Transcription Factor Networks in Dendritic Cell Development

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Abstract

Dendritic cells (DCs) are a heterogeneous population within the mononuclear phagocyte system (MPS) that derive from bone marrow precursors. Commitment and specification of hematopoietic progenitors to the DC lineage is critical for the proper induction of both immunity and tolerance. This review summarizes the important cytokines and transcription factors required for differentiation of the DC lineage as well as further diversification into specific DC subsets. We highlight recent advances in the characterization of immediate DC precursors arising from the common myeloid progenitor (CMP). Particular emphasis is placed on the corresponding temporal expression of relevant factors involved in regulating developmental options.

Keywords

Dendritic cell; Lineage commitment; Transcription factor; Flt3-ligand; Common dendritic progenitor

1. Introduction

The immune system comprises a large number of highly differentiated cell types whose functions emerge from coordinated interactions among developmentally heterogeneous cells, and are rarely observed in complete isolation. For example, phagocytic cells such as neutrophils or macrophages best exert their function through interactions with soluble components of the immune system such as antibodies secreted by B cells, or in their absence, mannose-binding lectins or deposition of complement protein C3. Perhaps the most elegant example of coordinated immunity is the generation of high affinity, isotype-switched antibodies capable of neutralizing a pathogen. Such a response requires not only the cell-intrinsic capacity of the B cell, but also linked recognition and activity of a differentiated form of the CD4^{+} T cell, the T follicular helper cell (Tfh), as well as contributions to T cell

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priming produced by cells of the phagocyte system called dendritic cells (DC). This three way interaction represents a highly organized system with each cell presenting checkpoints and barriers to improper activation as a means of guarding against autoimmunity. While the molecular mechanisms responsible for the development and diversification of T and B cells are well studied, those responsible for DCs are only beginning to be defined. This review will provide a synopsis of the known cellular and molecular events required for the development of DCs at steady-state.

2. Unique functions and subsets of DC lineages

DCs were discovered nearly 40 years ago by Steinman and Cohn, who identified a "large stellate cell" population among adherent splenocytes on particular glass and plastic surfaces [1]. Beginning with a strictly descriptive analysis, these cells were defined as a distinct lineage, separate from B and T cells as well as granulocytes and macrophages, on the basis of several criteria. Initially, their "dendritic" morphology and clear phagocytic capacity set them apart from lymphocytes. However, it was their profound capacity for stimulating T cells in a mixed lymphocyte reaction (MLR) that prompted the notion that these cells could play a unique role in directing adaptive immunity [2]. Additional hallmarks emerged from flow cytometric analyses that identified a distinct set of cell-surface markers separating DCs from macrophages and other myeloid lineages. Subsequent studies have demonstrated that, in addition to stimulating the immune response, DCs also possess a capacity to impose a tolerogenic state on T cells under appropriate conditions [3;4].

The first two decades of work on DCs largely considered this cell a homogenous population. In 1992, the first clear-cut example of subset differentiation within DCs was put forth, based upon the expression of the murine CD8-alpha marker. Clearly distinct from developing or recirculating thymic T cells, this subset represented a relatively small population within the thymus, and was thought to derive from an intrathymic lymphoid-restricted precursor rather than from bone marrow precursors [5;6]. As a byproduct of differences in identification and separation methods, the demarcation of various subsets has since been somewhat inconsistent [7]. Currently, the field considers four major subsets of lymphoid-resident DCs. In addition to the CD8-alpha positive DC, two other populations of conventional DCs (cDC) are defined by the presence or absence of the cell-surface molecule CD4 [8]. These two populations show a high degree of similarity with respect to function, development, and gene expression, and for simplicity will be grouped together and further referred to as CD4^{+} cDCs in this review [9]. A fourth population of plasmacytoid DCs (pDC) is defined by the expression of several distinguishing markers including B220, Siglec-H, and Bst2 [10-12]. Human counterparts for each of these DC subtypes have been identified, underscoring the conservation of DC lineage diversification across species, presumably owing to their important functional specialization [13-15]. As discussed briefly below, monocyte-derived cells sharing many features with DCs arise under inflammatory rather than steady-state conditions, although little is currently known regarding the transcriptional and molecular basis of their differentiation [16;17].

All subsets express high levels of the complement receptor CD11c, and also display a variety of surface receptors endowing them with the properties of growth factor

responsiveness, efficient phagocytosis, and antigen presentation. Figure 1 outlines the expression of some of these key surface proteins as DC subsets differentiate from hematopoietic precursors (discussed further below). While they are convenient markers in the murine system, there is no known function of the CD4 and CD8-alpha molecules in the execution of specific functional processes. In addition, these two markers are among the few that do not show conservation with subsets of DCs in the human system.

Beyond differences in cell-surface receptors as the basis for defining distinct subsets, non-overlapping functions for these populations are beginning to emerge. For example, pDCs appear to be a major source of type I interferon during viral infections. Recent models in which pDCs are selectively eliminated highlight their contributions in some, but not all, settings of infections by pathogens [18;19]. The cDC subsets appear to be primarily concerned with priming adaptive immune responses, particularly those of T cells, although important interactions with other immune cells, such as B cells and NK cells, have also been described [20-22].

Several years after their initial identification, a study by den Haan and colleagues indicated that one potentially unique function of the CD8^{+} DC is its capacity to cross-present exogenous antigens on MHC class I [23]. As a result, it was hypothesized that these cells are uniquely capable of cross-priming CD8^{+} T cells, a process first described in 1976 and now implicated as a critical response in viral and anti-tumor immunity [24-26]. By virtue of this unique function, these cells could provide an efficient mechanism to induce CD8^{+} T cells against pathogens that fail to infect DCs directly, and therefore evade classical mechanisms of inducing CTL (cytotoxic T lymphocyte) responses. In support of this role, CD8^{+} DCs are now recognized to reside in the T cell zones of the spleen, and are thought to traffic from areas of pathogen trapping into areas of T cell presentation [27;28]. Other studies have also proposed that these cells may provide functions independent of cross-presentation, such as the production of essential cytokines in response to various pathogens [29;30].

