**Review**

# Development of Dendritic Cell System

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The dendritic cell system contains conventional dendritic cells (DCs) and plasmacytoid pre-dendritic cells (pDCs). Both DCs and pDCs are bone marrow derived cells. Although the common functions of DCs are antigen-processing and T-lymphocyte activation, they differ in surface markers, migratory patterns, and cytokine output. These differences can determine the fate of the T cells they activate. Several subsets of mature DCs have been described in both mouse and human and the developmental processes of these specialized DC subsets have been studied extensively. The original concept that all DCs were of myeloid origin was questioned by several recent studies, which demonstrated that in addition to the DCs derived from myeloid precursors, some DCs could also be efficiently generated from lymphoid-restricted precursors. Moreover, it has been shown recently that both conventional DCs and pDCs can be generated by the Flt3 expressing hemopoietic progenitors regardless of their myeloid- or lymphoid-origin. These findings suggest an early developmental flexibility of precursors for DCs and pDCs. This review summarizes some recent observations on the development of DC system in both human and mouse. *Cellular & Molecular Immunology*. 2004;1(2):112-118.

## Introduction

Dendritic cells (DCs) are sparsely distributed bone marrow-derived, phenotypically and functionally hetero-geneous leukocytes. As the most potent antigen-presenting cells (APCs), DCs are specialized for the uptake, processing, transport and presentation of antigens to T cells (1-4), and are capable of priming naïve T cells (3). Although the development of DCs from early hemopoietic precursors is not fully understood (5, 6), terminal stages of DC deve-lopment and their life cycle during an immune response are well defined. Immature DCs reside in peripheral tissues and constantly capture antigens from the local environment, process and present them in association with surface major histocompatibility complex (MHC) molecules. The presence of microbial products or tissue damage in the environment that the DCs sample initiates their migration to peripheral lymphoid organs and their transition from antigen-capturing cells into antigen presenting cells. The migration and functional transition of DCs correlate with: decreased antigen uptake, increased half-life of surface

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MHC-peptide complexes, upregulation of co-stimulatory molecules, altered expression of chemokine receptors and production of cytokines that are crucial for effector T cell (helper or cytotoxic) differentiation (3). As a result, antigens captured by DCs are, in the cell bound form, transported to and concentrated in the peripheral lymphoid organs for presentation to antigen-specific T cells.

Not all DCs share this common life cycle. Several DC types with different biological features have been identified in different tissues, including Langerhans cells (LC) in the epidermis, interstitial DCs in various tissues, thymic DCs and DC populations found in lymphoid organs. Differences in the tissue distribution, phenotype and function indicate the existence of a heterogeneous population of DCs. DCs were originally considered to be of myeloid origin and closely related to monocytes, macrophages and granulo-cytes. Recent studies, however, suggest that DC can be generated along distinct developmental pathways and can originate from precursors of different hemopoietic lineages.

Another cell type, which belongs to the DC system, is the so-called plasmacytoid pre-DCs (pDCs). The pDCs were originally identified in human blood and lymphoid tissues as plasmacytoid T cells or plasmacytoid monocytes due to their morphological similarity to plasma cells and expression of certain T cell markers and MHC class-II molecules. These cells, also termed pDC2 in human, have a phenotype different from DCs (CD11c$^-$CD45RA$^{hi}$CD11b$^-$ MHC-II$^{lo}$IL-3R$^{hi}$CD4$^+$) and are efficient type I interferons (IFNs) producing cells (7). Recently, the mouse equivalent has also been identified in the mouse blood (8) and in all lymphoid tissues (9-12).

In this review we will discuss the heterogeneity of DCs and pDCs and their development from distinct hemopoietic precursors.

## Heterogeneity of DCs and functional diversity of DC subsets

Different subsets of DCs defined by their unique phenotypes and functional potentials, are present in lymphoid tissues. Five major DC subsets have been identified in lymphoid tissues of uninfected laboratory mice (summarized in Table 1). The CD11c and MHC class-II (MHC-II) molecules are expressed at high levels on all mature DCs in mice and co-expression of both markers is used to define DCs phenotypically (13). Four other markers are currently used to further define DC subsets: CD4, CD8$\alpha\alpha$ homodimer, CD11b (Mac-1) and CD205 (14).

