Protein Cell 2011, 2(8): 620–630
DOI 10.1007/s13238-011-1088-0

Protein & Cell

RESEARCH ARTICLE

The network of cytokines, receptors and transcription factors governing the development of dendritic cell subsets

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Received August 3, 2011 Accepted August 14, 2011

ABSTRACT

The pathways leading to the development of different dendritic cell (DC) subsets have long been unclear. In recent years, a number of precursors on the route to DC development, both under steady state and inflammatory conditions, have been described, and the nature of these pathways is becoming clearer. In addition, the development of various knockout mouse models and an *in vitro* system modelling DC development have revealed the role of numerous cytokines and transcription factors that influence DC development. Here, we review recent findings on the factors important in DC development in the context of the developmental pathways that have been described.

KEYWORDS dendritic cell, cytokines, receptors, transcription factors

DENDRITIC CELL SUBSETS

Dendritic cells (DC) are a heterogeneous population, consisting of multiple subsets, although they share the common function of antigen presentation. Each of these subsets plays a distinct functional role in an immune response. In the absence of inflammation, the DC network is comprised of plasmacytoid DC (pDC) and several subsets of conventional DC (cDC).

pDC

pDC circulate through the tissues, and are also known as interferon producing cells. When activated by inflammatory stimuli, pDC assume a dendritic morphology and acquire the ability to present antigen. pDC are rapidly recruited to a site of inflammation, where they produce large amounts of type I interferon (IFN) (Liu, 2005). Depletion of pDC following sterile injury has been shown to result in delayed wound healing (Gregorio et al., 2010). In addition, pDC may also be involved in maintaining tolerance (de Heer et al., 2004; Goubier et al., 2008), potentially by inducing the differentiation of antigen specific regulatory T cells. Paradoxically, pDC have also been implicated in the pathogenesis of autoimmune disorders (Isaksson et al., 2009).

cDC

cDC consist of both lymphoid tissue resident DC and migratory DC. Lymphoid tissue resident cDC (henceforth 'resident cDC') exist within the lymphoid tissues in the steady state in an immature form, surveying the blood and lymph for signs of infection (Wilson et al., 2003). In the mouse, resident cDC can be further subdivided into those cDC that express a homodimer of the CD8α chain (CD8αα$^+$, hereafter CD8$^+$), or those that lack this CD8 expression (CD8$^-$). The latter can be further subdivided into CD8$^-$CD4$^+$ (CD4$^+$) cDC or CD8$^-$CD4$^-$ (double negative, or DN) cDC (Vremec et al., 1992; Vremec et al., 2000). CD4$^+$ and DN cDC differ somewhat in their cytokine and chemokine expression (Proietto et al., 2004). However, they are largely functionally similar, and henceforth, termed resident CD8$^-$ cDC.

Migratory DC survey peripheral tissues, and upon maturation, capture antigen and migrate to the draining lymph node, in which they encounter T cells (Banchereau and Steinman, 1998). Migratory cDC can be subdivided into the 'resident cDC-like' subsets, which have been found in multiple

peripheral tissues such as the lungs, intestines and kidneys, and those such as Langerhans' cells, which are unique to a particular tissue. The 'resident cDC-like' migratory cDC share many functional and developmental characteristics of resident cDC. They can be further separated based on their expression of the integrin CD103 and the chemokine receptor CX_3CR1, into $CD103^+CX_3CR1^-$ ($CD103^+$ cDC) and $CD103^-CX_3CR1^+$ cDC ($CD103^-$ cDC)(Ginhoux et al., 2007; Poulin et al., 2007; Ginhoux et al., 2009).

$CD8^-$ and $CD103^-$ cDC are efficient at presenting antigen on MHC II molecules. These cDC are generally associated with the initiation of Th2 responses (Metlay et al., 1990; Agger et al., 1992; del Rio et al., 2007). Phenotypically, they are also characterized as $CD11b^+$ $Sirp\alpha^+$. In the literature, these cDC subsets are often referred to as $CD11b^+$ or $Sirp\alpha^+$ cDC.

