Decoding the Heterogeneity of Human Dendritic Cell Subsets

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Abstract

Dendritic cells (DCs) have been classified into distinct subsets based on phenotype and ontogeny. In the past few years, high throughput single-cell approaches have revealed further heterogeneity of human DCs, in particular at the transcriptomic level. Here we examine recent studies describing new human DC populations based on single-cell RNA-seq analysis and provide a unified view of these emerging DC populations. We also assess the features that define *bona fide* DC lineages as opposed to cell states of the same lineage. Finally, we examine where these newly described DC populations fit in the ontogeny-based classification of human DCs.

Key features defining dendritic cell subsets

Dendritic cells (DCs) (see Glossary) are recognized as the most efficient antigen-presenting cells and are present throughout the mammalian body in both lymphoid organs and peripheral tissues. Since the initial identification of DCs in mouse spleen by Ralph Steinman [1], methodological advances have gradually revealed the complexity of DC populations. Multi-color flow cytometry has shown the existence of several subpopulations of DCs displaying distinct surface phenotypes [2]. Transgenic mouse models have revealed the transcriptional control of DC lineages and specific functions of DC subsets [3]. Transcriptomic analyses have also evidenced a conserved gene expression profile for each DC subset across tissues and species [4,5]. The current classification of DC subsets is based on their cellular and molecular ontogeny: plasmacytoid DCs (pDC), type 1 classical DCs (cDC1), type 2 classical DCs (cDC2), and monocyte-derived DCs (mo-DC) [6] (Table 1).

To better understand the biology of human immune cells, an increasing number of groups are analyzing immune cells, including DCs, directly purified from human tissues using high-dimensional single-cell approaches. These single-cell analyses have revealed further heterogeneity within human DC subsets, in particular at the transcriptional level (Table 2). However, in this rapidly evolving field, there is still little consensus on the identification and naming of « new » DC populations. In this Opinion article, we aim to provide a unified view of the newly described human DC populations and to discuss the limitations of **single-cell RNA sequencing** (scRNA-seq) for identifying DC subsets. We argue that differential gene expression profiles are not sufficient to distinguish *bona fide* DC lineages from cell states of the same lineage. Finally, we examine the place of these newly described DC populations in the widely used ontogeny-based DC classification.

Transcriptional heterogeneity of human DCs revealed by single-cell approaches

DCs orchestrate immune responses and manipulating their properties holds great promise for therapeutic strategies to treat chronic inflammatory diseases, cancers, graft rejection, or to improve vaccine efficiency [7–9]. DC dysfunction may also play a crucial part in autoimmune diseases. Because each DC subset displays unique functions [3], it is vital to understand the complexity of human DC subsets to decipher their specific properties. Recent studies employing scRNA-seq have revisited this

issue with unprecedented resolution. However, results from this body of work can be confusing when different groups propose contradictory interpretations or different terminologies for apparently similar populations.

One important result from the recent series of human scRNA-seq studies is that **unbiased clustering** based on differential gene expression confirms the earlier classification of DCs into three main groups (pDC, cDC1 and cDC2). Another striking observation is that cDC1 display remarkable homogeneity as a subset in all tissues and pathological situations analyzed. While previous work suggested some level of heterogeneity in pDC and cDC2, scRNA-seq analyses have provided irrefutable evidence of the existence of different populations within these DC subsets.

AXL$^+$SIGLEC6$^+$ DCs : pDC-related cells or cDCs precursors ?

Human pDC do not express CD11c and are usually defined by their expression of CD123/IL3R, CD303/BDCA2 and CD304/BDCA4 [10]. Two studies have proposed the existence of subsets of pDC based on the differential expression of CD2 [11] or CD5 [12]. scRNA-seq analysis has shed new light on this question, showing the presence in human blood of a CD123$^+$ population with a mixed pDC/cDC gene expression profile, best characterized by high expression of *AXL* and *SIGLEC6*, which encode two surface molecules that can be used to distinguish this population [13,14]. This finding was consistent with previous observations of preferential *AXL* expression in blood CD2$^+$ pDC [11] and CD2$^+$ CD5$^+$ pDC [12]. High-dimensional cytometry further confirmed the phenotype of this population as being AXL$^+$SIGLEC6$^+$CD123$^+$CD2$^+$CD5$^+$ [14–16]. These AXL$^+$SIGLEC6$^+$ DCs do not secrete IFNa (a defining functional feature of pDC) but are efficient for T cell stimulation (a property classically associated with cDCs) [12–16]. They also display the typical morphology of cDC [13,14]. The identity of these cells remains unclear, as they show themselves some level of heterogeneity, in particular with a spectrum of expression for CD123, CD5 or CD11c [13–15]. AXL$^+$SIGLEC6$^+$ DCs are able to differentiate into cDCs (predominantly cDC2) when placed in *in vitro* culture systems [13,14,17], and therefore, were proposed to be DC precursors [14]. Putative mouse counterparts of AXL$^+$SIGLEC6$^+$ DCs were later identified, but these do not express either AXL or SIGLEC6 [16]. Because this population displays an intermediate phenotype between pDC and cDCs, both at the level of their transcriptome and surface markers, the term **‘transitional DCs’** was also proposed [16].