CD4^{+} cDCs for the most part have been considered poor cross-presenters in comparison to their CD8^{+} counterparts. These cells are largely considered to activate CD4^{+} T cells, rather than having any specialized role in the induction of CTLs [31]. However, the classical paradigm of functionally segregating cDC subsets based on the efficiency and quality of MHC class I and II presentation is not without controversy. For example, a recent study suggests that the distinction between CD8^{+} and CD4^{+} DCs may instead lie in the inability of the former to process antigens for MHC class II efficiently; and when controlled for antigen dose and delivery, both subsets are capable of efficient cross-presentation [32]. In fact, under certain inflammatory conditions, the monocyte-derived DC can present exogenous antigens more efficiently than either of the lymphoid-tissue resident cDCs [33;34]. Collectively, these seemingly conflicting observations may be explained by the type of antigen and the context in which it is being studied. Clearly, additional studies in which DC subsets are selectively ablated are required to substantiate the various competing claims.

3. Accurately defining the DC lineage

As discussed above, the criteria to mark the DC lineage has relied on a constantly evolving rubric, beginning with the purely descriptive analyses of Steinman and colleagues in the 1970's to a more functional and phenotypic characterization at present. Yet such seeming clarity has not resolved the topic of the exact contribution of DCs during an immune response, in part because of the shared expression of many surface markers between DCs and other myeloid cells. In fact, some of the seminal studies over the last decade that relied on genetic or antibody-based depletion of specific myeloid populations also affected several other cell types [35]. For instance, two independent reports have claimed that basophils, and not DCs, are critical for mediating T helper type 2 responses (Th2) [36;37]. However, these reports were disputed by another group who demonstrated that the antibody-based depletion of basophils in the previous studies also removed a key DC population, which actually appears to be necessary for initiating Th2 immunity [38]. More recently, a report claiming the importance of beta-catenin signaling in intestinal DCs to gut tolerance was based upon the selective deletion of this molecule in only DCs, a specious claim given there was also complete deletion in a sizeable fraction of macrophages [39]. These ambiguous results are the byproduct of a strict reliance on cell-surface markers to segregate myeloid populations. Instead, when considering any lineage, especially one as heterogeneous and plastic as DCs, more rigorous criteria need to be applied for its functional characterization in various contexts. Some authors have suggested defining DCs based on their anatomical location, origin from dedicated precursors, and antigen presenting properties in addition to phenotypic markers [40]. However, all of these measures can be inconsistent depending on the physiological context or method of measurement. Therefore, we suggest that the most succinct and reliable definition of the DC lineage is based on the expression of key lineage-specifying transcription factors which are unequivocally required for their development in all settings. Parsing these transcriptional networks will help resolve developmental and functional controversies that currently exist within the field.

4. The developmental origin of DCs

An issue with particular relevance to the transcriptional basis of DC development is their derivation from bone marrow progenitors. It is now clearly established that DCs are short lived and are continuously repopulated in the periphery, both within lymphoid and non-lymphoid tissues, from progenitors that arise in the bone marrow [41;42]. For some period of time, there was confusion about the origin of DCs, based primarily on the initial classification of immune lineages as broadly derived either from a common lymphoid-restricted progenitor (CLP), or from a common myeloid-restricted progenitor (CMP) [43;44]. Whereas other cells of the immune system, such as T cells, B cells or neutrophils, are derived strictly from the CLP or from the CMP, it is now clear that all DC subsets can be derived from either population [45-47]. This unexpected finding overturned the existing paradigm that CD8^{+} DCs were of lymphoid while CD4^{+} DCs were of myeloid origin [5;48]. Despite the ability of CLPs to generate DCs upon adoptive transfer into irradiated hosts, the mechanisms and cell intermediates through which this occurs is unclear. Moreover, recent lineage tracing studies by Schlenner *et al.* indicate that the contribution of this pathway to

the steady-state DC compartment is minimal [49]. Therefore, our focus will be on the CMP-derived pathway of DC development.

Fogg and colleagues identified the first precursor downstream of the CMP that still retained DC potential, termed the macrophage-dendritic cell precursor (MDP) [50]. As its name would indicate, the MDP strictly has the potential to generate macrophages and DCs whereas alternate myeloid lineages proceed through the granulocyte-macrophage precursor (GMP) [44]. Soon thereafter, two groups reported the identification of a purely DC-restricted bone marrow progenitor called the common dendritic cell progenitor (CDP), which is efficiently able to generate all DC subsets at a clonal level [51;52]. The CDP was shown to originate from the MDP following the loss of monocyte lineage potential [53]. Similar to the MDP, the CDP expresses relatively high levels of macrophage colony-stimulating factor 1 receptor (M-CSFR) and FMS-like tyrosine kinase 3-ligand receptor (Flt3), but lower levels of the stem cell factor receptor (c-Kit). Presently it is thought that both the MDP and the CDP derive exclusively from the CMP *in vivo*, strengthening the argument that DCs are minimally derived from lymphoid precursors. Finally, the terminal steps in diversification of DC subsets appear to begin at the CDP stage. As outlined in Figure 2, CDPs seem to be the immediate precursor of both pre-pDCs and pre-cDCs, which are cells restricted to their respective lineages but not yet fully mature [54;55]. pDCs complete their maturation in the bone marrow and circulate through the blood to secondary lymphoid organs. In contrast, pre-cDCs seed lymphoid and non-lymphoid tissues via the blood where-upon they complete their differentiation into either the $CD8^+$ or $CD4^+$ cDC subsets.

5. Extracellular cues supporting DC development

A number of extracellular cues induce the progression through the progenitor stages described above, mainly by activating several cytokine receptors. The major cytokine receptors influencing DC development are M-CSFR, granulocyte-macrophage colony-stimulating factor receptor (GM-CSFR) and Flt3, which are all expressed variably on early hematopoietic precursors but remain highly expressed on committed DC progenitors (Figure 1).