$CD8^+$ and $CD103^+$ cDC are able to cross-present exogenous antigens on MHC class I (den Haan et al., 2000; Pooley et al., 2001; del Rio et al., 2007; Bedoui et al., 2009; Henri et al., 2010) to activate CD8 T cells, and are major producers of IL-12p70 (Hochrein et al., 2001; Sung et al., 2006; Edelson et al., 2010). $CD8^+$ and $CD103^+$ cDC are associated with the initiation of Th1 responses. A recent report has described the cross-presentation activity of $CD8^+$ resident cDC to be concentrated in a $CD103^+$ marginal zone subset of the $CD8^+$ population. Whether this reflects true lack of cross-presentation activity or a lack of access to antigen in the $CD103^-CD8^+$ cDC subset remains unclear (Qiu et al., 2009). $CD8^+$ and $CD103^+$ cDC are sometimes referred to as $CD11b^-$ or $Sirp\alpha^-$ cDC. In addition, $CD103^+$ cDC express langerin (Henri et al., 2010), and may be referred to as $langerin^+$ or CX_3CR1^- cDC.

$CD8^+$ and $CD103^+$ cDC have also been implicated in the maintenance of peripheral tolerance. $CD8^-$ resident cDC and CX_3CR1^+ cDC in the gut migrate to the T-cell areas of lymphoid organs upon inflammation (De Smedt et al., 1996; Reis e Sousa et al., 1997; Schulz et al., 2009). In contrast, many $CD8^+$ cDC reside within the T-cell areas of lymphoid organs, and $CD103^+$ cDC in the gut constitutively migrate to lymph nodes in the steady state (Steinman et al., 1997; Schulz et al., 2009). Coupled with their ability to cross-present antigen, these localization and migration patterns give $CD8^+$ and $CD103^+$ cDC both the means and the opportunity to interact with both CD4 and CD8 T cells in the absence of inflammation. Further, evidence from a number of models suggests their importance in the maintenance of tolerance (O'Keeffe et al., 2005; Worbs et al., 2006; Coombes et al., 2007; Varol et al., 2009).

Langerhans' cells, first identified by Paul Langerhans' in 1868, were the first dendritic cells to be observed, although their antigen presentation function was not described until much later (Romani et al., 2003). Langerhan's cells were once phenotypically defined by their expression of CD207, or langerin (Valladeau et al., 2000). The function of Langerhans' cells has been questioned with the recent discovery of $CD103^+langerin^+$ dermal DC (Valladeau et al., 2002), evidence now suggesting that these dermal DC, rather than Langerhans' cells, are important in the initiation of immunity (Wang et al., 2008; Bedoui et al., 2009).

Langerhans' cells may rather be involved in the maintenance of tolerance. Selective depletion of Langerhans' cells, or deletion of IL-10 or MHC II in Langerhans' cells leads to a more severe inflammatory response in a hapten-induced model of contact hypersensitivity (Kaplan et al., 2005; Igyarto et al., 2009). The existence of Langerhans' cell-like tissue specific epidermal cDC in other organs, such as $CD103^+CD11b^+$ cDC in the gut and lungs (Bogunovic et al., 2009; Varol et al., 2009; Edelson et al., 2010), have also been described.

Inflammatory DC

An alternate class of DC subsets can differentiate from monocytes in the presence of inflammatory stimuli. For example, upon infection with listeria, a new subset of DC develops. These DC secrete high levels of tumour necrosis factor α (TNFα) and inducible nitric oxide synthase (Serbina et al., 2003). Inflammatory DC can secrete some IL-12, and can cross-present antigen to CD8 T cells, although with less efficiency than their steady state counterparts. Inflammatory DC are recruited in large numbers to sites of inflammation, and likely carry out a large part of the antigen presentation functions, such as cross-presentation and cytokine secretion, in the case of infection. The DC generated in GM-CSF cultures are thought to model such inflammatory DC subsets (Xu et al., 2007).

PATHWAYS TO DC

The study of DC development has been hampered by its plasticity, with numerous early precursors having been shown to have DC potential *in vivo*. However, in recent years, a number of DC precursors, and their roles along the pathway to DC development, have been described.

Monocytes were originally considered the immediate precursors of cDC. While this has been subsequently refuted (Naik et al., 2006; Varol et al., 2007; Xu et al., 2007), monocytes do give rise to certain DC subsets. In the steady state, monocytes have potential for migratory $CD103^-$ cDC (Jakubzick et al., 2008; Ginhoux et al., 2009; Varol et al., 2009). As pre-cDC also have this potential (Ginhoux et al., 2009), these pathways may collaborate to generate the pool of steady state migratory $CD103^-$ cDC, although it is unclear as to whether both precursors give rise to the same, or functionally divergent, subsets of $CD103^-$ cDC. The contribution of monocytes and pre-cDC to this population remains to be elucidated.

