

Minireview

# The Bloodline of CD8 $\alpha$ <sup>+</sup> Dendritic Cells

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The immune system is highly coordinated by various cell types. Dendritic cells (DCs) orchestrate immune responses at various stages and bridge innate immunity and adaptive immunity. DCs are a family of cells consisting of various subsets distinguished by surface markers, locations, and transcription factors that govern their development, differentiation, and homeostasis. The complexity of DC subset biology has hindered the understanding of the functional differences among DC subsets. The subset expressing the surface molecule CD8 $\alpha$  is of particular interest, due to the efficiency of this DC subset in priming CD8<sup>+</sup> cytotoxic T cells and cross-presenting exogenous antigens to CD8<sup>+</sup> T cells. CD8 $\alpha$ <sup>+</sup> DCs maintain tolerance to autologous antigens at steady state, but when activated secrete IL-12, polarizing T helper (Th) 1 responses. Recently, novel DC subsets were found to be present in peripheral tissues and the relationship between CD8 $\alpha$ <sup>+</sup> DCs in lymphoid organs and DC subsets in peripheral tissues has been revealed. This review describes the pedigree of CD8 $\alpha$ <sup>+</sup> DCs and related subsets, including a history of the discovery of DC subsets and their functional characterization.

## INTRODUCTION

Dendritic cells (DCs) are a group of heterogeneous myeloid cells that process antigens and present antigenic fragments to T lymphocytes, classifying them as professional antigen presenting cells. T cell activation and the differentiation of effector or memory cells require co-stimulatory molecules as well as the binding of peptide/MHC complexes to T cell receptors. The innate immune system decodes patterns imprinted in pathogens, called pathogen associated molecular patterns (PAMPs) via pattern recognition receptors and transfers accessory signals to T cells. Therefore, sensing molecular patterns is a determining factor in distinguishing between self and non-self. Numerous reports have elucidated that DCs have the unique ability to integrate signaling events downstream of pattern recognition receptors and to process and present antigens. Therefore, DCs are key mediators between the innate and adaptive arms. Since DCs are also important in maintaining tolerance to autologous antigens, their ability to play a dual role in immunity and tolerance is perplexing.

The identification of surface markers on DCs has enabled their classification into subsets, with multiparametric flow cytometry analysis making possible the more precise definition of new DC subsets. Questions have arisen regarding whether these DC subsets are heterogeneous in function, development and differentiation. The complexity of DC compartment has not been easily unveiled due to the plasticity of DC subsets. Their surface phenotype and function change depending on the milieu in which they are activated, making it difficult to precisely define DC subsets and determine precursor-progeny relationships among them. More recently, however, their phenotypic characterization has been combined with genome-wide expression profiling, providing a more definitive picture of the DC subset network. It has not yet been clarified whether individual DC subsets take different charges of immunity and tolerance or whether their involvement in immunity or tolerance is determined by the microenvironment which tips the balance toward one or the other.

DCs were originally distinguished from other cells in spleen isolates by their adherence and morphologic extension of dendrites (Steinman and Cohn, 1973). Subsequently, these phenotypically distinct cells were found to have a >100-fold greater potency in stimulating allogeneic T cells *in vitro* (Steinman and Witmer, 1978). The isolation of DCs, previously based on their morphologic characteristics, was replaced by the finding that they expressed the surface marker, CD11c, an alpha chain of integrin x expressed primarily by DCs. Thus, CD11c has been utilized extensively to identify and isolate DCs (Kraal et al., 1986; Metlay et al., 1990). It was not long before it was realized that DCs were heterogeneous, composed of several subsets. However, their relative paucity, the lack of reliable surface markers distinguishing these subsets, and the difficulty handling DCs *in vitro* hampered progress in understanding DC biology.