The expanding cDC2 family

In humans, cDC2 are usually characterized by their expression of CD1c/BDCA1. Based on their phenotypic characterization by flow cytometry, two studies have proposed the existence in blood and skin of two subsets of cDC2, $CD5^+$ and $CD5^-$ [18,19]. In addition, a population of $CD1c^+CD14^+$ cDC was reported in blood [20]. In these studies, $CD1c^+CD5^-$ DCs and $CD1c^+CD14^+$ cDCs both expressed genes classically associated with **monocytes** (such as *VCAN, FCN1, S100A8, S100A9*) [18,20]. These observations suggested the existence of a new DC subset displaying hybrid cDC/monocyte features, but could also be explained by the co-isolation of a mixed population of cDC2 and monocytes that were analyzed together by bulk transcriptomics. ScRNA-seq analysis confirmed the existence of a population of $CD1c^+$ DCs expressing at the same time cDC2 classical marker genes (such as *CD1c, FCER1A, CLEC10A*) and monocyte-related genes (such as *VCAN, FCN1, S100A8, S100A9, CD14, CD163*) [13]. These DCs were best defined as $CD1c^+CD163^+$ and were termed 'DC3'. Subsequent studies employing high-dimensional cytometry refined the phenotypic definition of blood cDC2 as $CD1c^+BTLA^+$ with low to high expression of CD5, and DC3 as $CD88^-CD1c^+CD163^+$ with low to high expression of CD14 [17,21,22]. Of note, DC3 were also described in human cord blood [23].

A recent scRNA-seq study has added another layer of complexity to the « cDC2 family ». Clec10A is considered a phenotypic marker of human cDC2 [24]. A population of $Clec10A^-$ cDC2 was described in human spleen but absent from blood. In this study, $CD1c^+Clec10A^-$ were termed 'DC2A' and $CD1c^+Clec10A^+$ 'DC2B', and were characterized mostly by similarity to their mouse putative counterparts, $Tbet^+$ 'DC2A' and $Tbet^-$ 'DC2B' [25]. Based on their higher expression of cDC2 markers Clec10A and CD1c, DC2B were proposed to correspond to the well-characterized classical cDC2 subset, while DC2A were thought to be a new population of cDC2-related DCs [25]. However, this population has not been reported by others so far. In a scRNA-seq analysis of human tonsil antigen-presenting cells [26], our group observed a population of $CD14^-$ cells which did not correspond to cDC1 nor cDC2, and which exhibited high expression of *LTB* and *RUNX3* – two genes enriched in the described DC2A population. We proposed that these LTB^+ cells were « precursor cells » because a proportion of them were mitotic. Of note, *AREG*, another proposed

DC2A marker gene, was more expressed in *Clec10A*$^{+}$ cDC2 in our dataset compared with *LTB*$^{+}$ cells [26]. Nevertheless, the gene profile of this putative human 'DC2A' remains ill defined.

A thin line between mo-DC and DC3

Fate-mapping techniques in mice have demonstrated that monocytes can differentiate into DC-like cells in mucosal or inflamed tissues [27–30]. Despite obvious methodological limitations, there is also accumulating evidence that this phenomenon can occur *in vivo* in humans [31]. We and others have initially used bulk transcriptomic analysis to identify human *in vivo*-generated mo-DC, and observed a transcriptomic similarity with blood monocytes or *in vitro*-derived mo-DC [32,33]. ScRNA-seq studies have confirmed the presence of a human DC population displaying a mixed cDC2/monocyte transcriptional profile (*CD1c, FCER1A, CLEC10A, VCAN, S100A8, S100A9, CD14*) in peritoneal tumor **ascites** [34], inflamed and uninflamed intestinal **lamina propria** [35], non-small cell lung cancer [36] and melanoma-draining lymph nodes [37]. Given that blood DC3 show an overall similar profile, DC3 and 'mo-DC' have been proposed as being the same DC subset [21]. A closer look at the gene expression patterns of the two populations suggests that expression of other genes such as *CD207* and *DAB2* might help to distinguish them. *CD207* is expressed by both mo-DC and cDC2 [35], but not by DC3 [21]. *DAB2* has comparable expression to mo-DC and **macrophage** clusters [34,35], and is detected in blood pDC but not DC3 [13]. A side-by-side comparison of blood DC3 and tissue mo-DC in the same scRNA-seq study would be needed to better define their respective gene signatures. Combining gene expression profiling with analysis of surface molecules might also be necessary to discriminate these. DC3 express CD163 [13,21,22] but not ascites mo-DC [38]. Of note, our group also recently identified surface LSP1 as a marker for ascites and *in vitro*-generated mo-DC, and not expressed by blood DC3 [39]. However, the possibility that differential surface expression results from differential localization (blood versus tissue) cannot be completely ruled out. More *in vivo* evidence is needed to clarify whether DC3 and 'mo-DC' represent distinct subsets ultimately displaying a similar phenotype, or variations of the same subset (for instance a blood state for DC3 versus a tissue state for the described mo-DC).

