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# Recent advances towards deciphering human dendritic cell development

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## Abstract

Dendritic cell (DC) populations are the orchestrators of immune responses and arise from hematopoietic progenitors. Studies to unravel DC ontogeny have been conducted mainly in mice due to historical and practical reasons. However, understanding DC development in humans is a prerequisite for manipulating this process for therapeutic design. Here, we review the advantages and limitations of methods used to study human DC development *in vitro* and *in vivo*. In particular, we examine the *in vitro* culture systems that support the differentiation of all or some DC subpopulations. We also review recent discoveries regarding human DC precursors and factors that regulate their differentiation.

**Keywords:** dendritic cells; human; development

## 1. Introduction

Dendritic cell (DC) biology, classification and development have been primarily described in mice due to historical and practical reasons. DCs have been divided into several subsets based on their ontogeny: plasmacytoid DCs (pDCs) and classical DCs (cDCs) derive from hematopoietic progenitors, while monocyte-derived DCs (mo-DCs) arise from monocytes. Langerhans Cells, which derive from embryonic self-renewing precursors, have

been re-classified as belonging to the macrophage family, and will not be covered in this review.

In the past ten years, analyzing human DCs directly extracted from tissues, and establishing correlations with mouse DCs, has gained considerable attention. Pioneer comparative transcriptomic studies have demonstrated the homology between mouse and human DC subsets (Croizat et al., 2010; Robbins et al., 2008). Human DC comprise pDCs (expressing CD123/IL-3RA and CD303/BDCA2/CLEC4C), cDC1 (expressing CD141/BDCA3/THBD, CLEC9A/DNGR1 and XCR1), cDC2 (expressing CD1b, CD1c/BDCA1, and FcεRI) and mo-DCs (expressing CD1a, CD1c and CD14). Moreover, recent high-resolution analyses have evidenced heterogeneity in human cDC2, with the identification of a previously overlooked DC3 subset (expressing CD1c, CD14 and CD163) in blood and bone marrow (Cytlak et al., 2019; See et al., 2017; Villani et al., 2017).

DC development from hematopoietic stem cells (HSCs) requires the coordinated action of cytokines and transcription factors (TFs). Mouse models have enabled major conceptual advances in our understanding of these processes. Efforts have also been made to translate these findings to the human situation. Here we review the experimental strategies employed to analyze DC development in humans, and summarize findings regarding human DC development, including the imprinting of DC lineage bias in early hematopoietic progenitors and the developmental pathway of the DC3 population.

## **2. Methods used to analyze human DC development**

Genetic fate mapping and lineage tracing experiments have considerably advanced our understanding of mouse DC ontogeny. For obvious reasons, these experimental approaches

are not transferable to human settings. In this context, alternative strategies have been developed to study the development of DCs in humans.

## 2.1 *In vitro* culture systems

*In vitro* culture is a powerful tool to assess the developmental potential of hematopoietic progenitors. Several culture systems supporting the simultaneous differentiation of all DC subsets have been developed (**Table 1**). They have been used to differentiate *in vitro* a variety of human progenitors: stem cells (HSC, in general defined as CD34<sup>+</sup>CD38<sup>-</sup>CD45RA<sup>-</sup> and ideally CD90<sup>+</sup>CD49f<sup>+</sup>), multi-lymphoid progenitors (MLP), pre-DCs or monocytes. Culture models differ in cytokine cocktails and/or co-culture with stromal cell lines. DC differentiation can be obtained by culturing progenitors with combinations of cytokines, mainly Flt3L but also stem cell factor (SCF), interleukin 7 (IL-7), thrombopoietin (TPO), granulocyte/macrophage colony-stimulating factor (GM-CSF) and/or interleukin 4 (IL-4) (Breton et al., 2015; Goudot et al., 2017; Harada et al., 2007; Proietto et al., 2012). One major weakness of this method is the need for an *a priori* knowledge of the cytokines to use. Cytokine cocktails potentially influence the outcome of the culture, and can bias fate decisions of the progenitors.

