Concise Review: Dendritic Cell Development in the Context of the Spleen Microenvironment

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ABSTRACT

The dendritic cell (DC) population in spleen comprises a mixture of cells including endogenous DC progenitors, DC precursors migrating in from blood and bone marrow, and DC in different states of differentiation and activation. A role for different microenvironments in supporting the dynamic development of murine DC of different types or lineages is considered here. Recent evidence for production of DC dependent on splenic stromal cells is reviewed in the light of evidence that cell production is dependent on cells comprising an endothelial niche in spleen. The possibility that self-renewing progenitors in spleen give rise to DC with tolerogenic or regulatory rather than immunostimulatory function is considered. STEM CELLS 2007;25:2139–2145

Disclosure of potential conflicts of interest is found at the end of this article.

INTRODUCTION

Dendritic cells (DC) are antigen-presenting cells capable of activating naïve T cells and initiating adaptive immunity. They also act as important regulators of self-tolerance by tolerizing peripheral T cells with self-reactive potential. DC are spread throughout the body as sentinel cells sampling local environments and in lymph tissues where they mediate T-cell responses [1]. DC vary in phenotype in their different locations, and phenotypic variation is evident by differences in cell lineage and states of maturation. For example, it is known that specific DC lineages are located in certain tissues such that the Langerhans cells (LC) in skin are identified by specific markers like Langerin (CD207) [2]. Functional DC resident in lymphoid tissues comprise a heterogenous mixture of cells with few distinct markers. This diverse population represents a combination of both in vitro and in vivo studies now describe several DC lineages and for different tissue origins. Indeed, different stromal cell microenvironments in different regions of lymphoid organs could contribute to the diversity of function among DC.

THE LINEAGE ORIGIN OF DC

DC are derived from hematopoietic progenitors in bone marrow. In mouse, the nature of immediate precursors for DC in most tissue sites is unclear. A multitude of reports based on a combination of both in vitro and in vivo studies now describe several DC precursors. Debate arises over whether there are separate precursors for the different DC lineages and for different tissue sites. For LC in skin, the identity of precursors has been established. Under inflammatory conditions, Gr1+ monocytes differentiate into LC in the skin [8]. However, LC can also develop from endogenous progenitors that continually replenish cells in the steady state [9]. The distinct lineage of pDC is found in all lymphoid organs, and cells are characterized by the expression of CD45RA (B220 in mouse) and the production of IFN-α upon stimulation [6]. Plasmacytoid-DC develop from an immediate precursor, a CD11c+B220+ p-preDC that is found in blood [10]. The development of pDC along with cDC has been characterized from bone marrow populations described as common myeloid progenitors (CMP) and common lymphoid progenitors (CLP), which respond to Flt3L [11, 12]. For the more common cDC subsets found in lymphoid tissues, there appear to be multiple sources of DC precursors. It

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was previously thought that a direct relationship existed between CD8α− DC and CD8α+ DC, supported by evidence from other DC subsets such as LC and pDC where CD8α was upregulated upon DC activation [6, 13]. However, it is now clear that CD8α− and CD8α+ DC subsets in spleen represent separate cell lineages with distinct function [14], with CD8α+ DC dominant in cross-presentation of antigen [15]. Together, these two apparent lineages make up the cDC population. Initially, CD11c+CD11b+CD8α− DC were shown to develop from myeloid progenitors induced with granulocyte macrophage–colony-stimulating factor (GM-CSF) [16], and CD8α+ DC were reported after CD45b+ precursors from adult mouse thymus were found to produce DC after culture with a cocktail of cytokines not including GM-CSF [17]. Further support for the “lymphoid-like” origin of CD8α+ DC came from reports that they could develop from intravenously transferred CD45b+ lymphoid precursors [18]. Subsequent studies have shown that both CD8α− and CD8α+ DC can be generated in vivo from CMP [11, 12], CD45b+ thymic progenitors [17], and CLP [11, 12] such that the terms “myeloid-like” and “lymphoid-like” DC become irrelevant. It now appears that plasticity inherent in DC differentiation is a characteristic of this hematopoietic cell type.