The importance of these cytokines in the differentiation of DCs was first established by early studies demonstrating the ability of GM-CSF, either alone or in combination with IL-4, to drive the formation of DCs from mouse bone marrow or human peripheral monocytes [48;56]. The unique potency of these cells for antigen presentation, combined with their expression of CD11c and MHC class II, confirmed their identity as *bona fide* DCs and provided a widely used model system for *in vitro* studies. Therefore, it was surprising when both GM-CSF- and GM-CSFR-deficient mice showed a largely unperturbed DC compartment, leading to the conclusions that GM-CSF is dispensable or redundant in steady-state DC development [57;58]. The recent identification of TNF-alpha- and iNOS-producing DCs under inflammatory conditions, and their dependence on GM-CSF, points to the relevance of this cytokine in infectious settings and likely explains its ability to induce DC development *in vitro* [32;59;60].

Nonetheless, these early results suggested that other cytokines were likely responsible for the steady-state development of DCs *in vivo*. In 1996, McKenna and colleagues reported that the administration of exogenous Flt3-ligand (Flt3L) potently induces the expansion of all DC subsets *in vivo* [61]. This cytokine was then also shown to support the development of all subsets *in vitro* [62]. Moreover, forced expression of Flt3 (receptor for Flt3L), or its downstream effector molecule STAT3, in progenitors committed to alternative lineages can re-direct their development into DCs [63]. The requirement for Flt3L *in vivo* was confirmed by the greatly reduced numbers of DCs in Flt3L-deficient mice and Flt3-deficient mice [64;65]. Interestingly, the absence of Ftl3L presented with a stronger DC phenotype than the absence of Flt3, suggesting that the ligand may interact with a hitherto undefined receptor. These findings have been reinforced by subsequent studies on STAT3-null mice, which appear to phenocopy Flt3L deficiency [66]. Recent evidence suggests that the activation of STAT3 is mediated by mTOR (mammalian target of rapamycin) signaling, and accordingly, chemical inhibition of mTOR with rapamycin perturbs the development of DCs *in vitro* [67].

While Flt3L appears to be the dominant cytokine controlling steady state DC development, GMCSF may contribute, as mice deficient in both Flt3L and GM-CSF have lower numbers of DCs than either single cytokine deficiency alone [57]. The precise degree of redundancy is still a matter of ongoing work. Much in the same manner as differential localization within the bone marrow has been proposed to regulate B cell development through various stages by distinct actions of cytokines and cell-surface proteins on stroma, a similar combinatorial regulation of DCs has recently been proposed [68;69]. First, as noted above, there is a compound defect in mice deficient in both Flt3L and GM-CSF, suggesting contributions of both cytokines to DC development. In addition, different cytokines appear to have varying effects on the development of specific DC subsets. For instance, GM-CSF favors the development of cDCs while inhibiting the development of pDCs, through a mechanism dependent upon activation of STAT5 [70]. In contrast, culturing bone marrow cells in M-CSF in combination with Flt3L has the opposite effect, favoring pDC development [51]. It is conceivable that specific stromal niches within the bone marrow could support distinct DC developmental outcomes through differential cytokine production. Indirect evidence supporting this model comes from clonal studies of the CDP, which noted that some cells within the population already appear to be biased toward either pDCs or cDCs [52]. The generation of unique, non-overlapping reporter systems for each of the abovementioned cytokines will greatly aid in the resolution of this outstanding question of niche-dependent effects.

6. Transcription factors control DC commitment and specification

Generally, cell fate specification occurs through the actions of transcription factors which may be induced or inhibited by initiating extracellular cues. Recently, a number of transcription factors have been identified that control commitment, specification, and survival of DCs. In this review, we will group these factors into two major categories. The first category consists of transcription factors that are required for the development of early DC progenitors, which for the most part influence all DC subsets. As one might expect with factors influencing such early decisions, the loss of these genes often affects a number of

additional immune lineages besides DCs. The second category is comprised of more lineage-restricted transcription factors, which regulate later stages of DC fate decisions. Deficiency of these factors usually results in more specific defects within the DC lineage. It is worth keeping in mind that the expression of some factors from the first category is not extinguished as the steps of commitment and specification unfold, and therefore may influence both progenitors and mature cell types in distinct ways. Importantly, unique developmental outcomes may emerge from transcriptional programs initiated by broadly expressed factors of the first category through the induction and then interaction with factors from the second.

6.1 Factors regulating early DC progenitor development

The landmark discovery of the indispensable role of the zinc finger transcription factor Ikaros in the development of all lymphoid lineages also represents the initial identification of a factor necessary for DC development. Specifically, Ikaros-null mice lack pDCs and CD4^{+} cDCs, and mice harboring a dominant-negative form exhibit a complete absence of all DC subsets [71;72]. Unexpectedly, Ikaros-null mice retain a residual population of CD8^{+} cDCs, although the function or origin of these cells has not been examined. The apparent mechanism by which Ikaros controls DC development is through its regulation of a large panel of receptors and transcription factors. Although classically considered to be required only for the priming of lymphoid lineages, gene expression analysis of the earliest cell stages of hematopoietic development revealed that the loss of Ikaros affects the expression of large gene sets associated with both lymphoid and myeloid lineages [73]. For example, the expression of Flt3, IL-7 receptor, Notch1, and the transcription factor Mef2c are all controlled by Ikaros, suggesting that it acts globally on many aspects of lineage specification [74].

Another well-known transcription factor influencing the early development of DCs is the ETS factor PU.1. Recently, PU.1 was unequivocally shown to be necessary for DC development, resolving earlier studies that resulted in conflicting conclusions [75;76]. Mice with a conditional deletion of PU.1 within the hematopoietic compartment fail to develop all DC subsets, but also show defects in other myeloid lineages including but not limited to macrophages and neutrophils [77]. Similar to Ikaros, PU.1 appears to control the induction of a broad range of genes necessary for development of many hematopoietic lineages. In particular, PU.1 controls the early expression of Flt3, GM-CSFR, and M-CSFR on progenitors, although only the absence of Flt3 was confirmed on DC precursors [78;79]. Notably, forced expression of Flt3 in PU.1-deficient progenitor cells did not rescue DC development, reinforcing the notion that PU.1 regulates other important programs aside from the signaling downstream of Flt3.