The addition of stromal cells can significantly improve the output of these cultures. In addition, it allows single-cell clonal cultures of progenitors to address their differentiation potential. OP9 is a mouse stromal cell line that has been used to facilitate differentiation of human pDCs (Schotte et al., 2004), while the stromal cell line MS5 supports other hematopoietic lineages in addition to DCs (Lee et al., 2015a). For instance, MS5 with Flt3L + SCF + GM-CSF supports the development of cord blood CD34<sup>+</sup> HSC into granulocytes, monocytes, lymphoid cells and *bona fide* DCs (Lee et al., 2015a). In addition, the combination of OP9 and MS5 together with Flt3L + SCF + GM-CSF allows the differentiation of DCs, granulocytes, monocytes, megakaryocytes, erythrocytes, NK and B

cells (Lee et al., 2017). Although stromal cell lines are widely used, the molecular mechanism by which OP9 or MS5 monolayers support the development of DCs remains unclear.

Another strength of *in vitro* cultures is the fact that it allows the manipulation of the system in order to address the role/importance of candidate molecules in DC development. An example is the silencing of TFs in human progenitors (Goudot et al., 2017; Poulin et al., 2012; Schotte et al., 2004). Manipulation of OP9 stromal cells (Dontje et al., 2006) has also revealed the role of Notch ligands in cDC1 differentiation (Balan et al., 2018; Kirkling et al., 2018).

While *in vitro* culture can provide definitive evidence of the differentiation potential of a putative progenitor, it remains unclear which *in vitro* system better recapitulates the *in vivo* development of human DCs. Caution should be taken to evaluate how closely the cells obtained *in vitro* resemble the *in vivo*-generated ones. Researchers have used comparative transcriptomics and functional assays to address this question (Balan et al., 2018, 2014; Goudot et al., 2017; Lee et al., 2015b; Villani et al., 2017).

## **2.2 Humanized mice**

Humanized mice can be used to address the developmental potential of human hematopoietic progenitors in an *in vivo* context. Human progenitors are injected into mice with severe immunodeficiency that limits the rejection of human cells. To achieve efficient engraftment, host murine NK cells are particularly limiting. A solution to this has been the injection of antibodies against IL2R $\beta$  (anti-CD122) to deplete murine NK cells (Ding et al., 2014) or the use of mice lacking NK cells by knockout of IL2R $\gamma$  (CD132), such as the nonobese diabetic–severe combined immunodeficiency (NOD-SCID)/IL2r $\gamma^{\text{null}}$  (NSG mice) (Ishikawa et al., 2007; Poulin et al., 2012) or the RAG-2 $^{-/-}$  $\gamma_c^{-/-}$  mice (Schotte et al., 2004) that also lack T and B lymphocytes. For example, sublethally irradiated NSG newborn mice are efficiently reconstituted by human CD34 $^{+}$  HSCs (Ishikawa et al., 2007; Poulin et al., 2012).

The different models of humanized mice are extensively reviewed elsewhere (Legrand et al., 2006; Li and Di Santo, 2019; Theodorides et al., 2016).

One major limitation is the lack of complete cross-reactivity between human and mouse cytokines, making it necessary to supplement mice with human cytokines. In particular, human HSC differentiation towards the monocyte/macrophage and DC lineages in these models is inefficient unless human cytokines are provided. For example, administration of human Flt3L enhances the differentiation of cDC subsets from CD34<sup>+</sup> progenitors (Ding et al., 2014). To circumvent this issue, NSG-SGM3 mice have been developed (NOD.Cg-Prkdcscid-IL2rg<sup>tmlWjl</sup> Tg(CMV-IL3,CSF2,KITLG)1Eav/MloySzJ), which constitutively express human IL-3, GM-CSF and SCF (Lee et al., 2017). One caveat of this approach remains the choice of specific cytokines and their doses, which may not be physiological and could bias progenitor differentiation towards one lineage at the expense of others.