CCR7^{+} DCs : the rediscovery of a well-known phenomenon

ScRNA-seq analysis of DCs isolated from human tissues other than blood has revealed another previously unnoticed group of DCs, expressing high amounts of genes associated with DC **maturation** and migration (such as *LAMP3, CCR7, FSCN1, CD83, CCL22*) [34]. *CCR7*$^{+}$ DCs, also referred to as 'activated DCs' or 'mature DCs enriched in immunoregulatory molecules' (mregDCs), were reported in liver tumors [40], non-small lung cancer [36,41], colon cancer [42], melanoma-draining lymph nodes [37], inflamed colon of Crohn's disease patients [35] and ulcerative colitis [42], skin of atopic dermatitis patients (He) as well as in inflammatory peritoneal ascites of cancer patients [34] and in the fluid of experimental skin blisters [43]. A similar population was also present in steady-state tonsils [26], spleen [25], thymus [44] and skin [45](He), indicating that this population was not specific to inflamed tissues. The fact that this population was detected in inflammatory fluids [34,43], which do not require dissociation prior to analysis, and by *in situ* staining [35,44,45] also shows that the expression of activation genes is not an artefact induced by tissue digestion.

While these cells all expressed a common gene signature of DC activation and maturation, a more detailed analysis showed that depending on context, this population also expressed genes associated with the transcriptional profile of cDCs [34], cDC1 [42], pDC [43], mo-DC [34], AXL^{+}SIGLEC6^{+} DCs [43] or those comprising both cDC1 and cDC2 [41,44]. Collectively, these results suggest that all human DC types may express the same transcriptional module upon activation *in vivo*. This is consistent with mouse studies showing that DCs of any subset undergoing maturation, either homeostatic or upon exposure to a danger signal, converge towards a common transcriptional program [5,46,47].

Distinguishing human DC lineages versus cell states

ScRNA-seq analysis represents a snapshot of transcriptional states, which can be dynamic for myeloid cells such as DCs that are constantly surveying their environment. Using scRNA-seq data to identify DC subsets purely based on differential gene expression can therefore be misleading, as illustrated by the *CCR7*$^{+}$ DC population which has been observed in many recent studies. In this case, a superficial description of cell clusters might conclude in the identification of a new putative DC subset. However, a more detailed analysis, by combining scRNA-seq

with surface staining of phenotypic markers [41] or by applying gene signatures analysis [44], has revealed that legacy markers of subset identity can also be detected, suggesting that *CCR7*$^{+}$ DCs may correspond to DCs undergoing maturation.

Another important consideration when studying human DCs is the potential variability between individuals, as exemplified by the diversity in CD1c^{+} DC phenotypes observed from different donors, using high-dimensional flow cytometry [15]. From a practical standpoint, if several donor samples cannot be analyzed via scRNA-seq analysis for practical reasons, identified populations may need to be validated in additional donors using other techniques.

What are the key features that define a *bona fide* DC subset (or lineage) as opposed to a transitory cell state? While distinct transcriptional programs might constitute a pre-requisite for discriminating distinct subsets, this criterion is not sufficient, as outlined above. Therefore, scRNA-seq analysis may provide a basis for DC subset identification but it also needs to be complemented by other approaches (see Box1). Numerous studies in mice have evidenced functional specialization of DC subsets. However, functional properties do not necessarily correlate with lineage identity. For example, immature or mature populations from the same cDC lineage will display a differential ability in activating T cells via co-stimulatory signals as shown *in vitro* for human DCs or *in vivo* in mice [47–49]. Therefore, distinct functional abilities might not necessarily define the attributes of DC subset identity. In line with the current DC classification based on ontogeny, we would instead argue that DC populations might only be considered distinct subsets if they possess distinct ontogeny, i.e separate developmental pathways controlled by specific transcription factors.

