Allergology International. 2007;56:215-223
DOI: 10.2332/allergolint.R-07-149

REVIEW ARTICLE

Dendritic Cells—Ontogeny—

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ABSTRACT

Dendritic cells (DC) play key rolls in various aspects of immunity. The functions of DC depend on the subsets as well as their location or activation status. Understanding developmental lineages, precursors and inducing factors for various DC subsets would help their clinical application, but despite extensive efforts, the precise ontogeny of various DC, remain unclear and complex. Because of their many functional similarities to macrophages, DC were originally thought to be of myeloid-lineage, an idea supported by many *in vitro* studies where monocytes or GM-CSF (a key myeloid growth factor) has been extensively used for generating DC. However, there has been considerable evidence which suggests the existence of lymphoid-lineage DC. After the confusion of myeloid-/lymphoid-DC concept regarding DC surface markers, we have now reached a consensus that each DC subset can differentiate through both myeloid- and lymphoid-lineages. The identification of committed populations (such as common myeloid- and lymphoid progenitors) as precursors for every DC subsets and findings from various knockout (KO) mice that have selected lymphoid- or myeloid-lineage deficiency appear to indicate flexibility of DC development rather than their lineage restriction. Why is DC development so flexible unlike other hematopoitic cells? It might be because there is developmental redundancy to maintain such important populations in any occasions, or such developmental flexibility would be advantageous for DC to be able to differentiate from any "available" precursors *in situ* irrespective of their lineages. This review will cover ontogeny of conventional ($CD8^{+/-}$ DC) DC, plasmacytoid DC and skin Langerhans cells, and recently-identified many Pre-DC (immediate DC precursor) populations, in addition to monocytes and plasmacytoid DC, will also be discussed.

KEY WORDS

dendritic cells, development, lineage, ontogeny, precursor

INTRODUCTION

Dendritic cells (DC) play key rolls in adoptive immunity, inducing antigen-specific immunity or tolerance. The functions of DC depend on DC subsets as well as their location or activation status. Understanding developmental lineages, precursors and inducing factors of each DC subset would help generating and/or activating "appropriate" DC subset *in vitro* or *in vivo* as a potential treatment for various diseases such as cancers and autoimmune diseases. However, despite extensive efforts, the precise developmental lineage, precursors of various DC, including skin Langerhans cells (LC), remain unclear and complex.[1] Although the ultimate goal would be to comprehend ontogeny of human DC, most findings of human DC ontogeny is considerably based on *in vitro* cell culture assay using limited hamatopoietic cell populations. Meanwhile in animal models, we can utilize *in vivo* labeling assay, reconstitution assay using wide variety of cell populations or various targeted gene knockout mice etc, findings of which have greatly contributed to understanding of DC ontogeny *in vivo*. Therefore, this review is mainly based on *in vivo* findings from animal studies, combined with those of *in vitro* (human and animals), although one could argue whether experimental techniques like donor cell reconstitution in irradiated recipients truly reflects normal DC differentiation in steady-state.

HISTORICAL BACKGROUND ON DEVELOPMENTAL LINEAGES OF DC

Because of their many functional similarities to macrophages, DC and epidermal LC, were originally thought to be of myeloid lineage, an idea supported by studies demonstrating DC generation from mono-

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Received 17 April 2007.
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Table 1 Various DC subsets and their unique functions

DC subsets	Functions	
Conventional DC		
CD8⁺ DC	・Th1 differentiation[10]	・Cross presentation[11] (constitutively)
CD8⁻ DC	・Th2 differentiation[10]	・Cross presentation[11] (upon activation)
Plasmacytoid DC	・Viral immunity[17] (upon activation)	・Tolerogenic potential[17] (constitutively)
Epidermal LC	・Primary APC in skin[22] (upon activation)	・Tolerogenic potential?[27,28]

DC, dendritic cells; LC, Langerhans cells; APC, Antigen-presenting cells

cytes using granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin (IL)-4 *in vitro*,[2] or that phagocytic monocytes could differentiate into DC *in vivo*.[3] Considerable evidence also suggested the existence of lymphoid-lineage DCs because $CD8^+$ thymic DC could be generated from $CD4^{low}$ early thymic precursors *in vivo* when intrathymically transferred.[4] Other studies further confirmed and extended the concept that $CD8^+$DC are of lymphoid origin[5,6] (and $CD8^-$DC are of myeloid origin). However, the simple concept was soon challenged by several studies where generation of both $CD8^+$ and $CD8^-$DC subsets from either lymphoid-,[7,8] or myeloid-committed progenitors[8] had been demonstrated *in vivo*.