### **2.3 Hematopoietic stem cell transplantation**

Transplantation of bone marrow cells in humans represents an opportunity for the *in vivo* study of the replacement kinetics of DCs. Recipient and donor cells can be distinguished based on mismatch, for instance HLA mismatch or gender mismatch by using fluorescent *in situ* hybridization (FISH) with X/Y probes (Haniffa et al., 2009; Richter et al., 2018). Patients undergoing HSC grafts are biopsied at different intervals, allowing the analysis of the replacement of short-lived recipient cells by donor cells while long-lived cells remain of recipient origin. Observations from these experiments remain very limited due to difficult access to patient samples. However, they provide valuable information on the life span of DCs in different tissues.

### **2.4 Human immunodeficiencies**

Genetic studies of human primary immunodeficiencies allow defining the contribution of specific proteins in the development of immune cells. The main advantage of this approach

is that it does not require any manipulation nor *a priori* knowledge to establish the relationship. Although the identification of patients clearly limits the use of this method, it has been successful in demonstrating the role of several TFs in the differentiation towards cDC, pDC and monocytes (Bigley et al., 2018; Cisse et al., 2008; Cytlak et al., 2018; Dickinson et al., 2011; Hambleton, 2011; Kong et al., 2018).

## **2.5 Injection of cytokines to healthy volunteers**

The injection of cytokines to human volunteers enables the *in vivo* study of the importance of certain cytokines (Breton et al., 2015; Dutertre et al., 2019; Maraskovsky et al., 2000; Pulendran et al., 2000). Volunteers receive daily subcutaneous injections of Flt3L for 5-14 days, and cell numbers are assessed in peripheral blood. These studies have confirmed the major role of Flt3L in sustaining cDC development. However, a major limitation of these experiments is that the injection of high cytokine concentrations does not resemble physiological conditions, which may induce artifacts that are difficult to control for.

## **2.6 Transcriptomic analysis**

RNA sequencing of bulk samples or single cells allows an unbiased comparison of DC subsets or progenitors in different states. Transcriptomic analysis should be systematically used to compare *in vitro*-differentiated DC subsets with their *in vivo* or mouse counterparts (Balan et al., 2014; Goudot et al., 2017; Lee et al., 2015b; Villani et al., 2017). scRNAseq can be used to identify rare populations in unmanipulated patient samples. When several cellular states are present simultaneously in a sample, scRNAseq data can inform on developmental pathways by analyzing trajectory inference in a pseudo-time. However, results can significantly vary according to the computational method used. To solve this problem, experts advise to compare several algorithms, depending on the topology of the expected trajectory, in order to get robust results and choose the most accurate methods (Saelens et al., 2019).

Certainly, the main advantage of scRNAseq is that unbiased analysis of biological processes may lead to unexpected findings.

### **3. DC development**

Human DCs have been described in lymphoid organs as well as in numerous peripheral tissues. Transcriptomic analysis has shown that DC subset identity is strongly imprinted by their ontogeny (Heidkamp et al., 2016). However, mucosal tissue DCs possess specific transcriptomic signatures, which likely result from the influence of micro-environmental signals on the final steps of their differentiation.

#### **3.1 Life cycle of human DCs**

DCs have a very limited life span and are constantly replenished in the periphery by hematopoietic progenitors. Study of skin antigen presenting cells after bone marrow transplantation showed that dermal recipient DCs are almost completely replaced after 40 days, while recipient macrophages persist in the dermis 1 year after transplantation (Haniffa et al., 2009). Similarly, intestinal recipient DCs are completely replaced 6 weeks after bone marrow transplantation (Richter et al., 2018). The homeostasis of DC subsets therefore relies on the migration and *in situ* differentiation of hematopoietic precursors. Of note, DCs have been identified in several fetal tissues (McGovern et al., 2017; Park et al., 2020; Popescu et al., 2019) after the development of HSCs and the onset of definitive hematopoiesis (in contrast to yolk-sac derived tissue macrophages or Langerhans Cells), suggesting that DC development starts early in life.