The methodological difficulties and available strategies for addressing experimentally the ontogeny of human DCs have been reviewed elsewhere [50]. With these technical limitations in mind, which of the « new » DC populations fulfill this criterion? AXL^{+}SIGLEC6^{+} DCs seem to be related to pDC in their ontogeny. They depend on E2-2/TCF4 for their development, as pDC do; this has been shown by the partial decrease in AXL^{+}SIGLEC6^{+} DC and pDC populations in the blood of **Pitt-Hopkins syndrome** patients bearing a loss-of-function mutation for *TCF4* [14]. In addition to *TCF4*, these cells express high amounts of *BCL11A*, *RUNX2* and *SPIB*, all of which are transcription factors involved in pDC development [16]. Analysis of chromatin accessibility by Assay for Transposase-Accessible Chromatin using sequencing

(ATAC-seq) also suggests that TCF4 and RUNX2 are active in AXL^{+}SIGLEC6^{+} DCs (Leylek 2020). Moreover, « transitional DCs » (the mouse counterpart of AXL^{+}SIGLEC6^{+} DCs) are severely decreased in *CD11C^{cre} Tcf4$^{fl/fl}$* deficient mice, but unaffected by *Irf8* deficiency in *CD11C^{cre} Irf8$^{fl/fl}$* mice, similar to pDC [16]. The transcription factor KLF12 is preferentially expressed in AXL^{+}SIGLEC6^{+} DCs and their mouse counterparts compared to other DC subsets, but whether it is involved in their differentiation remains unknown (Leylek 2020). Evidence for a unique transcriptional regulation of AXL^{+}SIGLEC6^{+} DCs, distinct from pDC, is still lacking.

Regarding DC3, two recent studies have shed light on their developmental pathway using *in vitro* culture models in which human hematopoietic progenitors can give rise simultaneously to cDC2, DC3 and monocytes [17,22]. In these cultures, purified monocytes or monocyte-commited precursors do not differentiate into DC3, but the IRF8^{low} fraction of granulocyte-monocyte and DC progenitors give rise to DC3 and monocytes along separate pathways, while pDC, AXL^{+}SIGLEC6^{+} DCs, cDC1 and cDC2 derive from IRF8^{high} progenitors [17,22]. Observations from patients with *IRF8* deficiencies also support this model. Specifically, patients with a total loss of *IRF8* activity exhibit a complete absence of DC and monocyte development [17]. However, patients harboring a heterozygous or dominant negative mutation in *IRF8* that results in reduced *IRF8* activity display decreased numbers of pDC, cDC1 and cDC2, but increased DC3 in blood [17], consistent with the idea that DC3 development is largely independent of IRF8. As shown in Batf3$^{-/-}$ mice in which related transcription factors Batf and Batf2 can induce an alternative cDC1 development program [51], such genetic deficiencies may result in compensatory phenomena, where the activities of other transcription factors with shared properties can compensate at the molecular level for the deficient molecule. Therefore, these observations should be interpreted with caution. Nevertheless, these data suggest that two distinct developmental pathways might exist for cDC2 and DC3 *in vivo*. Of note, both CD1c^{+}BTLA^{+}CD5^{+} and CD1c^{+}BTLA^{+}CD5^{-} DCs populations were reported to be reduced in the blood of patients with reduced *IRF8* activity, while CD1c^{+}CD163^{+}CD14^{+} and CD1c^{+}CD163^{+}CD14^{-} DCs were expanded [17], validating their classification as cDC2 or DC3 populations, respectively.

The case of DC2A and DC2B populations is less clear. While there is evidence for differential expression of some transcription factors between mouse DC2A and DC2B, the requirement for distinct transcriptional regulators for their development, to

our knowledge, has not been demonstrated [25]. In mouse, *in vivo* lineage tracing has shown that pre-DC give rise to both DC2A and DC2B, and DC2A appear to be enriched at mucosal sites while their numbers are decreased upon microbiota depletion by broad-spectrum antibiotics treatment; this suggested that signals from the tissue micro-environment could drive the expression of the DC2A phenotype in cDC2 [25]. Therefore, DC2A and DC2B might represent variations of the same subset rather than distinct lineages, although this remains to be further tested. This is also consistent with the heterogeneity of cDC2 expression profiles between tissues reported in mouse [5,52] and human [15,53], suggesting tissue adaptation in response to micro-environmental signals. More work is needed to better characterize the putative DC2A and DC2B populations, particularly in humans. While mouse models can be useful for understanding DC biology, caution should be exercised in extrapolating murine findings and conclusions to humans. In particular, marker genes are not always directly translatable, as in the case of $AXL^{+}SIGLEC6^{+}$ DCs and their mouse counterparts [16].