VARIOUS DC SUBSETS AND THEIR UNIQUE FUNCTIONS

All the DC subsets can uptake antigens and present them to T cells, but the detailed functions in immunity varies considerably, depending on the subset and presence/absence of inflammatory stimuli such as viral or bacterial infections. This review will focus on the following DC subsets (Table 1), discussing their developmental lineages and precursors.

CONVENTIONAL DC ($CD8^+$- AND $CD8^-$DC)

The first DC category is termed as conventional DC. In mice, these DC can be divided into two subsets, $CD8^+$- and $CD8^-$DC.[9] $CD8^+$ and $CD8^-$DC preferentially activate T cells toward Th1 and Th2 differentiation,[10] respectively. The $CD8^+$DC constitutively cross-presents antigens to T cells, while $CD8^-$DC will do so upon their activation.[11] These conventional DC reside within lymph nodes, spleen, thymus, but not in bone marrow (BM).[12] In thymus, most of the resident DC express CD8 and there is only small $CD8^-$DC population,[13] as compared to those of other organs. The question of whether these conventional DC subsets are developmentally distinct populations from each others or are merely presenting different maturation/differentiation steps will be discussed later.

PLASMACYTOID DC

Plasmacytoid DC is characterized by the round shape, plasmacytoid morphology and remarkable ability to secret copious amount of interferon-γ in viral or bacterial infection,[14-16] while they can also exhibit tolerogenic potential when not stimulated.[17] They reside within lymph nodes, spleen, thymus, and BM,[18-20] but the BM-plasmacytoid DC population does not seem to be developmentally equal to those of other organs since the BM-plasmacytoid DC population are capable of differentiating into conventional DC subsets.[21] Thus, some of plasmacytoid DC populations, as do monocytes, can serve as pre-DC (Immediate DC precursor) for conventional DC subsets, which will be discussed later.

EPIDERMAL LC (MIGRATORY DC)

Epidermal LC, the primary sentinels of the skin, is the first described DC subset, which can be characterized by the Langerin expression and cytoplasmic Birbeck's granules. Upon activation (and constitutively to some extent), epidermal LC migrate through dermis into regional lymph nodes to present antigens to T cells.[22] Therefore, this type of DC is categorized as "migratory DC". Other non-lymphoid tissue residing DC, such as dermal- and intestinal DC, both of which are not discussed in this review, would also fall into this category. In steady-state condition, LC have much long life span (labeled only ~25% of LC after 2 weeks) while most of other DC in other organs are labeled approximately by 3–10 days[23] Epidermal LC appear to self-renew themselves within skin and are hardly repopulated by circulating precursors unless their residing environment is disturbed.[24] Although LC cross-present viral antigen inefficiently (as compared to dermal DC),[25] a very recent study identified their potential contribution against HIV infection,[26] revealing the biological functions of Birbick granules for the first time. Possible tolerogenic capacity of LC have also been indicated.[27,28]

DC SUBSETS FOR TH1-, TH2-, TH17- AND REGULATORY T CELL DIFFERENTIATION IN MICE AND HUMAN

There has been considerable evidence which suggest that DC subsets may associate with differentiation of T helper 1 cells (Th1)-, Th2-, Th17- or regulatory T cell (Treg) in a subset specific manner. As partially mentioned elsewhere, murine $CD8^+$- and $CD8^-$DC appear to intrinsically induce Th1 and Th2, respectively.[10] However, it has been shown that tumor cells