#### **3.2 DC precursors**

The classical view of an ontogenic tree with a series of dichotomous fate decisions has been challenged in the past few years. Accumulating evidence shows that hematopoietic progenitors possess a developmental bias, observed already at early stages.



The classical model proposed that DCs arise from the common myeloid progenitor (CMP) that was thought to co-segregate with the lymphoid multipotent progenitor (LMPP) from the HSC multipotent progenitors (MPP). However, lymphoid progenitors are not lymphoid-restricted but also give rise to pDC, cDC1 and cDC2 *in vitro* and *in vivo* when transplanted to NGS mice (Doulatov et al., 2010; Helft et al., 2017; Ishikawa et al., 2007; Lee et al., 2017). DCs derived from “myeloid” and “lymphoid” progenitors are transcriptionally identical (Helft et al., 2017; Ishikawa et al., 2007), suggesting either that there are two DC developmental pathways or, more likely, that when defined by a restricted number of surface markers, DC-primed progenitors can phenotypically resemble both myeloid and lymphoid progenitors (**Figure 1**).

On the other hand, it was recently shown that the common myeloid progenitor (CMP) is divided into two subpopulations with different myeloid potential: a CD131<sup>+</sup> subset that expresses the TF Gata1 (CMP-Gata1<sup>+</sup>) and a CD114<sup>+</sup> subset (CMP-Gata1<sup>-</sup>). The former is restricted to the megakaryocyte/erythrocyte and mastocyte/basophil/eosinophil lineages, the latter is restricted to monocyte and neutrophil lineages (Drissen et al., 2019), while DC potential was not addressed. Human granulocyte-monocyte-DC progenitors (GMDP) transit towards monocyte-DC progenitors (MDP) and finally towards committed DC progenitors (CDP) that produce cDCs and pDCs (Lee et al., 2015b). In healthy individuals, CDP are found in cord blood and in the bone marrow (Lee et al., 2015b).

The DC lineage bias is observed from HSC-MPP, through LMPP, CMP and GMDP, and correlates with TF expression (**Figure 1**). For example, cDC1-lineage specification in HSC-MPP is enforced by IRF8/PU.1 ratio (Lee et al., 2017). Recent work shows that GMDP can be divided into subpopulations based on IRF8 expression. IRF8<sup>high</sup> GMDP are restricted to pDC, cDC1 and cDC2, while IRF8<sup>low</sup> GMDP transit to a “monocytic” pathway that also gives rise to DC3 (Cytlak et al., 2019). Therefore, phenotypically defined progenitors are not

considered anymore as homogeneous multipotent populations, but rather a mixture of pre-primed cells that share the same temporary phenotype.

Pre-cDCs are the immediate precursors of cDCs (Breton et al., 2015). These cells are found in diverse lymphoid tissues, such as blood, bone marrow, spleen and tonsils, but not in skin (Alcántara-Hernández et al., 2017). Initially they were described by direct comparison to their mouse counterpart. Pre-cDCs have also been identified by unbiased single cell transcriptomics of blood cells (See et al., 2017; Villani et al., 2017) and high-dimensional flow cytometry approaches (Alcántara-Hernández et al., 2017; Dutertre et al., 2019). Clonal analysis of hematopoietic progenitors shows that pre-cDCs derive from the CDP. Moreover, pre-cDCs are transcriptionally pre-committed to become cDC1 or cDC2 (Breton et al., 2016; Ma et al., 2019).