Further, when steady state conditions are perturbed, monocytes can differentiate into various DC subsets. Under inflammatory conditions, monocytes can differentiate into inflammatory DC (Naik et al., 2006; Xu et al., 2007), and in the

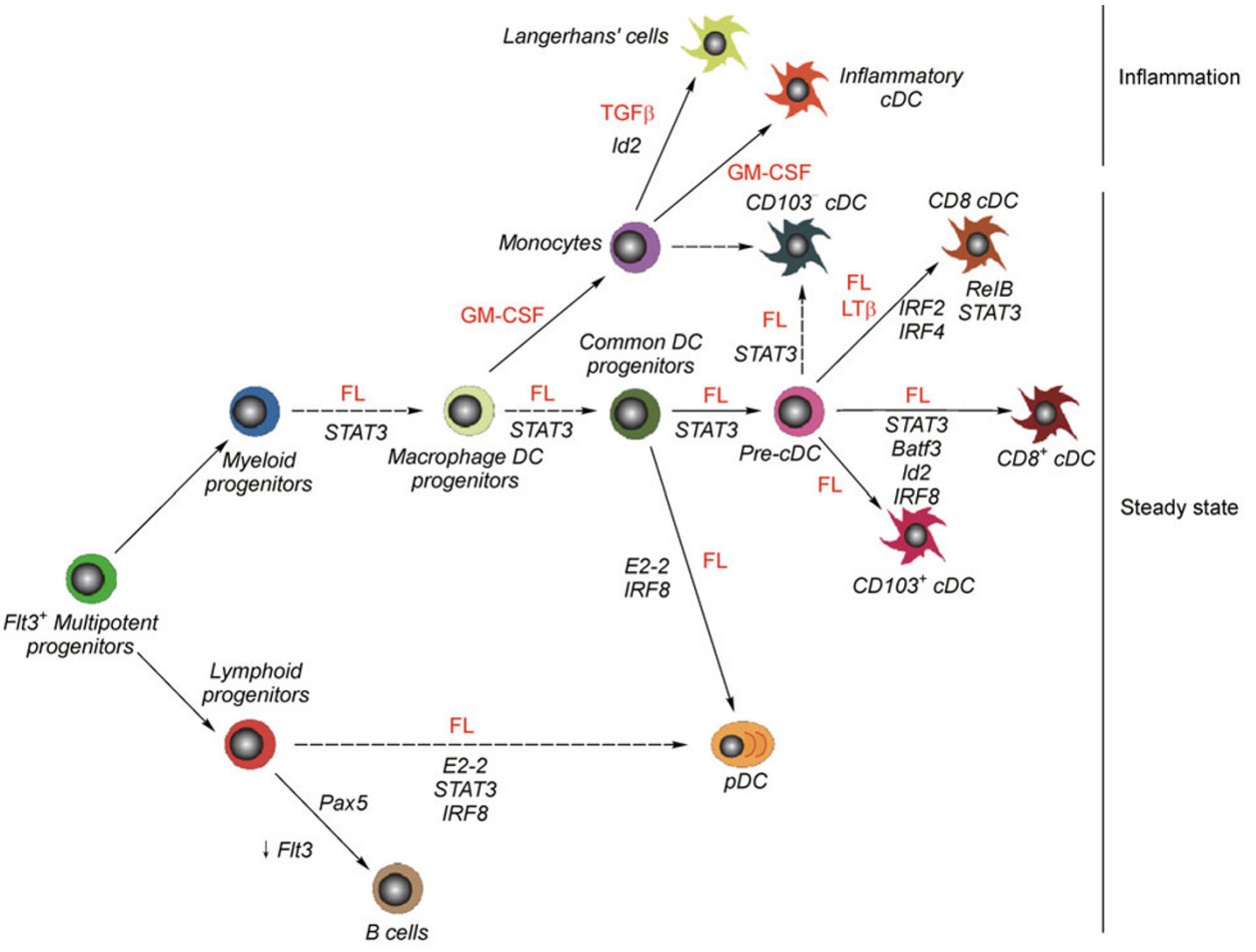

Figure 1. The transcription factors and cytokines required for development of DC subsets. Dashed lines indicate pathways that may, but have yet to be definitively shown to, operate in DC development.

case of severe skin injury, the Langerhans' cell population is reconstituted by monocyte precursors (Ginhoux et al., 2006).