Finally, Gfi1, a known transcriptional repressor, appears to play a role in the development of all DC subsets. Gfi1-null mice have a global reduction in lymphoid-organ DC compartments, albeit not as severe as either Ikaros- or PU.1-deficient mice [80]. Analysis of Flt3^{+} DC progenitors revealed a cell-autonomous role for Gfi1 in the activation of STAT3, suggesting that it may act downstream of Ikaros or PU.1. One proposed mechanism is through its ability to inhibit PIAS3 (protein inhibitor of activated STAT3), a specific

inhibitor of STAT3 [81]. In this model, loss of Gfi1 leads to the maintenance of PIAS3 activity and thus a reduction in transcriptional events mediated by STAT3. Additionally, studies in human monocytes have shown that Gfi1 can bind to a large number of myeloid gene loci such as JAK3, IL-8, and the CEBP family of transcription factors, indicating a potential role beyond its effect on STAT3 [82]. Interestingly, surviving Gfi1-deficient DCs closely resemble their counterparts from mice deficient in members of the NF-kappaB signaling cascade, suggesting that these two factors may converge on a common developmental pathway (see below) [83]. The dependence of DC development on both PU.1 and Gfi1 is in stark contrast to B cell ontogeny, during which these two factors act in opposition [84]. Whether Ikaros, PU.1, and Gfi1 act in concert or independently in DC development remains to be determined.

6.2 Factors regulating late DC progenitor development

Whereas the factors discussed above appear to be necessary during the HSC to CDP transition, particularly for the proper expression of key cytokine receptors, several transcription factors exert control over developmental options after commitment to the DC lineage has already occurred. These factors are expressed beginning in the CDP stage, and thus specify commitment into subsets or act on survival or proliferation programs in immediate precursors. For the most part, the factors acting in this realm of DC development have been evaluated for their actions on lymphoid resident DCs. However, it is clear that they likely affect the development of corresponding non-lymphoid DCs as well by mediating similar genetic programs in both compartments (Figure 2) [85;86].

The first transcription factor identified to affect a particular subset was RelB, a member of the NF-kappaB family of transcription factors, which also consists of RelA (p65), c-Rel, p50 and p52. In contrast to RelA and c-Rel, RelB selectively forms a heterodimer with p52 or the precursor form, p100 [87]. Expression of RelB is induced in the pre-cDC and then preferentially maintained in $CD4^+$ DCs. Consequently, mice deficient in RelB show a significant decrease in the $CD4^+$ population of cDCs, a defect determined to be cell intrinsic, while $CD8^+$ cDCs and pDCs appear to be unaffected [88;89].

Although studies investigating the mechanism by which RelB controls DC differentiation are lacking, one gene suggested to be a target of RelB is interferon regulatory factor 4 (IRF4), whose deletion also results in a large reduction in the number of $CD4^+$ DCs [90]. IRF4, one of nine IRF family members, contains an N-terminal DNA-binding domain (DBD) and a C-terminal IRF-associated domain (IAD). IRF4 is known to interact with a number of other transcription factors, including ETS factors and AP1 family members. In different settings, these interactions are critical in determining the DNA-binding specificity and functional nature of the IAD, either as an activator or repressor of transcription [91-93]. However, whether these interactions occur within the context of DCs remain to be defined, although PU.1 (ETS) and Batf3 (AP1) are attractive candidates.

Of the IRF family members, the member most functionally related to IRF4 is IRF8. IRF8 has also been demonstrated to play a critical role in DC differentiation. IRF8 deficiency leads to a complete loss of both $CD8^+$ cDCs and pDCs, and mice lacking both IRF4 and IRF8 exhibit essentially a compound phenotype, with a severe reduction in the total number

of DCs and an absence of all but a few $CD4^+$ and double negative cDCs [94;95]. This result suggests that these factors may provide an overlapping function in certain subsets, but for most DCs, these two factors display essentially non-overlapping patterns of expression. It is not yet known what signals are controlled by the IRF proteins, or what signals or transcription factors lead to the induction of IRF4 or IRF8 in their respective subsets.

In contrast to the selective expression of IRFs within DC subsets, Batf3 is expressed in both $CD8^+$ and $CD4^+$ cDCs, but loss of Batf3 leads to an abrogation in the development of only one of these subsets, the $CD8^+$ DC [96]. Batf3 is a member of the AP1 family of transcription factors that forms an obligate heterodimer with cJun or other Jun paralogs. However, unlike other AP1 members, Batf3 lacks a C-terminal extension that would provide a transcriptional activation domain, and instead consists only of a basic DNA-binding motif followed by a dimer-forming leucine zipper region [97;98]. Batf3 is most similar to a related factor Batf, which was recently found to be necessary for Th17 and Tfh differentiation as well as for isotype-switching in B cells [99;100]. These recent findings reverse the earlier notion that these minimal AP1 family members, Batf3 and Batf, were simply dominant-negative analogs of Fos, and instead suggest that these factors form unique heterodimers with Jun that posses distinct transcriptional activities. In the case of Batf3, this activity presumably includes the activation of genes selectively expressed by $CD8^+$ DCs.

Mice deficient in Batf3 have normal numbers of precursors, including CDPs and pre-cDCs [85], and appear deficient in only the most terminal stage of $CD8^+$ DC maturation. This final step is defined by the induction of genes important for the function of this DC subset, including Langerin and CD103. However, at present the immediate transcriptional targets of Batf3 are unknown.

The factors discussed above appear to regulate terminal steps of commitment to particular DC lineages. In contrast, two additional transcription factors appear to regulate the survival of terminally committed precursors or mature cell types. The transcription factor Rbp-j, a critical mediator of the Notch signaling pathway, was recently found to be required for the survival of $CD4^+$ DCs *in vivo* [101]. Mice deficient in Rbp-j lack more than 50% of their $CD4^+$ DCs. Detailed analysis revealed that commitment to this lineage appears to be unaffected since the population of splenic pre-cDCs in these mice is normal. Rather, higher levels of Annexin-V staining and BrdU incorporation in surviving $CD4^+$ DCs suggest increased apoptosis of the terminal cell stage.