### Table 1. Precursors of spleen DC identified in different tissue sites

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<th>Source</th>
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<th>Progenyt spleen DC</th>
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<td>BM</td>
<td>CD11c+ B220+Ly6C+</td>
<td>In vivo</td>
<td>pDC</td>
<td>Diao et al. [23]</td>
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<td>BM</td>
<td>CD11c+ B220+Ly6C+</td>
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<td>CD8α+ cDC</td>
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<td>BM</td>
<td>Lin− c-kit+sca−IL7R−CD16+32+Flt3+</td>
<td>In vitro culture with Flt3L</td>
<td>pDC</td>
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<td>Karsunky et al. [29]</td>
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<td>BM/blood</td>
<td>CD11c+ CD31+Ly6C−</td>
<td>In vivo</td>
<td>CD8α− cDC</td>
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<td>Blood</td>
<td>CD11c+CD11b+CD45RA+</td>
<td>In vitro</td>
<td>CD8α+ cDC</td>
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<td>pDC</td>
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<td>Spleen</td>
<td>CD11c+CD11b-CD8α+</td>
<td>In vivo</td>
<td>CD8α+ cDC</td>
<td>Wang et al. [26]</td>
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<tr>
<td>Spleen</td>
<td>CD11c+CD45RA+CD43+CD30+</td>
<td>In vivo</td>
<td>CD8α+ cDC</td>
<td>Naik et al. [27]</td>
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aAll subsets shown are also MHC-II+.
bIn vivo conditions reflect development after adoptive transfer by the intravenous route.

Abbreviations: BM, bone marrow; cDC, conventional antigen-presenting dendritic cell; DC, dendritic cell; GM-CSF, granulocyte macrophage–colony-stimulating factor; pDC, plasmacytoid dendritic cell; TNF, tumor necrosis factor.

**Spleen DC Precursors**

Recently, progress has been made in identification of specific precursors for murine spleen DC subsets (Table 1). These have now been identified using a combination of in vivo and in vitro studies. The first group of precursors present in bone marrow and blood has potential to differentiate into both DC and monocyte/macrophages. Initially, a CD11c+CD31+Ly6C+ DC/macrophage precursor population termed “preimmunocytes” was characterized in blood and bone marrow, which in the presence of GM-CSF developed into immature DC resembling splenic cDC [19]. Furthermore, fetal thymic organ cultures of these precursors led to CD8α− and CD8α+ cDC development [19]. Indeed, CD11c+CD31+Ly6C+ cells appear to be precursors to multiple DC types including pDC and cDC, at least under conditions of in vitro exposure to GM-CSF. An earlier bone marrow progenitor has now been identified with differentiative capacity for both monocytes and spleen cDC. Described as specifically Lin− c-kit+CXCR1+ macrophage/dendritic progenitors (MDP), these cells give rise to cDC of both CD8α− and CD8α+ type upon adoptive transfer into recipient mice [20]. However, since they do not produce pDC, they appear to represent a more restricted myeloid DC precursor. In this respect they are distinguishable from progenitors described within the Flt3+ subset of bone marrow-derived CMP and CLP. Unlike the MDP, this Flt3+ progenitor can develop into pDC as well as cDC following in vivo transfer or in vitro culture with Flt3L [11, 12, 21, 22].