Monocytes can also be direct precursors of DCs, referred to as mo-DCs (Sallusto and Lanzavecchi, 1994). Mo-DCs arise *in vitro* in different culture conditions (detailed in **Table 1**) and have been identified *in vivo* in mucosal or inflamed tissues (reviewed in Coillard and Segura, 2019). In contrast to pre-cDCs, monocytes are not transcriptionally pre-committed to become DCs versus macrophages (Goudot et al., 2017).

## **4. Regulation of DC development**

### **4.1 Cytokine requirements**

Flt3L is the most important cytokine in DC development and expansion. Initial observations in healthy volunteers injected with Flt3L showed a dramatic increase in the number of cDCs ( $\text{Lin}^- \text{HLA}^+ \text{CD11c}^+$ ) and pDCs ( $\text{Lin}^- \text{HLA}^+ \text{CD11c}^- \text{CD123}^+$ ) in peripheral blood (Pulendran et al., 2000), as well as  $\text{CD14}^+$  monocytes (Maraskovsky et al., 2000). In addition, injection of Flt3L also increases pre-cDC population (Breton et al., 2015) and circulating DC3 (Dutertre et al., 2019). In mice reconstituted with cord blood  $\text{CD34}^+$  cells and

injected with human Flt3L, cDC1 and cDC2 are expanded to a similar degree in blood and spleen, but in bone marrow mainly cDC2 are increased (Ding et al., 2014). Of note, patients with HSC and DC deficiency have 100-fold increased levels of circulating Flt3L (Bigley et al., 2011). This could happen because Flt3L is not consumed by DC or their progenitors and accumulates in the blood. However, the effect of DC versus HSC consumption cannot be separated in this study. Consistent with these observations, Flt3L expands DC progenitors *in vitro* better than SCF (Harada et al., 2007) and induces the differentiation of HSC into pDCs, unlike G-CSF (Blom et al., 2000). These observations demonstrate that Flt3L is essential in cDC and pDC development.

Other cytokines such as SCF, GM-CSF, IL-3 and IL-7 are widely used for *in vitro* culture systems that sustain DC development. Although the precise mechanisms are not well understood, these requirements have been used to define subpopulations within progenitor cells, using cytokine receptors as phenotypic markers. For example, the phenotype of human GMDP, MDP and CDP was refined based on the expression of the receptors for M-CSF, GM-CSF, and IL-3 (Lee et al., 2017), and Gata1<sup>-</sup> CMP was described based on the upregulation of the G-CSF receptor (Drissen et al., 2019).

Regarding mo-DCs, it is now clear that IL-4 drives their differentiation program *in vitro* (Goudot et al., 2017; Sander et al., 2017). However, the role of IL-4 *in vivo* for monocyte differentiation into DCs, and the requirement for M-CSF versus GM-CSF, remains to be characterized.

## **4.2 Transcription factors**

A combination of *in vitro* studies in culture systems and *ex vivo* analysis of genetically deficient patients has provided insight into the TFs involved in human DC development.

IRF8 is an essential TF for DC development. Patients with IRF8 deficiency have reduced or null numbers of blood cDC1, cDC2 and pDC, and dermal cDC2 and CD14<sup>+</sup> DC

(Bigley et al., 2018, 2011; Hambleton, 2011; Kong et al., 2018). Consistent with this, early expression of IRF8 in HSC-MPP imprints a bias towards the cDC1 lineage (Lee et al., 2017). CMP and GMDP are also heterogeneous for IRF8 expression. IRF8<sup>high</sup> progenitors give rise to pDC, cDC1 and cDC2, and IRF8<sup>low</sup> progenitors contain the monocytic, neutrophil and DC3 potential (Cytlak et al., 2019). A role for Flt3L in the development of DC3 has also been proposed (Dutertre et al., 2019). As GMDPs express CD135/Flt3 (Doulatov et al., 2010), an hypothesis to reconcile these observations would be that Flt3 expression is down-regulated in pre-monocytes but maintained in pre-DC3. Finally, while in pre-cDCs the expression of IRF8 or IRF4 is not mutually exclusive between pre-cDC1 and pre-cDC2, the IRF8/IRF4 ratio correlates with pre-cDC commitment to cDC1 (higher IRF8) or cDC2 (higher IRF4) (Ma et al., 2019).