Finally, by definition, mo-DC possess specific ontogeny. While monocytes differentiate into mo-DC *in vitro* (in human cultures and different models [38,48]) as well as *in vivo* in mice [27–30], it remains unclear whether the same phenomena occur *in vivo* in humans. Indeed, in an *in vitro* model, the developmental pathway of mo-DC is distinct from that of monocyte-derived macrophages, and silencing experiments have shown that it is dependent on IRF4, BLIMP1 and AHR [38]. While these findings have been corroborated in $Ahr^{-/-}$ mice [38], it remains to be formally demonstrated that monocytes differentiate into DCs along a specific developmental pathway *in vivo* in humans.

Concluding remarks

In the ontogeny-based human DC classification and based on the work discussed here, we propose that DC3 represent a new human DC subset, along with pDC, cDC1, cDC2 and mo-DC (figure 1). All these DC subsets express a similar transcriptional program upon activation, which dominates the gene expression profile of activated cells and corresponds to the recently described $CCR7^{+}$ DC populations. Evidently, further work is needed to classify $AXL^{+}SIGLEC6^{+}$ DCs and the proposed DC2A and DC2B populations (see also Outstanding questions).

Emerging observations that $AXL^{+}SIGLEC6^{+}$ DCs or DC3 are enriched in inflamed tissues or in the blood of patients with chronic diseases suggest that these previously overlooked DC populations may be important in immunopathologies. ScRNA-seq studies have brought new DC populations into the spotlight, but it appears now necessary to go beyond the description of transcriptomic profiles to address their functional properties. Identifying specific and unique cell surface markers for each DC subset will be a pre-requisite, allowing their isolation for functional assays, as well as a better profiling of the DC landscape in patients samples when scRNA-seq analysis is not feasible for practical reasons. This work will be essential for understanding the respective roles of these subsets in the initiation and progression of human diseases, and ultimately for manipulating them for improving patients treatment.

Outstanding questions

What is the heterogeneity of human cDC2 in peripheral tissues ? cDC2-related populations (DC3, DC2A, DC2B) have been identified in blood and in some studies in secondary lymphoid organs. It remains unclear whether these populations are present in peripheral tissues, and to what extent local signals imprint specific transcriptional signatures in cDC2. scRNA-seq analysis of purified cDC2, instead of whole tissue or all myeloid cells, might be a more powerful approach to address this question.

Can DC3 migrate to tissues and in which contexts ? While precursors of DC3 have been identified in bone marrow and $CD1c^{+}CD14^{+}$ DCs have been described in skin and tumor-draining lymph nodes, there is still no formal evidence that DC3 can be recruited into secondary lymphoid organs or peripheral tissues.

Is there a mouse equivalent of DC3 ? So far, mouse and human counterparts have been discovered for all DC subsets except for DC3. One potential candidate is the Notch2-independent $Esam^{low}$ cDC2-like population described in mouse spleen [54]. However, it remains unclear whether DC3 are present in human lymphoid organs. Studying mouse equivalents of DC3 might provide some clues about their dynamics and possible functions *in vivo*.

Are there specific markers of DC3 versus mo-DC that would distinguish them unambiguously ? Identifying unique surface markers for these two populations will be essential for better understanding their relationship and respective roles.

Can DC3 differentiate into mo-DC-like cells ? While it is clear that monocytes can differentiate *in vitro* into cells resembling the 'mo-DC' populations found in human samples, could some of these 'mo-DC' actually derive *in vivo* from DC3 instead of monocytes, along a convergent differentiation program ?

What transcription factors govern the differentiation of DC3 from $IRF8^{low}$ precursors ? In particular, do they depend on IRF4 and AHR, similar to mo-DC ? Perturbations of *in vitro* culture systems should provide a first line of evidence for this question.

Are $AXL^{+}SIGLEC6^{+}$ DCs a subpopulation of pDC or a distinct DC subset that stems from pre-pDC ? A side-by-side comparison of the gene regulatory network of pDC and $AXL^{+}SIGLEC6^{+}$ DCs may help identifying unique transcription factors regulating separate developmental pathways.

Can AXL^{+}SIGLEC6^{+} DCs differentiate *in vivo* into cDC, and if so, in which contexts ? Do they differentiate spontaneously or in response to specific signals ? AXL^{+}SIGLEC6^{+} DCs can differentiate *in vitro* into cDC2-like cells when placed in culture with cytokines. They are detected in inflamed tissues but whether they can differentiate *in situ* after their recruitment is unclear.