Other reports describe more restricted CD11c+ DC precursors that can be found in each of the bone marrow, blood, and spleen. A CD11c+MHC-II+Ly6c+ precursor population was identified in bone marrow. The B220+ subset of these cells produces splenic pDC after adoptive transfer into mice [23], whereas the B220− subset produces CD8α+ cDC [24]. Since cells of no other lineage are produced, this particular bone marrow-derived DC precursor appears to lack macrophage/monocyte potential. In blood, two distinct precursors for cDC and pDC were also identified as CD11c+CD11b+CD45RA− and CD11c+CD11b+CD45RA+ cells [17]. Upon stimulation with GM-CSF and tumor necrosis factor (TNF)-α or GM-CSF and CpG, these subsets differentiated into CD8α+ cDC and IFN-α-producing pDC, respectively [25], resembling known DC subsets in spleen. A CD11c+CD11b+CD8α+ DC precursor has also been identified in spleen, which, after i.v. transfer, develops into only CD8α+ DC [26]. Discovery of an immediate spleen cDC precursor, identified by its characteristic CD11c+CD43+CD30+ phenotype, now distinguishes the cDC and pDC lineages [27]. Termed pre-cDC, these spleen-derived precursors form all cDC subsets in spleen but not pDC, which appear to derive from precursors in blood [27]. At this stage it is not known whether these immediate spleen precursors arise from endogenous spleen progenitors or from precursors seeding spleen from blood.

Currently, it is difficult to define a clear relationship between progenitors found in different tissue sites, given that some experiments involve in vitro and others in vivo definition of differentiative potential. Lack of CD11c lineage marker expression suggests that the Lin− c-kit+CXCR1+ MDP may be the most primitive cell. However, its restricted differentiative potential for cDC and monocytes distinguishes it from other pre-
curators that have both cDC and pDC potential. Already this evidence suggests a division among DC precursors as either monocyte/cDC-restricted or pDC/cDC-restricted. Indeed, precursors that are pDC/cDC-restricted appear to be those that respond to Flt3L.

**FLT3L IN DC DEVELOPMENT**

Flt3L is a growth factor known to mobilize hematopoietic precursors into peripheral blood and can have a significant impact on DC development [28]. It has been used successfully both in vivo [29] and in vitro [11, 29] to expand DC populations. Although Flt3L is produced by bone marrow stromal cells, at this stage it is not yet known to what extent stromal cells that are present in other tissue microenvironments produce this factor. An analysis of commonly known cytokines produced by splenic stromal lines that support DC production versus lymph node stromal lines indicated no transcription of Flt3L by either stromal type, at least in the resting state [30]. The Flt3 receptor is expressed on short-term reconstituting hematopoietic stem cells (HSC) but not long-term HSC [31]. It is also expressed on subsets of CMP and CLP, although its expression is downregulated on more committed precursors like pro-B cells, pro-T cells, granulocyte/macrophage progenitors, and megakaryocyte/erythroid progenitors (MEP). The Flt3 receptor is absent on all major cell lineages (B, T, and natural killer cells, granulocytes, monocytes/macrophages) except DC. Analysis of spleen DC subsets has shown that CD8α+ DC, CD8α− DC, and, to a lesser extent, B220+ pDC all express Flt3 [29].

Flt3L therefore supports DC production from early bone marrow-derived progenitors. Although the development of splenic DC from CMP and CLP is well known, only recently have investigations in vivo revealed that the Flt3+ subset among these progenitors has greatest potential to develop into all subsets of spleen DC including cDC and pDC [11, 29].

![Image](https://via.placeholder.com/150)

**Figure 1.** Model for turnover of immunogenic dendritic cells (DC) within spleen under steady-state and inflammatory conditions. Precursors for cDC and pDC are known to exist in BM, blood, and spleen. Under steady-state conditions, they seed blood and spleen and provide immediate DC precursors of both the cDC and pDC lineages. Differentiation of immediate DC precursors will occur within the spleen environment, giving rise to both cDC and pDC. It is also proposed that Flt3+ progenitors in bone marrow will be expanded under inflammatory conditions, so seeding unknown intermediates of cDC and pDC into blood and spleen (dashed lines). Abbreviations: BM, bone marrow; cDC, conventional antigen-presenting dendritic cell; CLP, common lymphoid progenitor; CMP, common myeloid progenitor; pDC, plasmacytoid dendritic cell.
Bone marrow-derived Flt3+ progenitors are responsible for expansion of cDC and pDC in spleen following Flt3L treatment of mice [29]. Reconstitution of lethally or sublethally irradiated mice with congenic whole bone marrow combined with injection of Flt3L leads to an increase in donor-derived spleen DC cells, whereas host spleen DC cell numbers remain unchanged [29]. This specifically indicates the bone marrow rather than spleen origin of Flt3L-induced spleen DC. Flt3L treatment of mice also leads to increased DC numbers in lymph nodes, bone marrow, and thymus [35]. However, with evidence for a subset of CD11c+ MHC-II+ DC in spleen that are Flt3+ [29], it could appear that at least some DC precursors resident in spleen may not be Flt3L responsive.