Similarly, the zinc finger transcription factor Bcl6 appears to regulate the survival of cDC precursors [102]. Bcl6 is a BTB (bric-a-brac, tramtrack, broad) -domain containing repressor that also has important actions in Tfh differentiation and exerts a repressive action on plasma cell genes within germinal center B cells [103-105]. In DCs, Bcl6 deficiency causes a severe reduction in both types of cDCs. While *in vitro* Flt3L experiments suggest a more pronounced effect on $CD8^+$ DC equivalents, the *in vivo* results indicate a larger effect on $CD4^+$ DC frequency. In contrast to Rbp-j deficiency, Bcl6-null mice do appear to exhibit increased Annexin V staining in cDC precursors and accordingly, express markers of apoptosis such as p53.

7. The balance between E2-2 and Id2 determines the choice between cDC and pDC fates

A family of transcription factors known as class I basic helix loop helix (bHLH) factors plays an important role in the differentiation and survival of a number of lymphocyte populations [106]. Class I bHLH proteins, also referred to as E proteins, comprise a family of four members: E12, E47, E2-2, and HEB. These proteins function either as homodimers or heterodimers between family members, and bind to a conserved DNA motif referred to as an E-box. The DNA-binding activity of these heterodimers can be interrupted by formation of a complex between E proteins and members of the inhibitor of differentiation (Id) HLH protein family, which lack the necessary DNA-binding motif of the basic region. Of the four members of the Id protein family, Id2 and Id3 appear to be the major inhibitors of E2 protein activity during lymphocyte development.

The first indication of the importance of E proteins in DC development arose from the observation that over-expression of Id2 or Id3 inhibits the development of pDCs *in vitro* [107]. This was extended by the finding that Id2 expression is induced *in vitro* in response to GM-CSF, and that Id2 is required for *in vivo* development of CD8$^+$ DCs, but not other subsets [108]. Surprisingly, the frequency of pDCs is increased in these mice suggesting that Id2 may function to divert cDC precursors away from the pDC fate. This hypothesis is supported by the finding that DCs developing in Id2-deficient mice also show de-repression of many genes normally associated with B cells as well as pDCs, as they share a large common gene signature [109].

A major breakthrough in the understanding of DC subset development resulted from the discovery that E2-2 is required for the development of pDCs *in vivo* [54]. These findings also helped to establish the identity of a pDC-restricted precursor: a CD11c$^+$ Ly6C$^+$ SiglecH$^-$ cell termed the pre-pDC. Mechanistically, E2-2 regulates a large pDC gene program including the direct regulation of other key transcription factors associated with pDC development, including IRF8, Bcl11a, and Spi-B. Interestingly, E2-2 also appears to repress genes associated with cDC subsets [110]. This was shown using an inducible Cre-deletion system whereby E2-2 was deleted in mature pDCs. Terminal deletion of E2-2 results in the induction of a number of cDC-associated genes including the re-expression of Id2. However, only a small subset of cDC genes appears to be affected. Therefore, it is not clear whether the emergence of these genes represents a conversion of pDCs into *bona fide* cDCs, or instead into the recently reported alternative CD8$^+$ CX3CR1$^+$ DC lineage that appears to be more closely related to pDCs [111]. In any event, de-repression of Id2 caused by the loss of E2-2 expression now highlights an important regulatory circuit that controls the switch between pDCs and cDCs. In this circuit, induction of Id2 decreases E2-2 expression, and E2-2 activity suppresses Id2 expression. This arrangement represents a classical "flip-flop" circuit based upon mutual antagonism of alternately expressed factors. Presently, it is unknown what signals might control the induction of alternate states of this circuit.

8. Gene expression analysis of the CDP points to "pDC-priming."

Elaborating the transcriptional mechanism of T cell development was greatly aided by the identification of distinct cellular stages through which T cells progress. For example, the fate choice between T and NK cell lineages in the thymus was known to take place during the CLP to pro-T cell transition and to be dependent on Notch1 [112]. Careful gene expression analysis of intermediate cell stages allowed three groups to identify the downstream factor Bcl11b as a key mediator of this specification process [113-115] .

In contrast to T cell development, the specific intermediate stages of DC development have only recently been identified, as discussed above. In addition, the difficulty in identifying and isolating these progenitors, as well as their relative paucity in the bone marrow, has made them difficult to study. As a result, the relationships between the transcription factors that regulate DC development and the progenitor stages at which they act are still unclear. One plausible arrangement of the transcription factor network with respect to cellular differentiation is shown in Figure 3. In this scheme, we have diagrammed the potential relationships between key transcription factors, some of which were suggested in the original reports.

We propose that the CDP represents the key stage at which the choice between the pDC and cDC lineage is made. The CDP is the natural candidate for this choice, since it has extinguished its ability to form alternate lineages, but still retains the ability to generate all DC subsets. Recent studies, as well as unpublished studies from our lab, have provided expression profile data for each sequential stage of DC differentiation *in vivo* including the CDP [116]. Strikingly, analysis of transcription factors expressed in CDPs reveals that a number of pDC factors, including E2-2, Bcl11a, and Runx2, are already expressed at moderate levels (Figure 4). In contrast, factors associated with the cDC lineage, such as Id2, Batf3, and Bcl6, are expressed either at very low levels or not at all. One interpretation of this data is that CDPs may be set to "default" into the pDC lineage unless a trigger is received to induce Id2. Id2 becomes highly expressed at the precDC stage where-upon pDC-associated genes are lost. Such a default pathway could be likened to the strong skewing of naïve T cells towards the Th2 phenotype, which is mediated by an auto-activation of Gata-3 [117].

9. Concluding remarks

The transcriptional networks regulating the diversification of myeloid lineages are currently being elucidated. Arguably, DCs remain the most enigmatic and developmentally uncharacterized cell type within this system. However, recent studies have defined key steps in the DC differentiation pathway by identifying restricted bone marrow precursors. We hope that these advances will encourage the current transition of the field from a relatively descriptive state to one in which molecular mechanisms are emphasized. This shift in approach is needed to clarify confusion regarding the precise role of the DC lineage, and particularly specific DC subsets, in settings of infection and tolerance. For example, our understanding of the functional differences between pDCs and cDC subsets has greatly improved since the identification of transcription factors which are required for their

development, such as E2-2 and Batf3, respectively. The deletion of either gene leads to a model in which the exact function of the relevant cell can be interrogated.