In mouse, thymic DCs contain mainly CD8$\alpha^+$CD4$^-$CD205$^{hi}$CD11b$^-$ (~70%) and CD8$\alpha^{-/lo}$CD4$^-$CD205$^+$CD11b$^-$ (~30%) populations (14). Whereas in the spleen and lymph nodes (LNs), only about 20% of DCs are of the CD4$^-$CD8$\alpha^+$ phenotype. The majority of DCs in the spleen are CD8$\alpha^-$ and can be further divided into two subsets based on CD4 expression: a major CD4$^+$CD8$\alpha^-$CD205$^-$CD11b$^+$ subset and a minor CD4$^-$CD8$\alpha^-$CD205$^-$CD11b$^+$ subset. In comparison, the CD8$\alpha^-$ DCs in LNs can be divided into three subsets on the basis of CD4 and CD205 expression. The CD4$^-$CD8$\alpha^-$ CD11b$^+$ is the major DC population and can be further divided into CD205$^+$ and CD205$^-$ cells, while the CD4$^+$ CD8$\alpha^-$CD11b$^+$ DCs (the major DC subset in mouse spleen) form only a minor subset in LNs (15). One third of DCs from the skin-draining LNs and only a few DCs from mesenteric LNs and spleen are CD4$^-$CD8$\alpha^{lo}$CD11b$^+$ CD205$^{hi}$. Since these DCs also express langerin (CD207), the marker present on Langerhan's cells (LCs), and high levels of MHC-II and costimulatory molecules, it has been suggested that these cells are the post-migration mature form of LCs (15). These various DC subsets also differ with respect to their function and distribution in distinct microenvironments. The CD8$\alpha^+$CD205$^+$ DCs are mainly found in T-cell rich areas of the peripheral lymphoid organs such as the periarteriolar lymphatic sheaths (PALS) of the spleen (16) and paracortical regions of the LNs (17). In contrast, the CD8$\alpha^-$ DCs localize in splenic marginal zones (16) and in subcapsular sinuses and immediate peri-follicular zones of LNs (18), with a tendency to migrate to T-cell regions on stimulation with microbial products (19-21).

The presence of multiple DC subsets suggests that different DC subsets may be specialized for certain functions including induction of both immunological tolerance and effective immuno-stimulation. Indeed, there are numerous reports on functional differences between DC subsets. However, the clear-cut view of functional specialization among DC subsets is unsustainable due to both functional diversity and plasticity of these DC subsets. The CD8$\alpha^+$ DCs, for example, display immuno-regulatory or tolerogenic induction ability (22-25), but also exhibit the strongest ability to induce a T helper 1 (Th1) response (26, 27) by secreting high amounts of the Th1 polarising cytokine IL-12 (16, 28). In addition, DCs exhibit great functional plasticity, which is induced by the different tissue environments (29, 30), the response to different microbial stimuli (31, 32) or the duration of DC activation (33). Based on these observations, two distinct views have emerged on how all these phenotypic and functional varieties are created during the development of DC system. The specialized DC lineage model suggests that the point of commitment to phenotypically and functionally defined DC subsets happens very early in hemopoiesis, so that DC subsets develop as separate lineages with distinct functions. The functional plasticity model, however, supports the idea that DC subsets represent different activation states or alternative cell fates of a single lineage dictated by the local microenvironment. Currently, there is no conclusive evidence to support exclusively either model. Further studies are needed to reconcile these different views.

The mouse equivalent of human plasmacytoid DCs (pDCs) has been identified recently (9-12). These cells have a unique surface phenotype: CD45RA$^{hi}$B220$^+$CD11c$^{lo}$CD11b$^-$MHC-II$^{lo}$ (pDCs from Balb/c or Sv129 mouse strains also express Gr-1). Although similar to their human counterparts, mouse pDCs showed notable differences in the expression of a few surface molecules. In contrast to human pDCs which express high levels of IL-3R and are negative for CD8, mouse pDCs lack of high expression of IL-3R and some express CD8$\alpha$. Currently, the biological role of CD8$\alpha$ expression on mouse DCs and pDCs is not yet clear. Similar to human pDCs, mouse pDCs express Toll like receptor (TLR) 7 and 9 (34), and could produce large amounts of type I IFNs in response to viral stimulation or bacterial oligonucleotides containing CpG motifs. Moreover, the same stimuli could induce pDCs to

