulate cell growth (172). c-Myc regulates proliferation and metabolism (173) and increases transcription by interactions with RNA polymerases (174–176). Mature DCs lacked expression of c-Myc and N-Myc. The third paralog, L-Myc, had weaker effects on transformation (177); it was efficient in reprogramming fibroblasts into induced pluripotent stem cells (178) but dispensable for normal embryonic development (179). L-Myc expression is restricted to DCs and is regulated by Irf8. L-Myc expression first appears at the CDP stage concomitant to c-Myc reduction (171).

All DC subsets developed in *Mycl1*$^{-/-}$ mice. However, certain DC subsets, including migratory CD103$^+$ cDCs in the lung and liver, were significantly reduced. L-Myc is required in DCs for optimal in vivo T cell priming during *Listeria monocytogenes* and vesicular stomatitis virus infections. This implies that Myc transcriptional activity during infections may be provided first by c-Myc and later replaced by L-Myc in immature DCs (180).

CONCLUSIONS

Major questions remain regarding how DC subsets manifest their distinct effector functions at the molecular and genetic levels. For example, how do Irf8$^+$ cDCs and Notch-2-dependent Irf4$^+$ DCs regulate the selective production of IL-12 and IL-23? No mechanism explaining how the Klf4-dependent program within Irf4$^+$ cDCs operates in Th2 but not Th17 responses is known. Although a mechanism for Batf3 functioning in Irf8$^+$ cDC commitment has been uncovered, it reveals another problem: How is this lineage specified, and does Batf3 have other functions within the mature Irf8$^+$ cDC lineage besides maintaining Irf8 itself? It will also be important to test whether the corresponding human DC subsets operate along similar functional modules as DCs in the mouse, and whether the transcriptional basis for human DC diversification is conserved. Now that major differences between GM-CSF-derived DCs and cDC subsets developing in vivo are evident, it is important to reevaluate the potential for manipulating classical DCs as therapeutic vehicles or targets.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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Errata

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