Highlights

- ScRNA-seq analysis of human DC subsets has revealed unexpected heterogeneity within cDC2 and pDC subsets, leading to the identification of new populations
- $AXL^{+}SIGLEC6^{+}$ DCs display a mixed pDC/cDC transcriptional profile and can differentiate into cDC2 when placed in culture.
- DC3 have a mixed cDC2/monocyte gene expression profile and develop from precursors distinct from other DC lineages and monocytes.
- All human DC subsets express a convergent transcriptional program when they mature *in vivo* in lymphoid organs or peripheral tissues.
- In the ontogeny-based classification of human DCs, DC3 represent a new DC subset, along with pDC, cDC1, cDC2 and mo-DC.

Box 1. Challenges in the interpretation of scRNA-seq data to identify new DC populations

While scRNA-seq analysis is usually considered unsupervised, there are a number of analysis biases. Algorithms have been developed for the automated identification of cell types in scRNA-seq data [55]. However, automated identification is not suitable for cell types that closely resemble each other, such as DC subsets, and cell clusters are generally manually annotated. Prior knowledge therefore necessarily influences the identification of DC clusters, based on the presence of canonical markers among differentially expressed genes (Table1). A major challenge is to decide on how many clusters are present in the dataset. There is no consensus on how to predict the number of clusters, so it is left to the user to determine the number of clusters that are considered biologically relevant [56]. Under-clustering can hide a rare but biologically relevant population. By contrast, over-clustering can result in partitioning a population into several clusters that simply represent stochastic variations instead of biological states. A possibility for mitigating this problem might be to re-cluster a specific population of interest, as performed for cDC2 clusters [13,37], but such analysis will almost certainly reveal further heterogeneity that might be difficult to interpret.

Cluster annotation is usually based on the most differentially expressed genes. The output of such analysis is highly dependent on the populations being compared to one another. This can introduce some bias regarding the identification of marker genes for putative new populations. This difficulty is illustrated by the divergent interpretations of the level of expression of monocyte-related genes by DC3 [13,21]. One way to overcome this issue may be to assess the enrichment for transcriptomic signatures derived from independent datasets. This can be achieved by calculating an enrichment score for each individual cell [34,57], or by using Gene Set Enrichment Analysis-based methods [58] such as connectivity MAP (cMAP) which compares clusters to one another [14,59]. By taking into account the entire gene expression profile instead of the most differentially expressed genes, these approaches provide valuable complementary information.

Finally, another challenge lies in the interpretation of cellular changes that can superimpose on the core transcriptional program and dominate detected mRNA. This is particularly true for cell cycle genes, which are often regressed out to limit their weight in the analysis [56]. Recent scRNA-seq studies on human DCs have shown

that this is also the case for DC maturation genes. Such transcriptional changes can be dynamic for DC exposed to signals from their micro-environment, and can become a confounding factor for scRNA-seq data interpretation. New methods coupling surface protein stainings to scRNA-seq detection, such as cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq) [60], are useful to circumvent this issue [41].

Table 1. Known human DC subsets

	pDC	cDC1	cDC2	moDC
Marker genes	*CD123* *BCL11A* *TCF4*	*CADM1* *XCR1* *CLEC9A* *RAB32* *C1orf54*	*CD1C* *FCER1A* *CLEC10A* *CD1E*	*CD1C* *FCER1A* *CLEC10A* *S100A9* *S100A8* *VCAN* *CD14* *FCGR2B* *STAB1*
Surface markers	$CD123^+$ $CD304^+$ $CD303^+$	$CD141^+$ $Clec9A^+$	$CD1c^+$ $CLEC10A^+$	$CD1c^+$ $CD14^+$ $CD226^+$ $CD163^-$ $sLSP1^+$
Development	Dependant on E2-2/TCF4, IRF8, IKZF1/Ikaros, SpiB	Dependent on IRF8, BATF3, NOTCH	Dependent on IRF8	Dependent on IRF4, BLIMP1, AHR