The first distinction of DC subset was based on the expression of a surface marker, CD8 $\alpha$  (Ardavin and Shortman, 1992; Ardavin et al., 1993; Crowley et al., 1989; 1990; Maraskovsky et al., 1996; Nussenzweig et al., 1981; Vremec et al., 1992; 2000; Winkel et al., 1994; Wu et al., 1996). Unlike T cells, which express heterodimers of CD8 $\alpha$  and  $\beta$  chains, DCs express CD8 $\alpha$  homodimers. In secondary lymphoid organs, CD8 $\alpha$ <sup>+</sup> and CD8 $\alpha$ <sup>-</sup> DCs are present at a ratio of 1:2 and constitute so-called conventional or classical dendritic cells (cDCs). Spleen and lymph nodes contain another type of DCs, which express lower levels of CD11c and appear similar to plasma cells; they are

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therefore called plasmacytoid dendritic cells (pDCs) (Liu, 2005). These pDCs secrete high quantities of type I interferon upon activation, in particular following viral infection. Both cDCs and pDCs are found in all secondary lymphoid organs and believed to take up residence after getting out of blood circulation and parking directly in the lymphoid organs; they are therefore called 'resident DCs'. In addition, peripheral lymphoid organs that collect antigens from peripheral tissues contain a group of DCs called 'migratory DCs'. DCs in skin-draining lymph nodes consist of two subsets, Langerhans cells (LCs) and dermal DCs (dDCs) (Merad et al., 2008). LCs are the only hematopoietic cell type present in human epidermis and are distinguished from other cells by the presence of subcellular organelles, Birbeck granules. LCs and dDCs move through afferent lymphatics to draining lymph nodes, following the gradient of the chemokine ligands of CCR7. It is of note that CD8 $\alpha$  expression is upregulated on LCs migrating to the lymph nodes (Anjuère et al., 1999; Merad et al., 2000) and some pDCs express CD8 $\alpha$  (Smith et al., 2003). In addition, while expressed only by CD8 $\alpha$ <sup>+</sup> DCs in spleen, CD205 (DEC-205) is expressed by resident CD8 $\alpha$ <sup>+</sup> DCs and subsets of migratory CD8 $\alpha$ <sup>+</sup> DCs in lymph nodes (Henri et al., 2001). Therefore, care should be taken in isolating resident CD8 $\alpha$ <sup>+</sup> DCs by using a single surface marker, CD8 $\alpha$  or CD205.

In contrast to the aforementioned DCs present at steady state, circulating monocytes can be drafted to inflamed tissues and assume phenotypic similarities to DCs; these cells are therefore classified as 'monocyte-derived DCs (Mo-DCs)' or 'inflammatory DCs' (Cheong et al., 2010; Geissmann et al., 2003; Randolph et al., 1999). Mo-DCs may join the pool of DCs in the draining lymph nodes via two routes (León et al., 2007). In the first, Mo-DCs may enter the dermis and migrate via afferent lymphatics to draining lymph nodes. In the second, Mo-DCs migrate from the blood directly to the lymph nodes through high endothelial venules. Depending on inflammatory conditions, monocytes may reach the epidermis and become LC precursors (Ginhoux et al., 2006). TipDCs (TNF $\alpha$  and iNOS-producing DCs), identified in the spleen of *Listeria*-infected mice, have similar characteristics (Serbina et al., 2003) and are likely to be Mo-DCs.

## THE FUNCTION OF CD8 $\alpha$ <sup>+</sup> DC

Much attention has been paid to the functional specialization of CD8 $\alpha$ <sup>+</sup> and CD8 $\alpha$ <sup>+</sup> DCs in the context of CD4<sup>+</sup> T helper cell subset differentiation and the preferential activation of CD8<sup>+</sup> and CD4<sup>+</sup> T cells by each subset, respectively. Intracellular pathogens polarize naïve T cells to IFN- $\gamma$ -producing Th1 cells, whereas allergens and worms polarize naïve T cells to IL-4-producing Th2 cells. DC subsets can be distinguished by their ability to trigger differential programming of these T cell subsets (Maldonado-López et al., 1999; Pulendran et al., 1999). Difference in the generation of polarizing signals by DC subsets may be due to their specialization in decoding certain types of PAMP or in bestowing polarizing signals. Supporting this, CD8 $\alpha$ <sup>+</sup> DCs were shown to be the primary producers of IL-12, a facilitator of Th1 differentiation (Hochrein et al., 2001; Reis e Sousa et al., 1997).