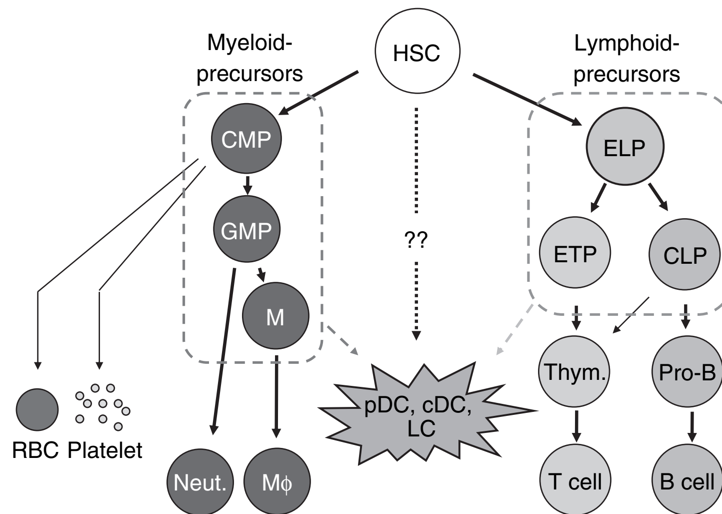

Fig. 1 Hematopoiesis and DC developmental pathways. All the DC subsets (pDC, cDC and LC) can derive from both myeloid- and lymphoid progenitor(s). RBC: red blood cells, CMP: common myeloid progenitor, GMP: granulocyte/macrophage progenitor, M: monocytes, Neut.: neutrophils, Mϕ: macrophages, HSC; hematopoietic stem cell, ELP: earliest lymphoid progenitor, ETP: early T-lineage progenitor, Thym. thymocytes, CLP: common lymphoid progenitor, Pro-B: Pro B cell, pDC: plasmacytoid dendritic cells, cDC: conventional dendritic cells, LC: Langerhans cells.

can convert $CD8^-$DC (or termed $CD11b^+$ myeloid DC) to induce Treg[29] or that $CD11b^+$ myeloid DC subset induces Th17 much more efficiently than other DC subsets in experimental autoimmune encephalomyelitis.[30] $CD11b^+$ myeloid DC can also induce Th1 when generated with GM-CSF and activated with LPS.[31] Plsasmacytoid DC show tolerogenic potential[17,32] but can also induce Th1.[31,33] In humans, DC subsets seem to be less heterogeneous and two distinct DC subsets are known to exist: $CD11c^+CD14^-CD1a^+$ myeloid DC (mDC) and $CD11c^-$ $BDCA2^+CD123^+$ plasmacytoid DC.[34] Human mDC and (the descendent of) plasmacytoid DC used to be termed DC1 and DC2 respectively, because the mDC and plasmacytoid DC were shown to preferentially induce Th1 and Th2, respectively.[35] However, the human DC1/DC2 concept was soon challenged by studies in which human plasmacytoid DC was shown to be able to induce Th1 response.[36,37] Furthermore, human plasmacytoid DC can also induce T reg.[38] LC was thought to induce Th1 because of its ability to produce bioactive IL-12, evidenced by a study using human LC.[39] However, LC has recently been shown to induce Th2[40] or to have a regulatory function in mice.[27] Overall, although there is a "tendency" of DC lineage- or subset-specific Th- or Treg induction, such induction is also highly dependent on the experimental condition or environmental factors such as cytokines, chemokines, stimuli through various Toll like receptors,[41] In other words, Th- or Treg differentiation by DC/LC depends on how they are generated/developed, matured or activated.

DEVELOPMENTAL LINEAGES AND PRECURSORS

HEMATOPIESIS AND DC DEVELOPMENTAL LINEAGES

All the DC subsets, like other hematopoietic cells, ultimately derive from hematopoietic stem cells (HSC). HSC self-renew themselves and can differentiate into myeloid- and lymphoid-committed hamatopoietic precursors (and other lineages like erythrocyte or megacaryocyte) (Fig. 1). In lymphopoiesis, common lymphoid progenitor (CLP)[42] used to be thought as the governing lymphoid-committed progenitor which could give rise to all the lymphoid lineages such as T cells and B cells. The CLP is characterized by its IL-7 receptor (R)-α expression, together with other hematopoietic progenitor markers such as c-kit and Sca-1. IL-7Rα knockout mice exhibited significantly impaired lymphopoiesis, supporting the idea of CLP might be a vital precursor for lymphopoiesis.[43] However, the current schema of hamatopoiesis is a little more complex. Other, more potent thymus repopulating progenitors, called early T-lineage progenitor (ETP) and the precursor have been identified within thymus[44] and BM[45] both of which are negative for IL-7Rα expression. Furthermore, earliest lymphoid pro-