**Table 1.** *DC subsets in mouse lymphoid tissues.*

| DC subsets | Spleen | Mesenteric lymph nodes | Skin-draining lymph nodes | Thymus |
|---|---|---|---|---|
| CD8$^+$CD4$^-$CD205$^{hi}$CD11b$^-$ | ++* | + | + | ++++ |
| CD8$^-$CD4$^+$ CD205$^-$CD11b$^+$ | ++++ | +/- | +/- | |
| CD8$^-$CD4$^-$CD205$^-$CD11b$^+$ | ++ | +++ | ++ | CD8$^{lo/-}$CD11b$^-$ (+)$^†$ |
| CD8$^-$CD4$^-$CD205$^+$CD11b$^+$ | +/- | ++ | ++ | CD8$^{lo/-}$CD11b$^+$ (+/-) |
| CD8$^{lo}$CD4$^-$CD205$^{hi}$CD11b$^+$ | - | +/- | + | |

*The relative frequency of DC subsets is expressed by the number of '+' symbols: 50-70% (++++), 30-50% (+++), 20-30% (++), 10-20% (+) and <5% (+/-).

† The five major DC subsets are not all present in the thymus.

adopt the DC morphology and to become CD45RA$^{lo}$ CD11c$^{hi}$MHC-II$^{hi}$CD8α$^{+}$ DCs *in vitro* (10, 12). However, these pDCs could not become DCs *in vivo* in the absence of microbial stimuli (12). These pDCs therefore may not serve as the precursors of conventional DCs under a steady state condition.

In human, direct analysis of DC subsets freshly isolated from different tissues has been limited. In addition, the phenotypic differences between human DCs and mouse DCs (e.g. absence of CD8α expression on all human DCs) make it difficult to directly compare the development and functions of DC subsets between the two species. Blood is the only readily available source of human DCs, but it is mainly a source of immature DCs (iDCs) and the more specialized pDCs. Human blood DCs are also hetero-geneous in their expression of a range of markers, but many of these reflect differences in maturation or acti-vation states of DCs, rather than delineating separate sub-lineages (2, 35). Most of the insights into human DC subsets and their developmental origins have come from studies of their development in culture from iDC or from pDC. These studies have led to the concept of distinct streams of human DC development. However, the corre-lations between the DCs generated in culture and naturally occurring DC subsets *in vivo* is not clear.

Similar to mouse DC subsets, the functional plasticity of a given human DC subset when exposed to different cytokines or pathogens makes it difficult to assign a fixed function to a particular DC lineage. Despite this plasticity, each human DC subset does appear to have a different functional bias. The evidence comes mainly from studies of the DC1 and DC2 populations generated from pre-cursors in culture (36, 37). Blood monocytes, termed pDC1, are the most commonly used precursor cells for generating human DC1 in culture. In the presence of M-CSF they will generate macrophages, but in the presence of GM-CSF and IL-4, the DC1 are generated after 6 days (38-40), and these DCs can be further matured to CD14$^{-}$CD38$^{+}$CD86$^{+}$ MHC-II$^{hi}$ DCs in the presence of TNF-α or LPS. The precursors for development of the second human DC subset, termed pDC2, are the interferon α/β producing plasmacytoid pDCs (41, 42) with a unique surface phenotype (CD4$^{+}$, IL-3R$^{+}$, CD11c$^{-}$). These pDC2 respond to viral and microbial stimuli by producing type I IFNs. They express intracellular but not surface MHC class-II. When cultured with IL-3 plus CD154, or with microbial stimuli such as bacterial CpG or human herpes simplex virus (HSV), they become mature DCs, namely DC2. The pDC1 and pDC2 themselves express different sets of pattern-recognition receptors (43) and show corresponding differences in reactivity to different microbial products. The pDC1 express TLR 1, 2, 4, 5 and 8 and respond to the appropriate microbial ligands, including peptidoglycan, lipoteichoic acid and LPS, while pDC2 express TLR 7 and 9 and respond to CpG oligonucleotides. The mature DCs generated from these precursors in culture also show functional specialization. CD154 activated DC1 were found to prime T cells to produce a Th1 response, whereas CD154 activated DC2 induced a Th2 response (42). The ability of DC1 to induce a Th1 response was associated with a high level of production of IL-12 p70, in response to the appropriate stimuli. Therefore, there does appear to be

substantial lineage specialization within human DC subsets.