**DC Development Within Spleen**

The ability to generate spleen DC from bone marrow and blood precursors is consistent with the existence of circulating DC precursors that continually seed spleen to replenish tissue-resident DC. Precursors for each of the cDC and pDC lineages have been identified in both bone marrow and blood (Table 1). Figure 1 identifies the relationship between incoming DC precursors and the cDC and pDC subsets in spleen. Further evidence in support of circulating DC precursors that lodge in spleen comes from the kinetics of DC development following transfer of bone marrow into irradiated recipients. In these mice, reconstitution of spleen cDC is rapid and sustained [36]. Bromodeoxyuridine labeling studies showed high turnover of spleen DC with a half-life of 2–3 days, suggesting rapid replenishment and generation of DC from blood-borne bone marrow precursors [36]. It is still questionable, however, whether maintenance of spleen DC subsets by continuous seeding from blood applies to all spleen DC subsets.

Another consideration is that some spleen DC appear to be produced from endogenous self-renewing progenitors that are maintained within the spleen environment. Analysis of mice that were joined in parabiosis such that they shared a blood supply produced from endogenous self-renewing DC progenitor arising from spleen [38].

Most splenic DC are immature in the steady state such that spleen cDC remain responsive to maturation and activation, display moderate levels of major histocompatibility complex (MHC)-II, and retain capacity to process and present antigen [39]. More recently, a separate class of regulatory DC was described originating from bone marrow-derived progenitors or immature DC cultured over spleen stroma [40–42]. An in vivo counterpart of regulatory DC derived from GM-CSF-induced immature DC has been described [43]. These DC share some characteristics with immature DC, including low levels of CD11c and MHC-II expression. They are, however, distinct from cDC and pDC by variable properties including expression of CD45RB, distinct or no response to signals that activate or mature cDC, and inability to activate alloreactive or CD4+ T cells [40, 42, 43]. These cells function to suppress T-cell responses either through regulatory T-cell formation [41, 42] or nitric oxide (NO) production [40, 42]. Since they lack antigen-specific function, they may play a nonspecific homeostatic role in immune regulation. Although the exact lineage origin of “regulatory DC” is yet to be clarified, their development in vitro has been reported in cultures of Lin− c-kit+ hematopoietic cells from bone marrow over spleen stroma of both fibroblastic and endothelial cell type [39, 41] (Fig. 2). This evidence is consistent with the hypothesis that seeding of spleen niches with bone marrow-derived progenitors represents a possible mechanism for regulatory DC development. However, the relationship between precursors of regulatory DC and other defined pDC and cDC precursors is not yet known. Their dependence on splenic stroma for development is one of their most defining characteristics. As yet, there is no reported evidence for the role of Flt3L in regulatory DC development dependent on stromal cells, and indeed other defining factors appear to be primarily responsible.