genitor (ELP)[46] have been identified and is supposed be in upstream of other lymphoid progenitors from the characteristics (c.f. ELP remains to have myeloid lineage potential). As for myeloid-lineage, common myeloid progenitor (CMP)[47] and their progeny (granulocyte-monocyte progenitor and monocytes etc) appear to be only myeloid-committed DC precursors. DC which are generated through these committed-precursors would be lymphoid- or myeloid-lineage DC. Indeed, some of plasmacyotid- and thymic DC (but not splenic DC, regardless of their CD8 expression) in normal mice have IgH D-J rearrangement,[48] which is a trace of lymphoid lineage.[49] Such location dependence of IgH rearrangement in DC might be because lymphoid- (thymic precursor) and myeloid progenitors are simply abundant in thymus and spleen, respectively, and because plasmacytoid DC population is initially generated in BM where precursors of both lineages (CMP and CLP etc) are abundant. The finding of lymphoid trace of steady-state DCs in normal mice may provide evidence that DC have both lymphoid- and myeloid origin *in vivo*, in addition to those obtained from cell transfer studies. The development of DC seems to be quite flexible since at least two distinct developmental pathways exist to form a particular DC subset. Then one might wonder if there are any other pathways for DC generation. There was a report indicating a common DC precursor population,[50] but the study was confounded by contamination of more than two populations.[51] So far, current findings have not achieved a clear consensus for such DC-restricted pathway yet.

ESSENTIAL FINDINGS FROM *IN VITRO* CELL CULTURE ASSAY

Human monocytes are first shown to differentiate into DC when cultured with GM-CSF+IL-4,[2] and can have some of LC characteristics when transforming growth factor (TGF)-β is added to the cytokine combination.[52] Human CD34+ cells (HSC-enriched population) can differentiate into DC or LC when cultured with GM-CSF and tumor necrosis factor (TNF)-α,[53] IL-3 and TNF-α,[54] or FMS-like tyrosine kinase-3 (FIT3) ligand (L) and TGF-β.[55] Mouse BM cells can differentiate into DC/LC when cultured with GM-CSF+TNF+stem cell factor (SCF),[56] or into plasmacyotoid-, $CD8^+$- and $CD8^-$DC when cultured with FLT3L.[57,58] There is an interesting report showing the importance of GM-CSF (+SCF) and IL-7 for DC generation from CMP and CLP *in vitro*, respectively.[59] Mouse spleen cells can differentiate into DC when cultured with GM-CSF+FLT3L (or+SCF).[60] Mouse thymic precursor populations can differentiate into thymic DC when cultured with IL-7-cytokine mix (+ IL-3) without GM-CSF, the myeloid lineage growth factor.[61] (Table 2)

From these findings, GM-CSF, IL-7 or FLT3L appears important for DC generation *in vitro*. The starting material tends to be cell populations which could be obtained from peripheral blood in human studies because of its easier access in many aspects, while cell preparations from various lymphoid organs are popular in murine studies, which makes direct comparison difficult between human and mouse studies.