## Ontogeny of the DC system

The maturation state of DCs has been suggested to be a determining factor for the induction of immune tolerance or immunity (44). It is well known that neonatal mice mount a limited immune response to infection and are more susceptible than adults to the induction of immunological tolerance following antigen exposure (45, 46). Understanding the mechanisms underlying this immunological feature is crucial in developing strategies for either enhancing the immune responses in the situation of microbial infection or suppressing it in the case of autoimmunity. The potential influence of DCs, the major inducers of effector T cell responses or tolerance (3, 44), on the functional state of the immune system at the early developmental stage has been studied. The recent studies have shown that DCs are present in lower numbers overall in the lymphoid tissues of neonatal mouse (47) and the relative incompetence of immune responses to microbial infection in the neonatal mouse is due to a low frequency of functionally mature DCs in mouse lymphoid tissues (48). It has also been shown that both CD11c$^{+}$ DCs and CD45RA$^{+}$ pDCs are detected in small numbers in mouse thymus as early as embryonic day 17, suggesting a role of DCs in thymocyte development. Significant but low numbers of DCs and pDCs are present in the spleen of day 1 newborn mice. The full complement of DC system is not acquired until 5 weeks of age. The composition of DC populations in the spleen of young mice differed significantly from that found in adult mice, with a much higher percentage of the CD4$^{-}$8α$^{+}$ DC population (50-60% compared to 20-25% in adult) and a much lower percentage of CD4$^{+}$8α$^{-}$ DC population (10-20% compared to 50-60% in adult) (47, 49). Although the pDCs of young mice showed a capacity to produce IFN-α comparable with that of adult mice, the conventional DCs of young mice were less efficient than their adult counterparts in IL-12p70 and IFN-γ production, and in antigen presentation (47). These observations suggest that the neonatal DC system is not fully developed and innate immunity is the dominant form of immune response. The complete DC system required for adaptive immunity in mouse is not fully developed until 5 weeks of age.

Similarly, it has also been shown that the DCs from human cord blood have a limited ability to induce either adult or cord blood T cells to proliferate in response to a given concentration of phytohemagglutinin or concanavalin A. Adult blood DCs, on the other hand, induce stronger mitogen responses of cord blood and adult blood T cells. This relative deficiency in T cell responses induced by cord blood DCs can be overcome by increasing the concentration of mitogen or the numbers of DCs in the culture. Therefore, the deficiency in neonatal primary immune responses may, in part, reflect the reduced function of DCs (50).

## Development of DCs from hemopoietic precursors

It is well known that DCs and pDCs are continuously

produced from bone marrow (BM) hemopoietic stem cells (HSC) (51). But, the exact precursors which can give rise to DCs and pDCs *in vivo* remain elusive. It has been widely accepted that all HSC-derived cells develop either as a part of lymphoid or myeloid pathway through a multipotent but lineage-restricted common lymphoid precursor (CLP) for B, T and NK cells, or common myeloid precursor (CMP) for granulocytes, monocytes, macrophages, erythrocytes and megakaryocytes, with subsequent commitment and differentiation along a single lineage (52, 53). Recent studies on DC development provide evidence that all DC subsets can be generated along both myeloid and lymphoid pathways, from CMP or CLP (54-56).