However, a model is now emerging in which most steady state DC appear to differentiate via a monocyte-independent pathway (Fig. 1). In this model, common myeloid progenitors give rise to a precursor population restricted to the macrophage and DC lineages (Fogg et al., 2006), the macrophage DC progenitor (MDP) (Liu et al., 2009). MDP then differentiate into common DC progenitors (CDP), DC restricted progenitors with potential for pDC and resident and migratory cDC. CDP then give rise to pDC and pre-cDC. As their name suggests, pre-cDC are restricted to differentiation into cDC. Pre-cDC have been observed in the bone marrow, blood (Liu et al., 2009), and peripheral tissues such as the kidneys, liver and lungs, suggesting these are the cDC-committed precursors that migrate into the peripheral tissues, before their differentiation into cDC.

The origin of pDC has long been a source of discussion. This is partly due to the unique nature of pDC. Given their similarity to both lymphoid-derived B-cells and myeloid-derived cDC, it was unclear whether pDC were products of the lymphoid or myeloid lineages. Recently, with the description of CDP, pDC have been assumed to have a myeloid origin. While other studies have suggested pDC differentiate via both lymphoid and myeloid routes (Corcoran et al., 2003; D'Amico and Wu, 2003; Shigematsu et al., 2004; Pelayo et al., 2005). These conclusions are yet to be confirmed *in vivo*.

CYTOKINES INVOLVED IN DC DIFFERENTIATION

A number of cytokines have been implicated in the development of various DC subsets. These range from those such as Flt3 ligand, which is required for the development of all steady state DC subsets, to those such as lymphotoxin β, which promote the differentiation of particular DC subsets. In line with the requirements of different cytokines for the development of various DC subsets, the precursors for these DC express different cytokine receptors (Table 1).

Table 1 Expression of cytokine receptors on DC precursors

	Flt3	M-CSFR	GM-CSFR
CMP	+/-	+	+
MDP	+	+	+
CDP	+	++	++
Pre-cDC	+	+	?

M-CSFR, macrophage colony stimulating factor receptor; GM-CSFR, granulocyte macrophage colony stimulating factor receptor; CMP, myeloid committed progenitors; MDP, macrophage dendritic cell progenitor; CDP, common dendritic cell progenitors.

Flt3 ligand

A number of studies have illustrated the importance of fms-like tyrosine kinase 3 (Flt3) ligand (FL) as the limiting cytokine in steady state DC development. The defining characteristic of a DC progenitor is the expression of the cytokine receptor Flt3 (Manz et al., 2001; D'Amico and Wu, 2003). Thus, precursors that lack Flt3 expression—such as megakaryocyte-erythroid progenitors (MEP)—have no DC potential. However, ectopic expression of Flt3 in MEP restores some DC precursor activity (Onai et al., 2006). Mice that lack functional FL, Flt3, or the downstream transcription factor, Stat3, have reduced numbers of steady state DC; conversely, mice that are treated with FL have increased numbers of steady state DC (Laouar et al., 2003; McKenna et al., 2000). Further, stimulation of total bone marrow with FL *in vitro* generates a heterogeneous population of DC phenotypically and functionally equivalent to those found in the mouse spleen (Brasel et al., 2000; Brawand et al., 2002; Naik et al., 2005).

The development and homeostasis of DC may also be regulated by the network of various immune cells. For example, depletion of regulatory T cells (Tregs) has been shown to lead to increased numbers of late DC precursors and DC in an FL-dependent manner (Kim et al., 2007; Liu et al., 2009). Interestingly, FL treatment promotes the expansion of Tregs *in vivo* in a DC and IL-2 dependent manner (Darrasse-Jeze et al., 2009; Swee et al., 2009). Thus, Tregs and DC form a feedback loop using FL and IL-2. However, it remains to be seen whether the increase in DC numbers following the depletion of Tregs is an attempt to compensate for the lack of Tregs by enhancing DC numbers, or whether Tregs are directly involved in the regulation of FL production.