**ROLE OF STROMA IN DC DEVELOPMENT IN SPLEEN**

Although it is known that Flt3L expands subsets of both CLP and CMP in bone marrow leading to production of new cDC and pDC in spleen [10, 28], the progression and location of progenitors that develop into spleen DC under these circumstances remain unresolved. It is also not known to what extent Flt3L treatment mimics normal steady-state DC development in spleen, and this issue was addressed in Figure 1. The common model predicts continuous seeding of committed DC precursors from bone marrow into blood and then into spleen. An alternative model is that Flt3+ multipotential progenitors, perhaps expanded and mobilized into blood due to Flt3L treatment, might migrate to spleen and localize there. These progenitors would then lodge in spleen and differentiate to give a continuous supply of DC (Fig. 1). By this model, DC development would be specifically regulated by stromal niche environments [43]. Although this model remains untested, it highlights a role for stromal niches in DC development that is now gaining wide attention.

In this lab, the importance of spleen stroma in DC development has been known and investigated for many years. Immature endothelial-like stromal monolayers can be grown from spleen that specifically support the development of dendritic-like cells from overlaid bone marrow or spleen [44]. LTC that continuously generate DC in a stromal cell environment from spleen-derived progenitors can be readily developed [45]. These support the development of both adherent and nonadherent DC-like cells and produce only DC and no other hematopoietic cell type. Nonadherent DC resemble immature myeloid-like DC in terms of phenotype and function. Their refractoriness to activation with common activators of cDC distinguishes them as an immature subset of DC also distinct from regulatory DC. Spleen LTC support the development of immature CD8α− DC from a population of small spleen-derived progenitors that can be maintained in culture [46]. If this small progenitor cell population is sorted and overlaid onto spleen stroma, it develops exclusively into this distinctly immature myeloid DC, confirming the ability of LTC to support both progenitor self-renewal and DC differentiation dependent on stroma [47]. A spleen stromal line, STX3, has been isolated from LTC, which can independently support the outgrowth of DC from progenitors among bone marrow or spleen cell suspensions cocultured above stroma [44].

More recently, other groups have confirmed a specific role for spleen stroma in DC development, and the combined evidence is summarized in Figure 2. Preparation of stroma, however, varies with different groups, and descriptions of stroma required for DC development range from predominantly fibroblastic cells to predominantly endothelial cells. Procedures for isolation of stroma are not well developed, and many cell
surface markers are commonly expressed on both fibroblastic and endothelial cells, with few markers specific for individual cell types. Human blood-derived CD34+ cells develop into CD1a+ HLA-DR+ DC after culture over splenic fibroblasts [48]. Murine spleen stroma described as predominantly fibroblastic with <5% endothelial cells has also been used to generate a population of CD11c+CD45RB+ regulatory DC from overlaid Lin c-kit+ HSC isolated from bone marrow [42]. In a similar report, when Lin c-kit+ murine HSC were overlaid onto isolated VCAM+ stroma described as endothelial, CD11c+CD45RB+ regulatory DC were also shown to develop [40]. Cells produced were shown to be DC with regulatory function mediated by production of NO. These were functionally distinct from regulatory DC derived from fibroblastic stroma, which were found to induce regulatory T-cell function [42]. Human umbilical cord endothelial cells stimulated with TNF-α have also been shown to support development of mature DC from cocultured CD34+ progenitor cells (Fig. 2) [49]. Since unstimulated endothelial cells did not support DC development, it is possible that inflamed endothelial cells may also represent a distinct niche environment for mature DC differentiation. Studies such as these, which vary in both cell origin and cell production, highlight the extent to which stromal cells can influence the development and function of DC in immunity.

The cell support capacity of spleen stroma does not appear to be restricted to early DC progenitors. Endothelial cells in spleen can induce mature DC cells to differentiate into regulatory DC (Fig. 2) [43]. Combined results from many reports now indicate that stromal cell microenvironments may regulate multiple stages in DC differentiation and development. In this lab, the STX3 splenic stromal line, which supports DC development, has been cloned to produce a range of fibroblastic and endothelial-like cell lines, only some of which support DC development from overlaid bone marrow or spleen cell populations or progenitors within these populations [44]. Extensive characterization of cloned lines has now identified supportive lines as endothelial with immature characteristics reflecting an endothelial niche for DC hematopoiesis (Fig. 2). Among these, some stromal lines support DC hematopoiesis of early progenitors dependent on stromal cell contact [47], whereas other lines provide soluble stromal factors that appear to act on more committed DC precursors [44]. These lines are now under study for their capacity to support DC hematopoiesis from different progenitor/precursor subsets and for their production of supportive growth factors. However, evidence to date indicates that common factors used by others to induce DC development in vitro, like Flt3L, GM-CSF, or TNF-α, do not contribute to stromal cell support of DC hematopoiesis [30].