Table 2. Newly described human DC populations

	AXL^+ $SIGLEC6^+$ DC	DC3	DC2A	DC2B	$CCR7^+$ DC
Presence in blood	Cord blood [23] Peripheral blood [11–15,21]	Cord blood [23] Peripheral blood [13,17–22]	No	Corresponds to classical cDC2	No
Presence in lymphoid organs	Spleen [14,15,25] Tonsil [12,13,15] Bone Marrow [12,14]	Bone Marrow [17]	Spleen [25]	Spleen [25]	Tonsil (cDC) [26] Thymus (cDC1 and cDC2) [44] Spleen [25] Melanoma-draining Lymph Node [37]
Presence in peripheral tissues	Absent from uninflamed skin [15]	Skin ? [19,20]	?	?	Ascites (moDC) [34] Skin blisters (AXL^+ $SIGLEC6^+$ DC and pDC) [43] Liver tumor [40] Lung tumor [36] Lung tumor (cDC1 and cDC2) [41] Colon tumor (cDC1) [42] Skin (He)
Pathological situation	Recruited to inflamed skin blisters [43] and inflamed lung [61]	Increased in blood of SLE patients [21] and melanoma patients			Increased in skin lesions of atopic dermatitis patients (He)

		[20]			
Marker genes	*AXL* *SIGLEC6* *CD123* *SIGLEC1* *LYZ* *BCL11A* *TCF4* *KLF12*	*CD1C* *FCER1A* *CD14* *VCAN* *S100A8* *S100A9* *FCN1* *CLEC4E* *CD36* *CD163*	*CLEC4A* *AREG* *LTB* *CD3E*	*CD1C* *FCER1A* *CLEC10A*	*LAMP3* *CCR7* *CD83* *CCL22* *BIRC3* *FSCN1* *IDO1*
Surface markers	$CD5^{+}$ $CD2^{+}$ $CX3CR1^{+}$ $CD11c^{+/-}$ $CD123+/^{low}$	$CD1c^{+}$ $CD5^{-}$ $BTLA^{-}$ $CD26^{-}$ $CD14^{low/+}$ $CD163^{+}$	$CD1c^{low}$ CLEC10 A^{-}	$CD1c^{+}$ $CLEC10A^{+}$	$CCR7^{+}$
Development	E2-2/TCF4 dependent Differentiate into cDC2 *in vitro*	Derived from $IRF8^{low}$ precursor	?	?	Cell state induced by maturation

Glossary

ATAC-seq: Next Generation Sequencing method providing the DNA sequences of open chromatin regions; this allows the inference of regions that are accessible for transcription factor binding

Ascites: abnormal infiltration of fluid in the abdomen

Dendritic cell: immune cell specialized for antigen presentation and T cell stimulation; can migrate from peripheral tissues to lymphoid organs

Fate-mapping: method to trace cell lineages, for instance by transfering purified precursor cells or inserting a genetic mark such as a fluorochrome in precursor cells

Lamina propria: mucosal connective tissue that lies between the epithelium and underlying tissues

Macrophage: antigen presenting immune cell residing in peripheral and lymphoid tissues and specialized in ingesting pathogens and dying cells

Maturation: process by which dendritic cells modify their morphology and expression of molecules (including surface), making them for more efficient to interactwith T cells

Monocyte: immune cell that circulates in the blood and massively infiltrates tissues upon inflammation