M-CSF

As DC express the macrophage colony stimulating factor (M-CSF) receptor (M-CSFR), and were originally thought to be derived from monocytes, it seemed logical that M-CSF would play an important role in DC development. Surprisingly, $CSF1^{op/op}$ mice, which lack functional M-CSF, showed no deficiencies in the generation of resident DC (Dai et al., 2002), although Langerhans' cells were reduced in these mice (Ginhoux et al., 2006). However, M-CSF treatment has been shown to induce generation of cDC and pDC both *in vitro* and *in vivo* in an FL-independent manner (Fancke et al., 2008). M-CSFR deficient mice have a decreased proportion of migratory $CD103^-$ cDC in the skin, lungs and kidneys compared to their wild type counterparts (Ginhoux et al., 2009). Whether this reflects a contribution to $CD103^-$ cDC from monocyte precursors, or a role for M-CSF in commitment to this lineage, remains to be determined. The pronounced phenotype produced by the deletion of M-CSFR, in contrast to the $CSF1^{op/op}$ mice, suggested the activity of another ligand for the M-CSFR. It has since been shown that interleukin-34 also binds the M-CSFR (Lin et al., 2008).

GM-CSF

The original culture systems used to generate DC for functional analysis involved culturing monocytes, or total bone marrow, with granulocyte macrophage colony stimulating factor (GM-CSF) and interleukin-4 (IL-4) (Sallusto and Lanzavecchia, 1994; Caux et al., 1996). Thus, it came as a surprise when GM-CSF deficient mice were shown to have little to no defects in resident cDC development (Vremec et al., 1997; Hikino et al., 2000). GM-CSF levels in the blood, undetectable in the steady state, are upregulated under inflammatory conditions (Baiocchi et al., 1993; Cebon et al., 1994; Cheers et al., 1988), and GM-CSF has been implicated in the development of inflammatory cDC from monocytes (Naik et al., 2006; Varol et al., 2007).

The addition of GM-CSF to FL stimulated cultures blocked pDC development from lin^-Flt3^+ progenitors, via an Stat5 dependent mechanism (Esashi et al., 2008). This abrogation of pDC development was shown to be the result of suppression of Irf8, a crucial transcription factor in pDC development, by Stat5. The authors of this study concluded that GM-CSF thus actively suppressed pDC development. However, the study also showed a lack of $CD11b^-$ cDC development in these cultures. This may be the result of GM-CSF induced Irf8 suppression in DC progenitors, as Irf8 is also required for the development of $CD11b^-$ cDC (Aliberti et al., 2003b). Alternatively, GM-CSF may be acting on early progenitors, diverting them towards the production of monocytes and inflammatory DC, rather than steady state DC.

Mice deficient in GM-CSF or its receptor show no significant deficiencies in the development of steady state resident DC (Vremec et al., 1997; Kingston et al., 2009). Interestingly, the absence of GM-CSF showed no impact on the number of splenic pDC (Kingston et al., 2009), suggesting that if GM-CSF does inhibit pDC development, this does not appear to be a critical mechanism in the steady state. Mice lacking both GM-CSF and FL had significantly decreased numbers of the DC progenitors MDP and CDP, compared to mice lacking FL alone.

However, conflicting reports have been published on the role of GM-CSF in the development of peripheral DC. One report showed no change in the frequency of dermal $CD11b^-$ cDC in the absence of GM-CSF, but found a significant reduction in the number of $CD11b^+$ dermal cDC (Kingston et al., 2009). Interestingly, $CD11b^+$ dermal DC were significantly reduced in $GM\text{-}CSF^{-/-}FL^{-/-}$ mice, as compared to $FL^{-/-}$ mice alone (Kingston et al., 2009), perhaps, as discussed above, reflecting the contribution of monocytes to this subset. However, another report described a reduction in the frequency of $langerin^+CD11b^-CD103^+$ dermal DC, but no change in the frequency of $langerin^-CD11b^+$ dermal DC (King et al., 2010). It should be noted that GM-CSF has been shown to induce CD103 expression both *in vitro* (Jackson et al., 2011; Sathe et al., 2011) and *in vivo* (Zhan et al., 2011), although in the absence of Batf3, this subset does not cross-present antigen (Jackson et al., 2011). Thus, in the latter report (King et al., 2010), which measured the frequency of $CD11b^-CD103^+$ dermal cDC, it is unclear whether the lack of GM-CSF has led to a decrease in the frequency of the Batf3-dependent cross-presenting dermal DC subset, or simply an absence of CD103 expression in this subset. Indeed, it is telling that the frequency of $CD11b^-CD103^-$ dermal cDC rises in the absence of GM-CSF (King et al., 2010). The exact role of GM-CSF in the development of peripheral DC subsets thus remains to be established.