### Development of DCs from myeloid precursors

DCs were originally believed to be of myeloid origin, based on certain similarities to monocytes/macrophages in terms of morphology, phenotype, endocytic potentials and enzymatic activities. Indeed, in the presence of GM-CSF, DCs and granulocytes or macrophages can be produced in cultures from mouse blood and BM proliferating MHC class-II-negative precursors (57, 58). All three cell types can develop from a single BM-precursor derived colony grown in semisolid medium – a confirmation that they share a common precursor. In contrast to phagocytic cells, DCs generated under these conditions can home to T-cell regions of lymph nodes and strongly activate naïve T cells in MHC-mismatched mixed leucocyte reaction (MLR). In human, it was also demonstrated by clonal assay that a single BM bi-potential CD34$^+$ precursor could form mixed colonies of monocyte/macrophages and DCs in the presence of GM-CSF and TNF-$\alpha$ (59, 60). The DCs generated, however, resembled Langerhans cells (LCs). Additional support for the myeloid origin of DCs came from the observation of their direct differentiation from peripheral blood monocytes, without proliferation and under various experimental conditions. Mouse monocytes can be induced *in vitro* by GM-CSF and IL-4 to differentiate into DCs that are capable of inducing T cell proliferation in MLR (61). Human blood monocytes can also serve as precursors for the *in vitro* generation of DCs in the presence of GM-CSF and IL-4 (38-40). The direct evidence for the myeloid origin of some DCs came from the studies on BM CMP as mentioned previously (54). It has been shown that all the major DC populations found in mouse lymphoid tissues can be generated *in vivo* by the CMP (55, 56).

The epidermal LCs are recognized as a separate DC subtype and can be distinguished by the expression of langerin (CD207), E-cadherin and the presence of Birbeck granules. When mouse BM c-kit$^+$ lineage marker negative cells were cultured in the presence of GM-CSF, stem cell factor (SCF) and TNF-$\alpha$, two independent DC precursor populations were generated (62). The CD11b$^{-/dull}$CD11c$^+$ precursors differentiated into E-cadherin$^+$ LC-like cells in the presence of GM-CSF and TNF-$\alpha$, while CD11b$^+$CD11c$^+$ precursors differentiated either into CD11b$^-$CD8$^-$ DCs in the same cultures or into macrophages when induced by M-CSF. This indicates a developmental separation between LCs and DCs. Similarly, two different developmental pathways are identified for the production of LCs and DCs from human CD34$^+$ progenitor cells in the

presence of GM-CSF, SCF and TNF-$\alpha$. One pathway gives rise to LCs via a CD14$^-$CD1a$^+$ intermediate (63), while the other gives rise to DCs through a CD14$^+$CD1a$^-$ bi-potent intermediate capable of differentiating into either DCs (64) or macrophages (63, 65). However, the lymphoid or myeloid lineage origin of the LCs has not yet been clearly defined.

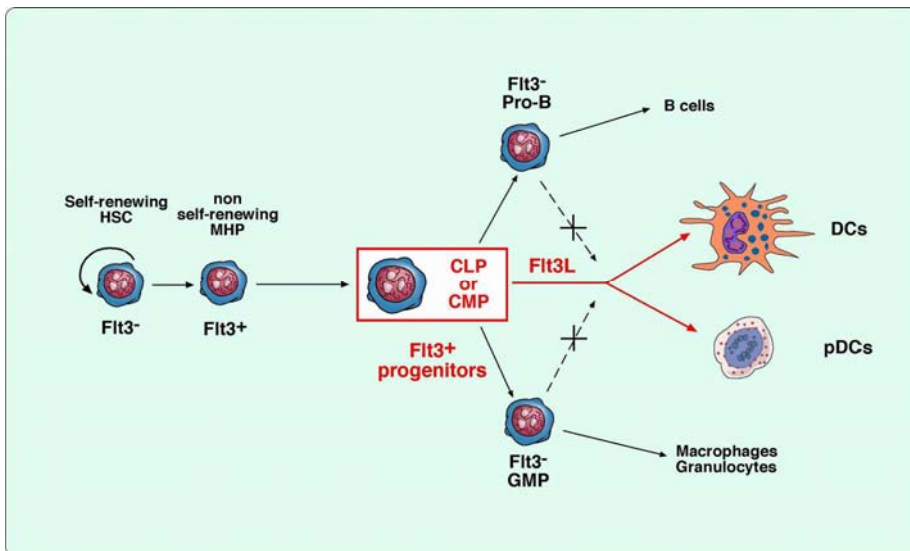### Development of DCs from lymphoid precursors