Clearly, many questions regarding the transcriptional regulation of DC fate decisions still remain unanswered. The basis for the initial commitment to the DC lineage or its split from the monocyte pathway downstream of the MDP is unknown. Similarly, the mechanisms regulating the transition from the cDC precursor into either the CD4$^+$ or CD8$^+$ subset are also unclear. Another key issue is whether the combinatorial action of cytokines within distinct stromal niches plays any role in influencing these fate choices. Answers to these outstanding questions will allow for the arrangement of the current transcription factor network into a resolvable structure that can be used for manipulation of DCs in clinical settings.

Acknowledgments

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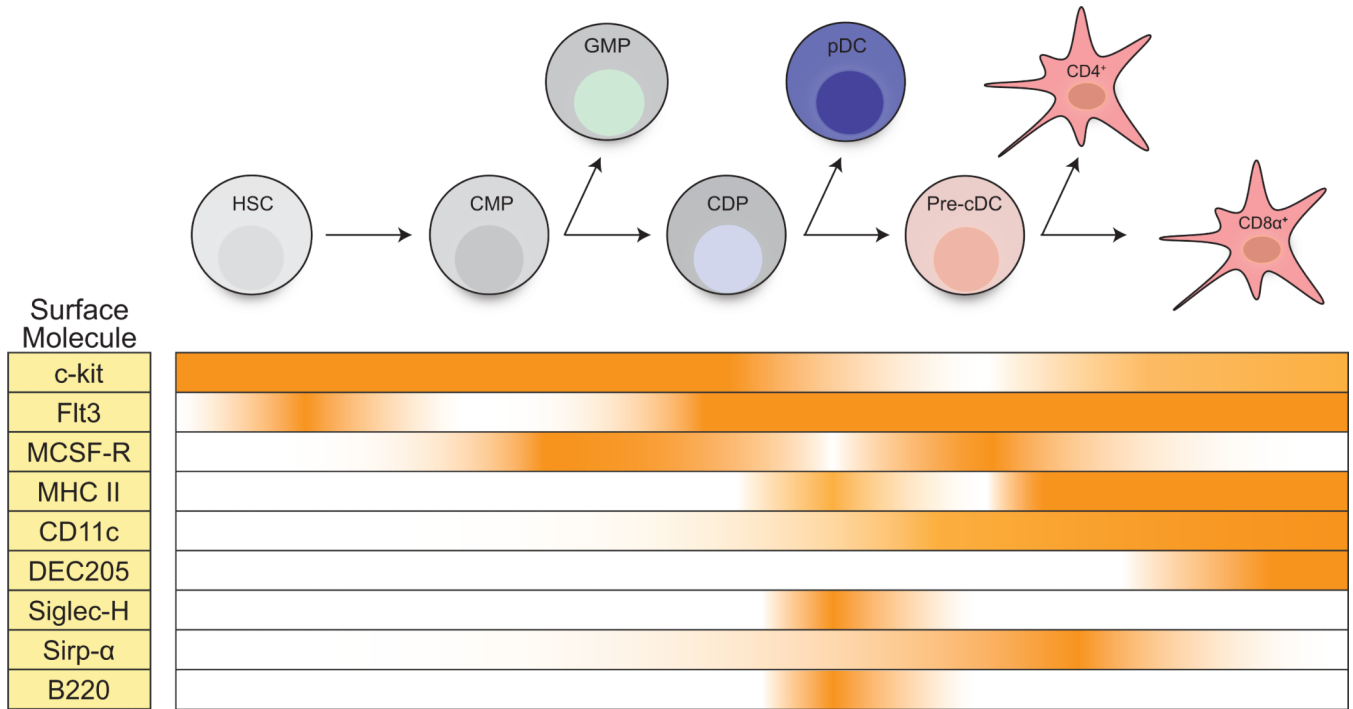
Figure 1. The temporal expression of genes in DCs and their precursors