IL-6

Interleukin-6 (IL-6) has been implicated in both the differentiation and maturation of dendritic cells. Treatment with IL-6 promoted the differentiation of macrophages, rather than inflammatory DC, from monocytes (Chomarat et al., 2000), and IL-6 secreted by mesenchymal stem cells suppressed the differentiation and maturation of DC (Djouad et al., 2007). Further, IL-6 deficient mice showed enhanced DC maturation, while $gp130^{F759/F759}$ mice, with enhanced IL-6 signaling, had defects in DC maturation (Park et al., 2004). The numbers of immature resident DC were unchanged in these mice, indicating a role for IL-6 in maturation, rather than in development, of DC. In contrast, another study described an enhancement in both the number and function of GM-CSF derived DC upon treatment with anti-gp130, which induced gp130 signaling (Wang et al., 2002). In these studies, DC differentiation was tested by the stimulation of bone marrow or monocytes with GM-CSF. Further, gp130 is shared by a number of cytokines, such as leukemia inhibitory factor and IL-11, and gp130 has been implicated in mediating IL-3, stem cell factor, and FL signal transduction. A more definitive indication of the role of IL-6 in DC differentiation comes from the analysis of FL cultures with blocking antibody to IL-6, or with bone marrow from IL-6 deficient mice, which show deficiencies in the generation of DC (Brasel et al., 2000).

Lymphotoxin-β

Lymphotoxin-β (LTβ), a member of the tumor necrosis factor superfamily, has been shown to be important in the growth and survival of resident $CD8^-$ cDC. Mice deficient in LTβ have reduced numbers of splenic $CD8^-$ cDC, as well as reduced proliferation of $CD8^-$ DC, while overexpression of LTβ increased numbers of $CD8^-$ splenic cDC (Kabashima et al., 2005a).

TRANSCRIPTION FACTORS IN DC DIFFERENTIATION

Signal transducers and activators of transcription (Stats)

Stat3 has been shown to be a key component of the FL signaling pathway. Stat3 deficient mice show significant reductions in steady state DC numbers (Laouar et al., 2003), while enforced expression of Stat3 in megakaryocyte-erythroid progenitors restores some DC potential, mirroring the effects of deletion or overexpression of Flt3 (Onai et al., 2006). Stat3 deficient hematopoietic stem cells differentiate into common lymphoid or myeloid progenitors, but fail to produce DC committed CDP, indicating Stat3 acts early in the process of commitment to the DC lineage (Laouar et al., 2003). A similar effect is noted in the absence of Gfl-1, which is involved in the activation of Stat3.

Stat5 has been shown to be downstream of GM-CSF. As discussed above, it has been suggested that Stat5 activation suppresses the differentiation of pDC (Esashi et al., 2008). Stat5 deficient mice show slight reductions in lymphoid organ DC (Vremec et al., 1997; Esashi et al., 2008), echoing the phenotype of GM-CSFR deficient mice.

PU.1

PU.1 is expressed in both cDC and pDC and in the precursors for DC. As with Stat3, enforced expression of PU.1 restores some DC precursor activity in MEPs (Onai et al., 2006). Further, enforced expression of Flt3 upregulates PU.1 expression, suggesting PU.1 forms part of a positive feedback loop in the Flt3 signalling pathway. Subsequent work has shown PU.1 to be essential for both the induction and maintenance of Flt3 expression in a dose-dependent manner (Carotta et al., 2010). Restoration of Flt3 expression in PU.1-deficient progenitors did not restore DC development potential, suggesting the role of PU.1 in DC development extends beyond its influence on Flt3 expression.

Interferon regulatory factors (Irfs)

A number of the Irf family members, originally described for their involvement in regulating gene transcription in response

to interferon signaling, have been implicated in the development of particular DC subsets. Mice deficient in Irf2 have reduced numbers of resident $CD8^-$ cDC and Langerhans' cells (Ichikawa et al., 2004), while Irf4 deficient mice lack $CD8^-CD4^+$ cDC, and show a reduction in pDC numbers (Suzuki et al., 2004). Irf8 deficient mice have reduced numbers of pDC, resident $CD8^+$ cDC and Langerhans' cells, while $CD8^-$ cDC are unaffected (Schiavoni et al., 2002; Aliberti et al., 2003b; Tsujimura et al., 2003; Schiavoni et al., 2004). Subsequent work has shown Irf8 to be an important factor in the development of migratory $CD103^+$ cDC (Edelson et al., 2010).