Other important TFs for cDC development are NOTCH, BATF3 and GATA2. NOTCH activation through DL1 promotes cDC1 differentiation (and less strikingly cDC2) from bone marrow and cord blood precursors (Balan et al., 2018; Kirkling et al., 2018), and refines the phenotype and function of *in vitro*-differentiated cDC1 compared to their blood counterpart (Kirkling et al., 2018). Silencing of BATF3 in HSC impairs cDC1 differentiation *in vitro*, but not in humanized mice (Poulin et al., 2012). Finally, patients possessing a non-functional mutation of GATA2 lack DCs, monocytes, B cells and NK cells (DCML deficiency patients) (Dickinson et al., 2011). These data indicate that GATA2 is important for maintenance/differentiation of hematopoietic progenitors with different potentials (DC, monocytic and lymphocyte), while NOTCH and BATF3 participate in later differentiation events of the DC lineage.

The development of pDCs is positively regulated by Ikaros, E2-2, SpiB, and negatively regulated by NOTCH. Patients with IKZF1 (Ikaros) deficiency have reduced numbers of pDCs and decreased IFN $\alpha$  production (Cytlak et al., 2018). By contrast, patients

bearing monoallelic loss-of-function mutations or deletions of E2-2 (Pitt-Hopkins syndrome patients) have normal pDC numbers in the blood, but displaying an altered phenotype and function (Cisse et al., 2008). Forced expression of E2-2 in thymic CD34<sup>+</sup> progenitors enhances the development of pDC *in vitro*, while silencing E2-2 decreases pDC output (Nagasawa et al., 2008). In addition, co-expression of E2-2 and SpiB is necessary to promote pDC development in this system (Nagasawa et al., 2008). Silencing SpiB completely blocks pDC development *in vitro* and *in vivo* in humanized mice (Schotte et al., 2004). Actually, Spi-B expression is downregulated by NOTCH1 signaling (Dontje et al., 2006), which inhibits pDC development *in vitro* (Balan et al., 2018).

Finally, the differentiation of monocytes toward mo-DCs involves IRF4, BLIMP1 and Aryl Hydrocarbon Receptor (AHR), as demonstrated by gene silencing in an *in vitro* culture model (Goudot et al., 2017). The nuclear receptor corepressor 2 (NCOR2) was also proposed as an orchestrator of mo-DC differentiation in a different culture model (Sander et al., 2017).

## **5. Conclusion and perspectives**

Despite inherent limitations to working with human samples, successful experimental approaches have been developed to study human DC ontogeny. This work has confirmed that the same principles apply to mouse and human DC development. Human DCs have a short life span and are constantly replenished from circulating precursors originating from the bone marrow. Lineage bias is imprinted early in human hematopoietic progenitors, and correlates with TF expression.

Differences between mouse and human may lie in the molecular regulators involved. Although Flt3L is the key DC-promoting cytokine in both mouse and human, it is not sufficient to generate human DCs *in vitro* from BM progenitors without additional cytokines, contrary to the mouse. The precise role of some TF could also differ, such as IRF8. IRF8

deficiency in humans affects all cDC and pDC populations (Hambleton et al., 2011; Kong et al., 2018), while cDC2 development is independent of IRF8 in the mouse (Sichien et al., 2016).

Methodological developments are needed to refine our understanding of human DC development *in vivo*. Such methods will undoubtedly include clonal genetic tracing, either using artificial barcoding of single hematopoietic stem cells (Naik et al., 2013; Perié et al., 2014) or using spontaneous somatic mutations as genetic labels for progenitors (Lee-Six et al., 2018; Woodworth et al., 2017).