ESSENTIAL FINDINGS FROM *IN VIVO* CELL TRANSFER ASSAY

In vivo cell transfer studies have significantly contributed to the understanding of DC lineages. Unlike the case of *in vitro* studies, where many myeloid-differentiation of DC had been shown, the first *in vivo* evidence of DC differentiation *in vivo* was that of lymphoid-lineage. A study demonstrated that thymic precursor population (with lymphoid-restricted differentiation capacity) can give rise to DC and T cells when intrathymically transferred.[4] Meanwhile, the first evidence of myeloid-lineage DC differentiation *in vivo* was given later in a study where phagocytic monocytes differentiated into DC.[3] Generation of both $CD8^+$- and $CD8^-$DC have been shown after intravenous transfer of lymphoid-committed ($CD4^{low}$ thymic precursor[7] or CLP[8]) or myeloid-committed precursor (CMP[8]), which have challenged the $CD8^+$DC = lymphoid-lineage DC concept. Generation of plasmacytoid DC from $FLT3^+$ fractions of both CLP and CMP has also been shown.[62] In the case of LC, LC differentiation through lymphoid-lineage (thymic precursor[63] and CLP[64]) and myeloid-lineage (CMP[64] and BM monocyte precursor[65]) has been shown *in vivo*. (Table 3) Overall, these *in vivo* cell transfer studies have suggested the DC develop through both lymphoid- and myeloid pathways, indicating the developmental flexibility of DC. CLP is more potent than CMP at DC generation in a per cell basis, but since there is an excess of CMP over CLP in BM,[66] perhaps both the committed-progenitors significantly contribute to DC generation as a whole. Interestingly, most of the DC precursor populations have receptors such as GM-CSFR, IL-7Rα, FLT3 or c-kit (SCFR), reflecting the findings of cytokine requirement in *in vitro* studies.

FINDINGS FROM KNOCKOUT MICE (LINEAGE RESTRICTION OR DIFFERENTIATION FACTORS?)

The findings in knockout mice are complicated (Table 4). The data from knockout mice that show hematopoietic lineage restriction has been thought to readily indicate DC lineages. For example, Ikaros DN mice have showed significantly impaired lymphoid development and absence of certain DC subsets (splenic $CD8^+$- and $CD8^-$DC and thymic $CD8^+$DC),[67] by which these absent DC subsets are considered to be of lymphoid lineage. However, there have been many inconsistent findings in other knockout mice

Table 2 Essential findings from in vitro cell culture assay

Precursors	*Differentiation factors*	*Induced DCs*	*Ref.*
Human monocytes	GM-CSF + IL-4	DC	2
	GM-CSF + IL-4 + TGF-β	LC	52
Human CD34$^+$ cells	GM-CSF + TNF-α	DC, LC	53
	IL-3 + TNF-α	LC	54
	FLT3L + TGF-β	DC	55
Mouse bone marrow	GM-CSF + TNF-α + SCF	DC	56
	FLT3L	pDC, CD8$^{+/-}$ DC	57, 58
Mouse splenocytes	GM-CSF + FLT3L (or + SCF)	DC	60
Mouse thymocytes	IL-7-mix (without GM-CSF)	Thymic DC	61

GM-CSF, granulocyte/macrophage colony-stimulating factor; IL, interleukin; TNF-α, tumor necrosis factor-α; SCF, stem cell factor, FLT3L, FMS-like tyrosine kinase 3; pDC, plasmacytoid DC

Table 3 Essential findings from *in vivo* cell transfer assay

Transferred populations	*yielded DCs*	*Ref.*
(CD4^{low}) thymic precursor	CD8$^{+/-}$ DC, LC	4, 7, 63
Monocytes	LC	3, 65
CLP	CD8$^{+/-}$ DC, LC	8, 64
CMP	CD8$^{+/-}$ DC, LC	8, 64
FLT3$^+$ fractions of CLP or CMP	pDC	62
pDC (in bone marrow)	CD8$^{+/-}$ DC	85

CLP, common lymphoid progenitor; CMP, common myeloid progenitor; FLT3, FMS-like tyrosine kinase 3; pDC, plasmacytoid DC