Nuclear factor-κB (NF-κB) family

Mice deficient in the NF-κB/Rel family member, RelB, show dramatically decreased numbers of $CD8^-$ resident cDC (Wu et al., 1998). A similar phenotype has been observed in mice lacking Traf6 (Kobayashi et al., 2003). This may be due to its role in mediating LTβ signaling; as discussed above, LTβ is important for maintaining the $CD8^-$ resident cDC compartment (Kabashima et al., 2005a).

Inhibitors of DNA binding (Id)

The Id proteins belong to the family of basic helix-loop-helix (HLH) transcription factors, and are involved in inhibiting other members of the HLH family. Id2 has been demonstrated to be important in cDC development. Mice lacking Id2 have dramatically reduced numbers of resident $CD8^+$ cDC, migratory $CD103^+$ cDC, and Langerhans' cells (Hacker et al., 2003; Edelson et al., 2010). pDC numbers in these mice are increased. Conversely, ectopic expression of Id2 or Id3 in human $CD34^+$ progenitors inhibits pDC development, but does not alter cDC development. Id2 thus appears necessary for cDC development, while inhibiting pDC development (Spits et al., 2000). Interestingly, transcriptional profiling of Id2 deficient mice showed a role for Id2 in the suppression of many B-cell related genes (Hacker et al., 2003). The transcriptional similarities between pDC and B-cells may therefore form the basis for Id2-mediated suppression of pDC development.

Ikaros

The zinc finger DNA binding protein ikaros has been shown to be an integral component of DC development. Mice deficient in this transcription factor have numerous hematopoietic defects, including a significant reduction of DC in the spleen and thymus. $Ikaros^{-/-}$ mice were unable to produce DC upon adoptive transfer (Wu et al., 1997). Recently, it has been reported that mice lacking functional Ikaros fail to generate DC in response to FL (Brugmann and Winandy, 2010). Interestingly, GM-CSF stimulated bone marrow was able to produce DC normally, suggesting Ikaros is important in the development of steady state, rather than inflammatory DC. Further, progenitors from Ikaros null mice were reported to have decreased levels of expression of Flt3, Irf4, Irf8 and SpiB, suggesting Ikaros acts upstream of these factors that are known to be important in DC development.

Batf3

The basic leucine zipper transcription factor, ATF-like 3 (batf3), is expressed in resident $CD8^+$ and $CD8^-$ cDC (Hildner et al., 2008), as well as $CD103^+$ and $CD11b^+$ migratory cDC (Edelson et al., 2010). Mice deficient in Batf3 lack $CD8^+$ and $CD103^+$ cDC, while $CD8^-$ cDC and $CD11b^+$ cDC remain unaffected (Edelson et al., 2010), indicating a specific requirement for Batf3 activity in the cross-presenting steady state cDC subsets.

E2-2

The transcription factor E2-2 is part of the family of E proteins, a group of the HLH family whose effects are antagonized by the Id proteins. Deletion of E2-2 in early progenitors abrogates pDC development (Cisse et al., 2008). E2-2 deletion in differentiated pDC induces reversion of these cells to a cDC-like phenotype (Ghosh et al., 2010), indicating E2-2 is essential in not only the development, but also the maintenance, of the pDC population.

SpiB

SpiB is expressed at high levels in pDC. A deficiency in SpiB leads to impaired development of pDC from human $CD34^+$ hematopoietic progenitors (Schotte et al., 2004). Interestingly, SpiB, while expressed in murine pDC, does not appear to be necessary for their development. Mice deficient in SpiB show normal levels of splenic pDC (Reizis, 2010), perhaps due to redundancy in the transcriptional factor network.

THE NETWORK DRIVING DC DEVELOPMENT

A complex network of cytokines and transcription factors appears to govern the development of the various DC subsets from early progenitors. Although the earliest hematopoietic progenitors, long-term hematopoietic stem cells, are $Flt3^-$, they upregulate Flt3 expression upon differentiation into non self-renewing multipotent progenitors. From here, progenitors may differentiate into $Flt3^-$ megakaryocyte-erythrocyte progenitors, $Flt3^+$ common lymphoid progenitors (CLP), or myeloid progenitors, which are heterogeneous for Flt3 expression.