The expression of key surface markers used for the identification of mature dendritic cells (DCs) and their bone marrow precursors is depicted. In addition, the expression of important growth factors required for the development of DC subsets is shown.
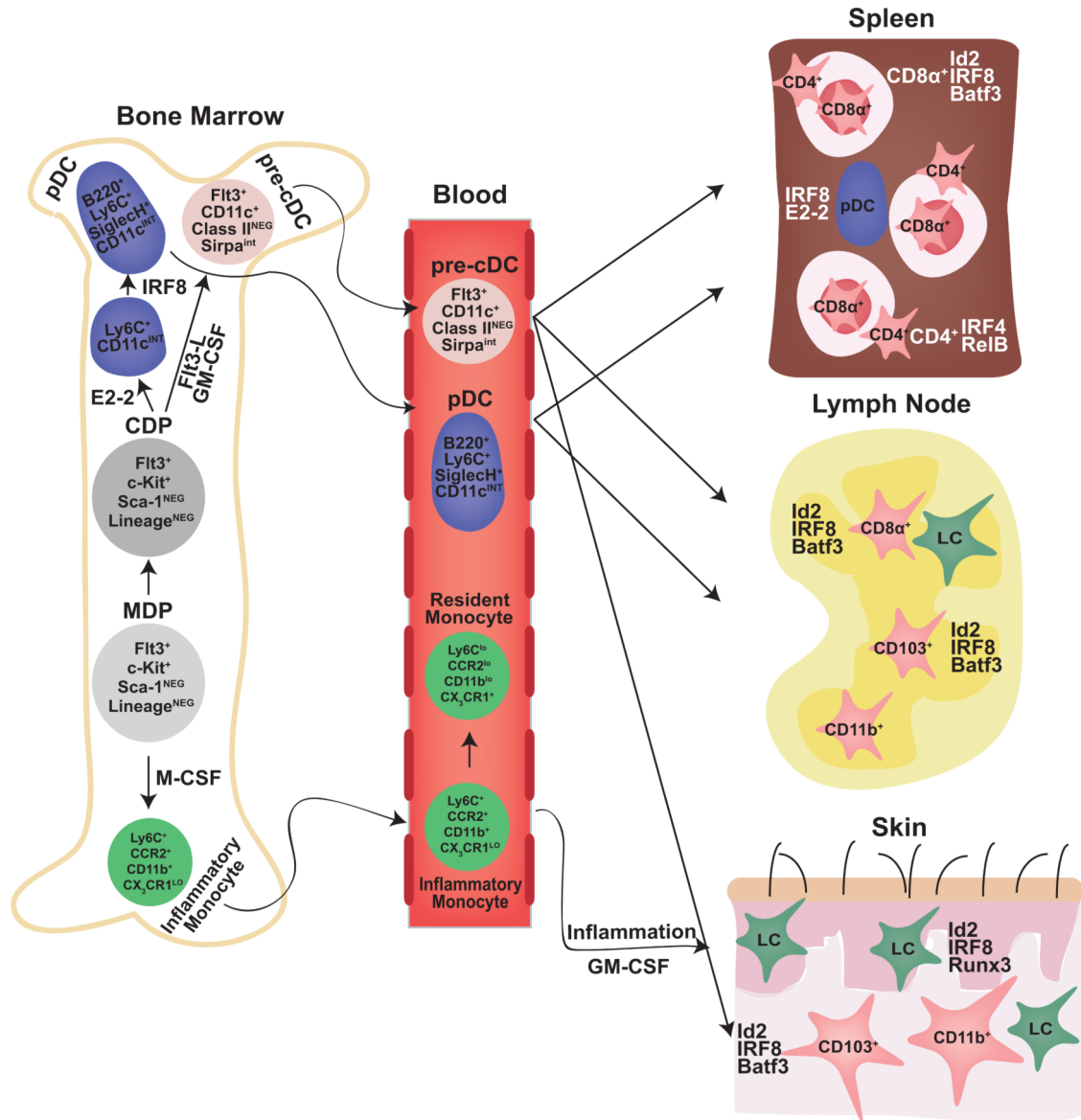
Figure 2. Stages of DC development

DCs derive from bone marrow–resident hematopoietic progenitors. Following differentiation of the hematopoietic stem cell (HSC) into the common myeloid progenitor (CMP), alternate myeloid fates are extinguished in the subsequent macrophage-dendritic progenitor (MDP). Commitment to the DC lineage occurs at the next stage termed the common dendritic progenitor (CDP). The CDP has the potential to develop into both plasmacytoid dendritic cells (pDC) and conventional dendritic cells (cDC). This choice occurs as the CDP transitions into either of the intermediates for each lineage: the pre-pDC (not shown) or the pre-cDC. pDCs complete their maturation in the bone marrow and then circulate to secondary lymphoid organs through the blood. In contrast, pre-cDCs seed both lymphoid and non-lymphoid organs and undergo one final fate choice into either CD8$^+$ or CD4$^+$ DCs; or CD103$^+$ or CD11b$^+$ equivalents in the periphery, respectively. The developmental similarity between lymphoid-organ and peripheral DC subsets has been shown using mice deficient in the transcription factors Id2, IRF8, and Batf3. Additionally, monocytes can give rise to DCs in inflammatory settings although the transcriptional mechanism for this process is poorly characterized.
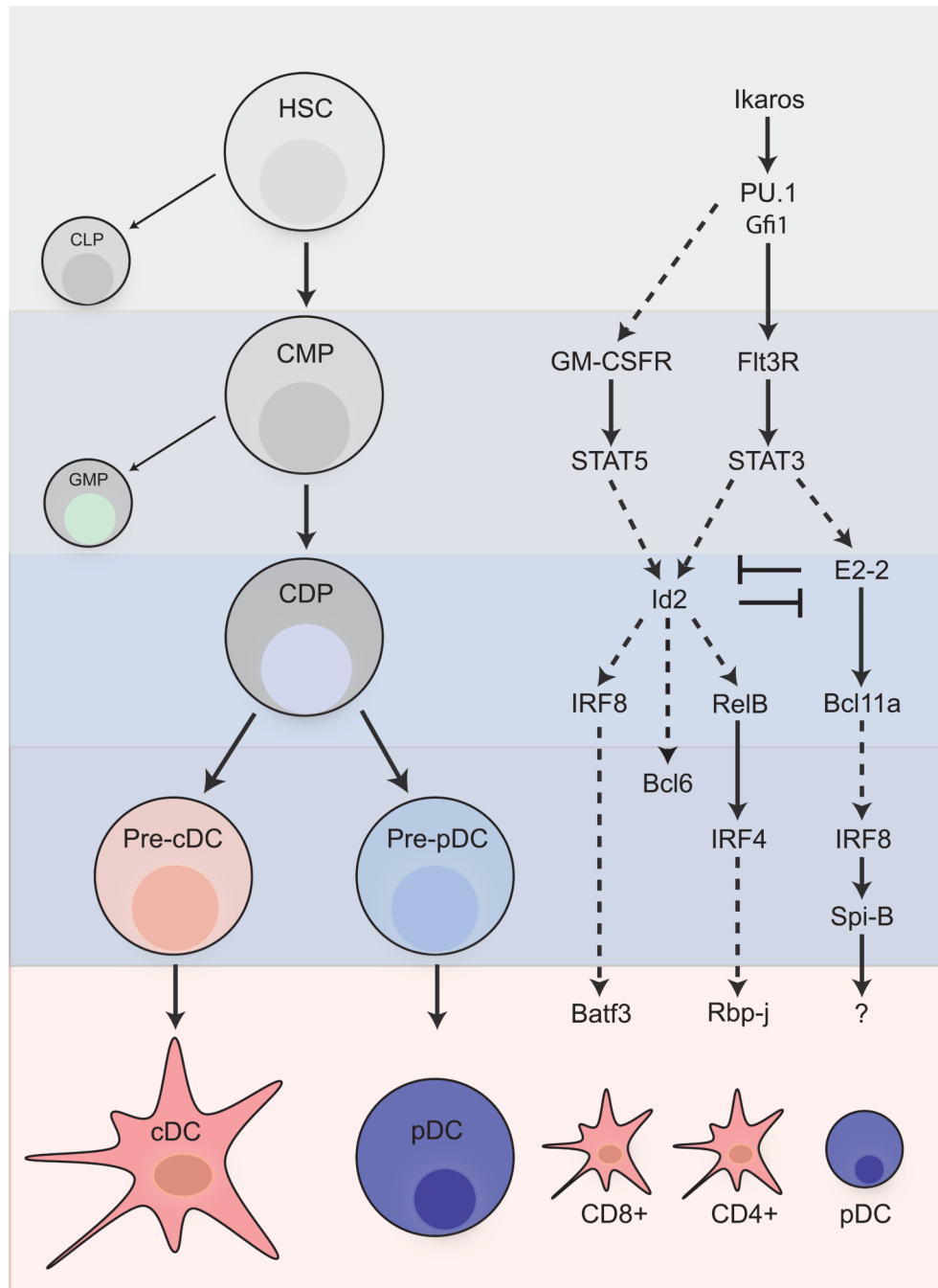
Figure 3. Transcription factor networks control DC development