that also show impaired lymphopoiesis, such as mice deficient for Ikaros C[68] or Common γ-chain[69] where development of only limited (only CD8$^-$DC) to no DC subsets are affected. Furthermore, CD8$^-$DC is absent in Relb knockout mice[70] where myeloid hyperplasia is observed, while the same CD8$^-$DC subset is again absent in PU.1 knockout mice where development of B- and myeloid lineage is deficient.[71,72] Meanwhile, plasmacytoid DC and CD8$^+$DC are absent, accompanied with defective myeloid development in mice deficient for ICSBP (IRF8),[73,74] which form transcription complexes with PU.1. These findings might simply indicate complicated regulation of lymphopoiesis, but they might as well indicate the existence of indispensable differentiation factor for certain DC subsets regardless of their passed lineages. At least the absence of LC in TGF-β knockout mice,[75] which have normal development of both lymphoid- and myeloid-lineage, probably favors the latter idea. LC are also absent in mice deficient for Id2,[76] a transcription factor regulated by TGF-β, or Runx3[77] which mediates TGF-β responses. Meanwhile, LC and monocytes are absent in macrophage colony-stimulating factor (M-CSF) R knockout mice,[65] which may provide evidence that LC are of myeloid lineage. Paradoxical finding is that GM-CSFR knockout mice show normal DC development[78] or that IL-7Rα knockout donor cells can reconstitute various DC subsets, including epidermal LC, in lymph nodes, spleen, thymus or BM.[79] However, given the developmental flexibility of DC, the absence of signals in a DC differentiation pathway would have been compensated by those of the other pathways. Absence of these cytokines (GM-CSF or IL-7 etc) appears to be compensated by FLT3L,[57,58] although addition of GM-CSF to FLT3 improves yields of DC as compared to those with either cytokine alone.[80] The FLT3 knockout mice show low DC numbers[81] as do mice deficient for STAT3,[82] which is a key factor in FLT3 signal cascade. Furthermore, FLT3L exceptionally increase DCs in mice[83] and human[84] when systemically administered.

PRE-DCs

Distinct DC subsets possess different functions, but their developmental independency is not perfectly clear. As previously mentioned, plasmacytoid DC has been shown to be able to differentiate into conventional DC.[85] Thus, plasmacytoid DC, at least those in bone marrow, can serve as pre-DC for cDC in an inflammatory condition. Many pre-DC populations, or immediately DC precursors, have been identified so far in blood, spleen and BM (Table 5). In BM, a pre-DC called “pre-immunocyte” (B220$^+$CD11c$^+$CD31$^+$Ly-6C$^+$)[86] has been found to generate immature DC and macrophage under aegis of M-CSF or GM-CSF and also to generate both CD8$^+$- and CD8$^-$ conventional D in thymic organ culture. In addition, the pre-immunocyte population immediately produce high level of interferon-α mRNA as do plasmacytoid DC. A few years later, 2 phenotypically distinct populations (B220$^+$CD11c$^+$MHCII$^-$Gr-1$^+$M-CSFR$^-$ and B220$^-$CD11c$^+$MHCII$^-$Gr-1$^-$M-CSFR^{int})[87] have been identified. The B220$^+$ pre-DC give rise to both plasmacytoid DC, conventional DC, while the latter B220$^-$ pre-DC give rise to only conventional DC. From the surface phenotype and generating DC subsets, the former population might be equal or closely related to the pre-immunocyte population. BM-monocyte population (Ly-6C^{high}CD11b$^+$CD11c$^-$B220$^-$CD24^{int})[88] also differentiate into splenic conventional DC, although

Table 4 Findings from knockout mice

Knockout genes	*Phenotypes*	*Absent DCs*	*Ref.*
Ikaros DN	Deficient T-, B-, NK-lineage	$CD8^{+/-}$ DC	67
Ikaros C	Deficient T-, B-, NK-lineage	$CD8^{-}$ DC	68
Common γ-chain	Deficient T-, B-, NK-lineage	None	69
Relb	Myeloid hyperplasia	$CD8^{-}$ DC	70
PU.1	Deficient myeloid-, B-lineage	$CD8^{-}$ DC, thymic DC	71, 72
ICSBP (IRF-8)	Deficient myeloid-lineage	pDC, $CD8^{+}$ DC	73, 74
TGF-β	Normal myeloid-, lymphoid-lineage	LC	75
Id2	Deficient NK-lineage	LC, $CD8^{+/-}$ DC	76
Runx3	Deficient T-lineage	LC	77
M-CSFR	Deficient monocytes	LC	65
STAT3	Increased macrophage	pDC, $CD8^{+/-}$ DC	82