Depending on external signals, CLP may upregulate expression of the transcription factor, leading to the down-regulation of Flt3 expression (Holmes et al., 2006) and

differentiation into B cells (Nutt et al., 1999). Alternatively, they may sustain Flt3 expression and activate the transcription factor E2-2 (Cisse et al., 2008). E2-2 subsequently activates downstream transcription factors, including SpiB and Irf8, culminating in the differentiation of CLP into pDC.

In the meantime, a subset of those myeloid committed progenitors with Flt3 expression may differentiate into the so called MDP (Fogg et al., 2006), while those which have lost Flt3 expression give rise to alternate lineages, such as granulocytes.

These MDP, upon encountering cytokines such as FL, M-CSF or GM-CSF, differentiate into either CDP or monocytes. These monocytes then enter the circulation, and can give rise to macrophages, $CD103^-$ cDC, or under inflammatory conditions, to inflammatory DC (Serbina et al., 2003; Xu et al., 2007).

CDP may activate E2-2 (Cisse et al., 2008; Ghosh et al., 2010), which activates and sustains the developmental program that drives pDC differentiation. Alternatively, upon upregulation of Id2, and the retention of high levels of PU.1 expression, CDP can differentiate into pre-cDC. Exposure of CDP to GM-CSF, and the subsequent activation of Stat5 and suppression of Irf8, which actively suppresses differentiation of pDC and the equivalent of $CD8^+$ DC (Esashi et al., 2008), lead to the production of DC similar to monocyte-derived DC. Irf8 has also been shown to have a role in the development of $CD8^+$ and $CD103^+$ cDC (Schiavoni et al., 2002; Aliberti et al., 2003a; Schiavoni et al., 2004; Edelson et al., 2010), both of which develop via pre-cDC. GM-CSF mediated Irf8 suppression may be important in commitment to pre-cDC, while other factors such as increased Flt3 signaling, or Batf3 activation in pre-cDC may override the effect of GM-CSF mediated Irf8 suppression and promote the differentiation of $CD8^+$ and $CD103^+$ cDC.

Finally, pre-cDC leave the bone marrow and migrate into peripheral lymphoid tissues, such as the lymph nodes and spleen, or peripheral non-lymphoid tissues, such as the kidneys, lungs or skin (Naik et al., 2006; Ginhoux et al., 2009; Liu et al., 2009; Varol et al., 2009). The factors that determine the migration patterns of pre-cDC remain unknown; their final destination may be a stochastic effect, determined by the random differential expression of chemokine receptors, or pre-cDC in the bone marrow may be predestined to migrate to certain tissues. At their final destination, pre-cDC may be exposed to cytokines such as lymphotoxin β (Kabashima et al., 2005b) or M-CSF, and differentiate into $CD8^-$ or CX_3CR1^+ cDC. Alternatively, driven by factors that are as yet unknown, pre-cDC may upregulate Irf8 and Batf3 to give rise to $CD8^+$ or $CD103^+$ cDC.

CONCLUSION

A wide and complex array of factors governs the development of DC subsets. Ultimately, the subsets produced reflect an interplay of different families of proteins, including cytokines and their receptors, and transcription factors (Fig. 1). How these factors—the production of each of these cytokines, the expression of their receptors on DC progenitors, and the expression of different transcription factors—are regulated is as yet unknown, although some clues are emerging.

A number of cytokines, including GM-CSF and FL are upregulated upon inflammation, promoting the development and expansion of both steady-state type and inflammatory DC. It is evident that the network governing DC development is malleable, changing to meet the needs of the immune system. How these processes are regulated in both the steady state and under inflammatory conditions, remains to be revealed.

ABBREVIATIONS

cDC, conventional DC; CDP, common DC progenitors; CLP, common lymphoid progenitors; CMP, myeloid committed progenitors; DC, dendritic cells; FL, fms-like tyrosine kinase 3 (Flt3) ligand; GM-CSF, granulocyte macrophage colony stimulating factor; Id, inhibitors of DNA binding; Irfs, interferon regulatory factors; LTβ, lymphotoxin-β; M-CSF, macrophage colony stimulating factor; MDP, macrophage DC progenitor; MEP, megakaryocyte-erythroid progenitors; NF-κB, nuclear factor-κB; pDC, plasmacytoid DC; STATs, signal transducers and activators of transcription; TNFα, tumour necrosis factor α

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