Open questions to be addressed in humans include the ontogeny of DC3. More work is also needed to better understand the final steps of DC differentiation and the role of tissue-derived signals.

### **Author Contributions**

JV and ES wrote the manuscript.

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### **Competing interests statement**

The authors declare no competing interests.

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**Table 1. *In vitro* culture models for the differentiation of human hematopoietic progenitors into dendritic cells.** Some models include a step for “progenitor expansion” without stromal cell lines.

Progenitor		Culture conditions					Output	Ref
		Progenitor expansion		Differentiation				
Phenotype	Tissue origin	cytokines	Time	stromal cell line	cytokines	Time		
HSC (CD34 <sup>+</sup> )	bone marrow	-	-	-	GM-CSF + IL-4 + TNF- $\alpha$ + Flt3L	14d	DCs (CD1a <sup>+</sup> CD86 <sup>+</sup> HLA-DR <sup>+</sup> )	(Maraskovsky et al., 2000)
HSC (CD34 <sup>+</sup> CD38 <sup>-</sup> )	fetal liver cells	-	-	OP9	IL-7 + Flt3L	7d	pDCs (CD303 <sup>+</sup> CD123 <sup>hi</sup> ) pro-B cells(CD10 <sup>+</sup> CD19 <sup>+</sup> ) Myeloid cells (CD14 <sup>+</sup> CD11c <sup>+</sup> )	(Schotte et al., 2004)
HSC (CD34 <sup>+</sup> CD1a <sup>-</sup> )	postnatal thymus	SCF + IL-7	1d	OP9	IL-7 + Flt3L	7d	pDCs (CD303 <sup>+</sup> CD123 <sup>hi</sup> )	(Dontje et al., 2006)
HSC (CD34 <sup>+</sup> Flt3 <sup>+</sup> )	blood from patients with non-Hodgkin's lymphoma	-	-	-	GM-CSF + TPO + Flt3L	14d	DC precursors (CD14 <sup>+</sup> CD1a <sup>+</sup> ) DCs (CD1a <sup>+</sup> CD11c <sup>+</sup> )	(Harada et al., 2007)
MLP (CD34 <sup>+</sup> CD38 <sup>-</sup> Thy-1 <sup>neg</sup> - <sup>hi</sup> CD45RA <sup>+</sup> Flt3 <sup>+</sup> CD10 <sup>+</sup> )	cord blood	SCF + TPO + IL-7 + Flt3L	7d	OP9	GM-CSF + IL-4	7d	DCs (CD14 <sup>+</sup> CD1a <sup>+</sup> CD11c <sup>+</sup> )	(Doulatov et al., 2010)
HSC (Lin <sup>-</sup> CD34 <sup>+</sup> )	cord blood	SCF + Flt3L + IL-3 + IL-6	7-11 d	-	SCF + GM-CSF + IL-4 + Flt3L	12-14d	DCs (Lin <sup>-</sup> HLA-DR <sup>+</sup> : CD141 <sup>+</sup> CLEC9A <sup>+</sup> or CD11b <sup>+</sup> )	(Poulin et al., 2012)
HSC (CD34 <sup>+</sup> )	G-CSF mobilized cells from peripheral blood	-	-	-	Flt3L + TPO	21d	pDC (CD123 <sup>+</sup> ) cDC (CD1b/c <sup>+</sup> or CLEC9A <sup>+</sup> )	(Proietto et al., 2012)
pre-cDC (CD34 <sup>+</sup> CD117 <sup>+</sup> CD135 <sup>+</sup> CD116 <sup>+</sup> CD45RA <sup>+</sup> CD115 <sup>-</sup> HLA-DR <sup>+</sup> )	cord blood	-	-	-	Flt3L + SCF + GM-CSF	7d	DC1 (CD141 <sup>+</sup> ) DC2 (CD1c <sup>+</sup> )	(Breton et al., 2015)