pDC, plasmacytoid DC, M-CSFR, macrophage colony-stimulating factor

Table 5 Pre-DCs

populations	*Surface phenotypes*	*Yielded DCs*	*Ref.*
bone marrow			
Pre-immunocyte	Ly-$6C^{+}$ $CD11c^{+}$ $CD31^{+}$ $B220^{+}$	$CD8^{+/-}$ DC, pDC?, (Mϕ)	86
$B220^{+}$ DC precursor	$B220^{+}$ $CD11c^{+}$ $MHCII^{-}$ Gr-1^{+} M-$CSFR^{-}$	$CD8^{+/-}$ DC, pDC	87
$B220^{-}$ DC precursor	$B220^{-}$ $CD11c^{+}$ $MHCII^{-}$ Gr-1^{-} M-$CSFR^{int}$	$CD8^{+/-}$ DC	87
Ly-$6c^{high}$ monocyte	Ly-$6c^{+}$ $CD11c^{-}$ $MHCII^{-}$ $CD24^{int}$	$CD8^{+/-}$ DC, (Mϕ)	88
blood			
Ly-$6c^{low}$ monocyte	Ly-$6c^{low}$$CD11b^{+}$ NK1.1^{-} SSC^{low}	$CD8^{-}$ DC	89
spleen			
Splenic pre-cDC	$CD11c^{int}CD45RA^{low}CD43^{int}$ SIRP-$\alpha^{int}CD4^{-}$ $CD8^{-}$	$CD8^{+/-}$ DC	89

pDC, plasmacytoid DC; cDC, conventional DC; M-CSFR, macrophage colony-stimulating factor; Mϕ, macrophage.

repopulating only very small percentage (2%) of total splenic DC. Recently, a group identified a much more efficient splenic DC-repopulating population within spleen, called "intrasplenic pre-cDC" ($CD11c^{int}CD45RA^{low}CD43^{int}$SIRP-$\alpha^{int}CD4^{-}CD8^{-}$MHC II^{-}).[89] The intrasplenic pre-cDC repopulates splenic DC in steady-state most efficiently while monocyte populations hardly do so. The pre-cDC population does not respond M-CSF, therefore they appear different from monocytes. Blood- or splenic monocytes (Ly-$6C^{low}$ $CD11b^{+}$NK1.$1^{-}SSC^{low}$) can give rise to splenic DC in inflammatory conditions, but they form only a distince ($CD8^{-}$) DC population. Since most of known pre-DC (plasmacyitoid DC or monocytes) differentiate into DC with inflammatory stimuli or inflammatory growth factors such as GM-CSF. Although there have been many studies using monocyte-derived or GM-CSF-induced DC, these protocol might not be suitable to examine DC functions in steady-state, or tissue-residing DCs.

Incidentally, it has been reported that $CD8^{-}$DC can differentiate into $CD8^{+}$DC,[90] indicating precursor-progeny relationship between the two conventional DC subsets. However, this might be because of the existence of direct $CD8^{+}$DC precursor (but yet negative for CD8 expression) within $CD8^{-}$DC population like others pointed.[91]

THE ONTOGENY OF DC—THE PLURAL DEVELOPMENTAL REGULATION—

As mentioned, the DC development is complex and quite flexible, unlike other hamatopoietic lineages like T-, B-, NK cells, neutrophils or monocytes etc. One might wonder why such flexibility exists in DC development. The speculations here are as follows. The flexibility might simply be a redundant mechanism to maintain the important hamatopoietic populations, DCs, in any situations. Alternatively, since the existence of tissue-residing DC and their functions are relevant in various lymphoid organs (thymus, lymph nodes, spleen etc) or in non-lymphoid organs (skin, gut etc) to induce immunity and/or tolerance (such that within thymus),[92] it would be advantageous for a DC subset to be able to differentiate in situ from certain "available" precursors such as organ-seeding- or self-repopulating populations in the organ according to the required functions (or DC subset), irrespective of the committed-lineages of DC precursors.

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