**DEVELOPING A NICHE MODEL FOR DC HEMATOPOIESIS IN SPLEEN**

One unifying model predicts that the development of different types of DC is regulated by different stromal niches in spleen [30]. Some data suggest that cDC in spleen may arise predominantly from more committed CD11c+ precursors produced in bone marrow (summarized in Table 1). Another possibility is
that they arise from circulating Flt3+ DC progenitors mobilized from bone marrow into blood and spleen after Flt3L stimulation, perhaps during inflammation (Fig. 1) [11, 29]. Incoming cells would lodge in stromal cell niches, which direct differentiation into immediate DC precursors, immature DC, and mature DC. Development of immature and mature DC from immediate precursors would be expected to involve the action of soluble stromal-derived factors [44], since there is currently no evidence for the stroma-dependent development of cDC or pDC in spleen. In contrast, regulatory DC that have been reported to develop in vitro from bone marrow-derived GM-CSF-induced DC depend on contact with fibronectin on endothelial stroma and factors like IL-10 for their development [40]. Figure 3 shows an in vivo mechanism for their development in an endothelial niche. Regulatory DC involved in regulatory T-cell induction are also reported to arise from distinct fibroblastic stromal niches seeded with bone marrow-derived ckit+ HSC (Fig. 3) [42]. A distinct myeloid dendritic-like cell developing from endogenous spleen and bone marrow progenitors in the context of endothelial stroma is also shown in Figure 3 [46, 47]. These stroma-dependent CD11c+CD11b+CD8α+ MHC-II+ dendritic-like cells differ from cDC in that they are refractory to lipopolysaccharide and TNF-α activation [50], very weakly immunogenic, and are distinct from described populations of regulatory DC due to complete absence of surface CD45RB and MHC-II expression [47, 50]. The possibility that they reflect precursors of regulatory DC or DC developing in spleen from endogenous self-renewing progenitors is yet to be determined. A weight of recent evidence now characterizes spleen as a site for derivation of nonimmunogenic or regulatory DC. This would be consistent with the need to compartmentalise tolerogenic or regulatory DC development within spleen for maintenance of peripheral tolerance.

The development of spleen DC of conventional, plasmacytoid, and regulatory type needs to be reconciled in terms of progenitor origin and triggers for differentiation. For each DC type, it is important to consider whether progenitors are bone marrow-derived or endogenous to spleen, the role and type of stroma involved, and mechanisms involving both cell-cell contact and soluble factors. Endogenous spleen DC progenitors, perhaps equivalent to those maintained in LTC, could maintain a steady-state supply of DC precursors in spleen. However, the extent to which this occurs in relation to DC development from blood-derived precursors is not known. In line with DC development in spleen LTC, a model proposing development of immature DC from endogenous self-renewing progenitors within the spleen microenvironment is shown in Figure 3. This attempts to reconcile evidence for the production of regulatory DC from HSC cocultured above spleen stromal cells. Mobilization of HSC or other DC progenitors into blood, perhaps mediated by Flt3L, could also lead to progenitor seeding in spleen. Such a model would be consistent with the derivation of regulatory DC following culture of HSC over endothelial or fibroblastic stroma [40, 42]. Development of regulatory DC could thus be distinguished from development of immunogenic DC subsets.

**DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST**

The authors indicate no potential conflicts of interest.
References