HSC (CD34 <sup>+</sup> )	cord blood	-	-	MS5	Flt3L + SCF + GM-CSF	14d	pDCs (CD303 <sup>+</sup> CD1c <sup>+</sup> ) DC1 (CD141 <sup>+</sup> CLEC9A <sup>+</sup> ) DC2 (CD141 <sup>+</sup> CD1c <sup>+</sup> ) Monocytes (CD14 <sup>+</sup> ) Granulocytes (CD66b <sup>+</sup> ) B cells (CD19 <sup>+</sup> ) NK cells (CD56 <sup>+</sup> )	(Lee et al., 2015a, 2015b)
Monocytes (CD14 <sup>+</sup> )	peripheral blood	-	-	-	M-CSF + IL-4 + TNF $\alpha$	5 d	mo-DC (CD1a <sup>+</sup> ) mo-Mac (CD16 <sup>+</sup> )	(Goudot et al., 2017)
MLP (Lin <sup>-</sup> CD34 <sup>+</sup> CD38 <sup>-</sup> CD45RA <sup>+</sup> CD10 <sup>+</sup> )	cord blood	-	-	MS5	Flt3L + SCF + GM-CSF + IL-4	12d	DC1 (CD141 <sup>+</sup> CLEC9A <sup>+</sup> ) DC2 (CD1a <sup>+</sup> ) Monocytes (CD14 <sup>+</sup> )	(Helft et al., 2017)
Monocytes (CD14 <sup>+</sup> )	peripheral blood	-	-	-	GM-CSF + IL-4	6d	mo-DC (CD14 <sup>+</sup> CD209 <sup>+</sup> )	(Sander et al., 2017)
HSC-MPP (CD34 <sup>+</sup> CD38 <sup>-</sup> CD45RA <sup>+</sup> CD90 <sup>+</sup> and CD90 <sup>-</sup> )	cord blood	-	-	MS5 + OP9	Flt3L + SCF + GM-CSF	2-3 weeks	DCs (DC1, DC2 and pDC) Granulocytes, Monocytes, Megakaryocytes, Erythrocytes NK and B cells	(Lee et al., 2017)
HSC (CD34 <sup>+</sup> )	bone marrow	-	-	OP9-DL1	GM-CSF + Flt3L + SCF	14-21 d	DC1 (CD141 <sup>+</sup> CLEC9A <sup>+</sup> ) DC2 (CD1c <sup>+</sup> CD11c <sup>+</sup> ) pDC (CD303/4 <sup>hi</sup> CD123 <sup>hi</sup> ) Monocytes (CD14 <sup>+</sup> CD11c <sup>+</sup> )	(Kirkling et al., 2018)
HSC (CD34 <sup>+</sup> )	cord blood	Flt3L + SCF + TPO + IL-7	7d	OP9 + OP9-DL1	Flt3L + TPO + IL-7	18-21 d	pDCs (CD303 <sup>+</sup> CD123 <sup>hi</sup> ) DC1 (CD141 <sup>+</sup> CLEC9A <sup>+</sup> )	(Balan et al., 2018)

## Figure legend

**Figure 1. Unified model of human dendritic cell development.** Hematopoietic stem cells (HSCs) give rise to dendritic cells (DCs) by a sequential diversification of increasingly restricted progenitors. Lymphoid multipotent progenitors (LMPPs), deriving from the HSCs, give rise to a Gata1<sup>-</sup>CD114<sup>+</sup> common myeloid progenitor (CMP). Monocytes and DC3 arise from the IRF8<sup>low</sup> fraction of the granulocyte-monocyte-DC progenitors (GMDP or GMP), while conventional DC1 and DC2 and pDC differentiate from committed DC progenitors (CDP). Colors represent lineage potential observed in each progenitor. Proportions of each lineage in a given progenitor are arbitrary.