The first suggestion that DCs might be related to lymphoid lineage came from the finding that the CD8, CD2, CD25 and BP-1, typical lymphoid markers, were expressed on the surface of mouse thymic DCs and a subset of splenic DCs (66, 67). The first evidence came from the hemopoietic reconstitution studies with the earliest intrathymic lymphoid-restricted CD4$^{lo}$c-kit$^+$Sca-1$^+$Sca-2$^+$ (CD4$^{lo}$) precursors (68, 69), which showed the development of predominantly the CD8$^+$ DCs (70, 71) in addition to T, B and NK cells (72) and limited development of CD8$^-$ DCs and myeloid cells in mouse spleen. In contrast, BM HSC generated both CD8$^+$ and CD8$^-$ DCs in the spleen after reconstitution (71). These results led to the hypothesis that the CD8$^+$ DC subset might represent a lymphoid DC lineage. The intrathymic CD4$^{lo}$ precursors can also generate DCs in cultures, with a 70% cloning efficiency, in the presence of cytokines (TNF-$\alpha$, IL-1$\beta$, IL-3, IL-7, SCF, Flt-3L and CD40L) that support DC development (73, 74). However, GM-CSF, the essential cytokine required for DC development from myeloid precursors in culture, is not required for generation of DCs from these CD4$^{lo}$ precursors.

The recent studies on mouse BM CLP provided direct evidence for the lymphoid origin of some DCs. The BM CLP can generate all DC populations identified in lymphoid tissues, although there is a strong bias to the development of CD8$^+$ DC subset (55, 56). In comparison to CMP, CLP are more potent in DC production on a per cell basis. However, the CLP are less numerous than CMP in normal mouse BM. Therefore, the overall contribution of CLP- and CMP-derived DCs may be similar in mouse lymphoid organs. The functional capabilities of DCs derived from different hemopoietic precursors are yet to be investigated.

Human progenitor cells with lineage restrictions similar to those of the CLP in mouse have also been described and were shown to generate DCs *in vitro* (75). However, it is not clear if these precursors can generate all human DC subsets.

### The Flt3 expressing hemopoietic precursors are the precursors of both DCs and pDCs

The fact that both CMP and CLP can generate all the DC populations suggests plasticity in developmental potentials of these early precursors (76). It also suggests that the CMP and CLP that can give rise to DCs may share some common features. Flt3 ligand (Flt3L) has been shown to act as a growth factor for hemopoietic progenitors (77-79) and it can promote the expansion of both DCs and pDCs *in vivo* and *in vitro* (80-88). However, the cells responding to Flt3L treatment and subsequently giving rise to DCs and pDCs had not been fully characterized. Recent studies further examined the different mouse BM hemopoietic

**Figure 1.** The development of dendritic cells (DCs) and plasmacytoid dendritic cells (pDCs) from mouse bone marrow precursors that express Flt3. HSC: hemopoietic stem cells; MHP: multipotent hemopoietic progenitors; CLP: common lymphoid precursors; CMP: common myeloid precursors; GMP: granulocyte and macrophage precursors; pro-B: early precursors for B lymphocytes.

precursor populations for the surface expression of Flt3 (the receptor for Flt3L) and tested them for early DC and pDC precursor activity. It was demonstrated that most DC and pDC precursor activity was within the BM hemopoietic precursors expressing Flt3 (89, 90). The majority of mouse BM CLP express high levels of Flt3 and these are the most efficient precursors of both DCs and pDCs (89). In contrast, only a small proportion of the CMP express Flt3, but the precursor activity for both DCs and pDCs is within this minor Flt3$^+$ CMP fraction (89). The downstream lineage committed precursors, including granulocyte and macrophage precursors (GMP) and pro-B cells do not express Flt3 and have no DC or pDC precursor activity. These findings demonstrate that the early precursors for all DC subtypes and for pDCs are within the BM Flt3$^+$ precursor populations, regardless of their lymphoid or myeloid lineage orientation, and Flt3 signaling is required for the development of both DCs and pDCs (Figure1).

## Conclusion

In this review, we summarized the evidence from recent studies on DC subsets and their development from hemopoietic precursors. The evidence leads to the current view that both DCs and pDCs in mouse can develop from the Flt3 expressing hemopoietic precursors regardless of their myeloid or lymphoid origin (as summarized in Figure 1). Similar developmental pathways have been suggested for human DC populations. Further studies are required to determine the cytokines and environmental factors required for inducing the specialized functions of different DC subsets.

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