A number of transcription factors regulate the commitment and survival of dendritic cells at various stages of development. However, the coordinated action of these factors with respect to one another as well as to relevant progenitor stages has not been well defined. One plausible network of these factors is depicted alongside the cellular stage in which they likely exert their functions. Solid arrows indicate connections that have been proven or suggested within original reports. Dotted arrows indicate hypothesized relationships.
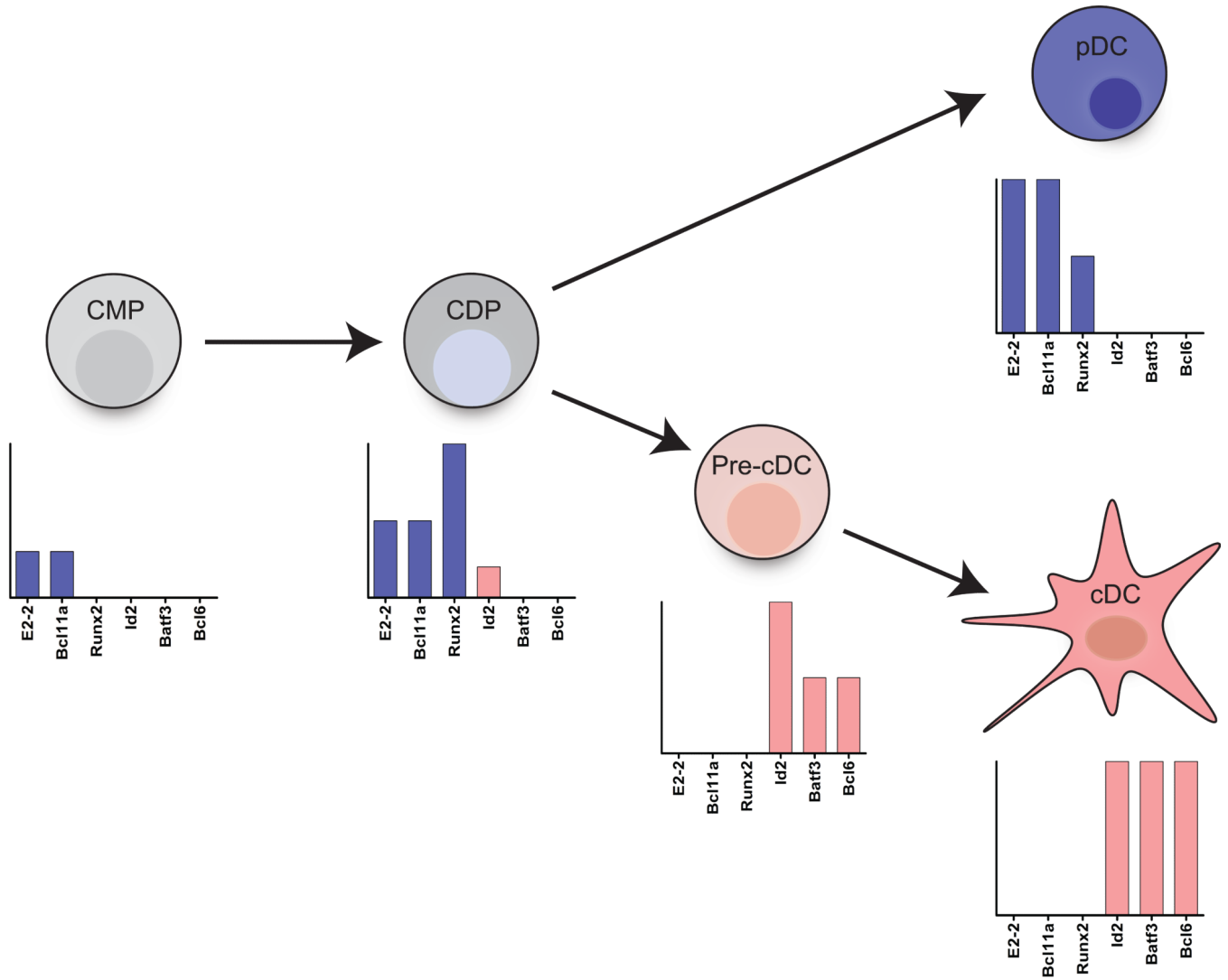
Figure 4. CDPs are "pDC-primed."

CDPs represent the last common precursor for both cDCs and pDCs, and thus a likely stage of specification into either lineage. This decision is dependent on a mutually antagonist balance between the transcription factors E2-2 and Id2. Gene expression analysis of progenitor populations reveals that CDPs express a number of pDC-specific transcription factors. Shown is the expression of pDC factors (E2-2, Bcl11a, and Runx2) in comparison to cDC factors (Id2, Batf3, and Bcl6). Expression of pDC factors and the lack of expression of cDC factors in the CDP points to a "default" pDC pathway during DC differentiation. Re-direction into the cDC lineage requires the induction of Id2 and repression of pDC factors in the pre-cDC.