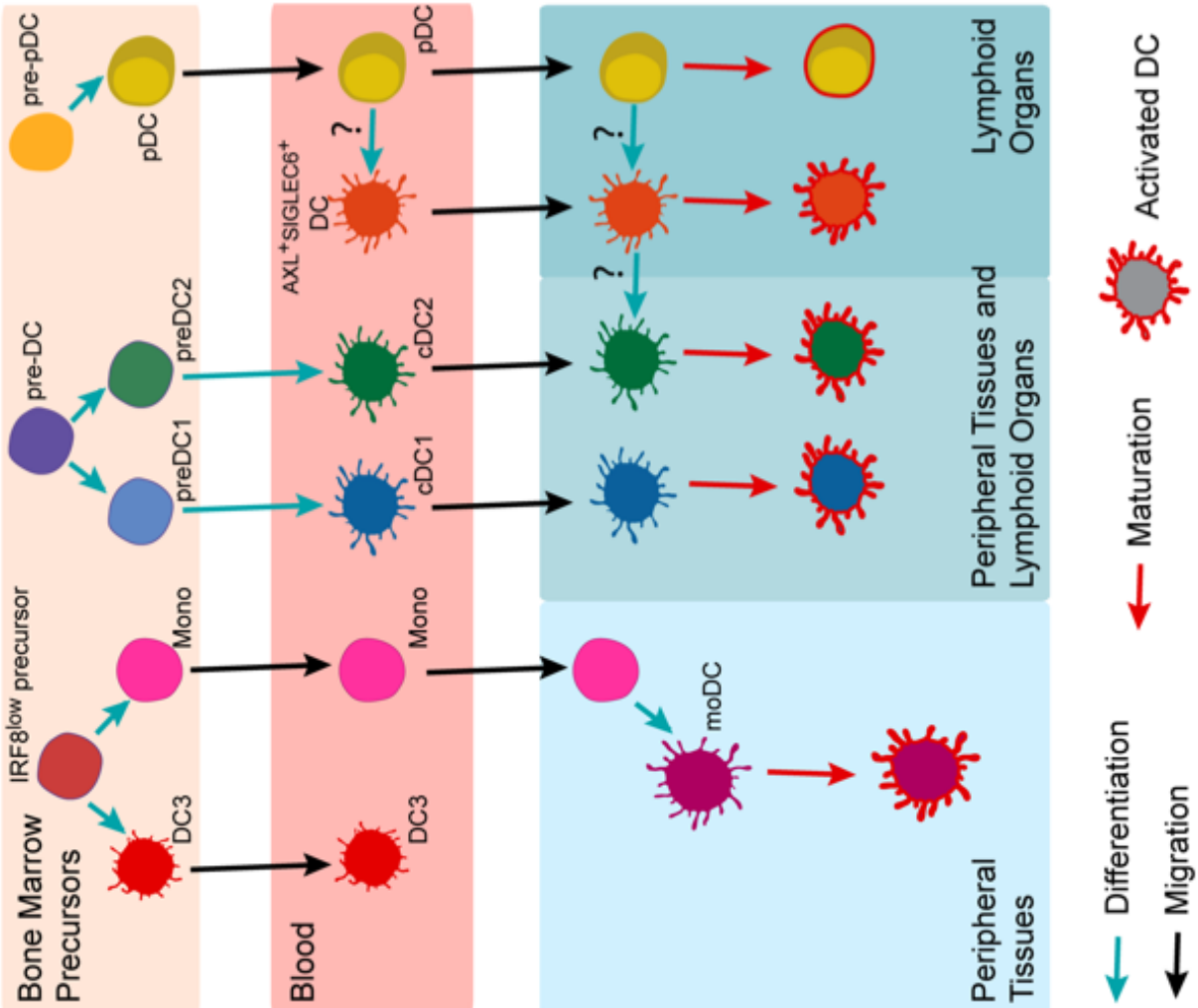
Pitt-Hopkins syndrome: rare genetic disorder due to the loss of function of the transcription factor 4 (TCF4), characterized by a moderate to severe intellectual disability. Patients show an impairment of the pDC compartment in terms of number, phenotype and function

Single-cell RNA sequencing: Next Generation Sequencing method providing RNA expression profiles from individual cells

Transitional DCs: population of DCs displaying an intermediate phenotype inbetween cDC and pDC

Unbiased clustering: (in single-cell RNAseq) grouping of a set of cells that are more similar to each other than to the others groups of cells, based on their expression data. Unbiased clustering (also termed unsupervized clustering) is performed without consideration of prior knowledge about the cells attributes. Common clustering methods include hierarchical clustering, graph-based clustering and *k*-means clustering.

Key Figure 1. Proposed updated ontogeny-based classification of human dendritic cell (DC) subsets. Plasmacytoid DCs (pDC), type 1 classical DCs (cDC1), type 2 classical DCs (cDC2), type 3 DCs (DC3) and monocyte-derived DCs (mo-DC) represent distinct human DC subsets. $CD14^+$ monocytes (Mono) and DC3 derive from $IRF8^{low}$ bone marrow precursors along distinct pathways. Bone marrow pre-DC give rise to preDC1 and preDC2, which differentiate into cDC1 and cDC2 in the periphery. Pre-pDC differentiate in the bone marrow into pDC. The developmental pathway of AXL^+ $SIGLEC6^+$ DCs remains to be confirmed, they may derive from pre-pDC or directly from differentiated pDC in the periphery. Monocytes, cDC1, cDC2, pDC and AXL^+ $SIGLEC6^+$ DCs circulate in the blood and can migrate to peripheral tissues and lymphoid organs. Whether blood DC3 can migrate to tissues is unclear. In mucosal tissues or upon inflammation, monocytes can differentiate *in situ* into mo-DCs. AXL^+ $SIGLEC6^+$ DCs may differentiate into cDC2 directly in tissues. Upon homeostatic or inflammation-induced maturation, all DC subsets express a convergent activation program.


Bone Marrow Precursors
Blood
Peripheral Tissues
Peripheral Tissues and Lymphoid Organs
Lymphoid Organs
pre-pDC
pDC
pre-DC
preDC2
preDC1
IRF8low precursor
Mono
DC3
AXL+SIGLEC6+ DC
cDC2
cDC1
moDC
?
Differentiation
Migration
Maturation
Activated DC

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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