CD8 $\alpha$ <sup>+</sup> and CD8 $\alpha$ <sup>+</sup> DCs have been reported to be present at different locations in the spleen. The anatomical localization of DCs was initially characterized using the anti-CD11c antibody, N418. Initially, N418-positive staining was observed in cells interdigitating within white pulp but was more strongly detected at the sites of central arterioles (Metlay et al., 1990). The T cell

containing region surrounding the central arterioles breaches the rim of the marginal zone and has been called the marginal zone bridging channel (Agger et al., 1992; Leenen et al., 1998). Marginal DCs located in this area migrate into the deep T cell area upon endotoxin stimulation (Reis e Sousa and Germain, 1999). CD8 $\alpha$ <sup>+</sup> and CD8 $\alpha$ <sup>+</sup> DCs are located in the marginal zone, red pulp and T cell area (McLellan et al., 2002), but skewed localization of CD8 $\alpha$ <sup>+</sup> DCs in the T cell zone and CD8 $\alpha$ <sup>+</sup> DCs in the red pulp and marginal zones was observed. Furthermore, CD8 $\alpha$ <sup>+</sup> and CD8 $\alpha$ <sup>+</sup> DCs express different sets of molecules involved in MHC class I and II antigen processing and presentation (Dudziak et al., 2007). In the spleen, CD8 $\alpha$ <sup>+</sup> DCs are more specialized in activating CD8<sup>+</sup> T cells whereas CD8 $\alpha$ <sup>+</sup> DCs preferentially trigger CD4<sup>+</sup> T cells. In the lymph nodes, resident CD8 $\alpha$ <sup>+</sup> DCs were shown to be potent stimulators for priming T cells specific for viruses and intracellular bacteria (Belz et al., 2004; 2005; Smith et al., 2003). This was further corroborated using bone marrow chimeras, in which radioresistant LCs were left intact to present viral antigens but were unable to mount virus specific CD8<sup>+</sup> T cell responses, indicating that LCs alone cannot function as direct antigen presenters (Allan et al., 2003; 2006).

Probably the characteristic that most distinguishes between CD8 $\alpha$ <sup>+</sup> and CD8 $\alpha$ <sup>+</sup> DCs is the ability of the former to present exogenously added antigens to CD8<sup>+</sup> T cells (den Haan et al., 2000; Pooley et al., 2001; Schnorrer et al., 2006). This process, called cross-presentation, is physiologically important in generating immune responses against tumors and viruses that do not invade the cytosol of DCs. The unique ability of CD8 $\alpha$ <sup>+</sup> DCs to cross-present antigens has been the most important functional criterion to classify CD8 $\alpha$ <sup>+</sup> DC-related subsets. This attribute of CD8 $\alpha$ <sup>+</sup> DCs may originate from their competence to take up dead cells (Iyoda et al., 2002; Schulz and Reis e Sousa, 2002). Although these CD8 $\alpha$ <sup>+</sup> DCs were thought to capture both apoptotic and necrotic cells efficiently, a recent study showed a molecular link between necrotic cell uptake and cross-presentation. Clec9A, a C-type lectin-like molecule specifically expressed on CD8 $\alpha$ <sup>+</sup> DCs, was shown to increase cross-presentation upon ligation by necrotic cells, although it is not involved in necrotic cell uptake *per se* (Sancho et al., 2009). Further detailed studies are required to determine how the downstream signaling events activate cross-presentation. More importantly, the contribution of Clec9A to various immune responses awaits further testing. CD8 $\alpha$ <sup>+</sup> DCs express TLR3, which serves as a receptor for West Nile virus (Wang et al., 2004) and enhances cross-priming (Schulz et al., 2005) when stimulated with the double-stranded viral RNA analog, poly I:C. Thus, CD8 $\alpha$ <sup>+</sup> DCs are well-equipped with various signaling components that link the sensing of an aberrant condition and the presentation of associated antigens. In contrast to inflammatory settings such as virus infection, CD8 $\alpha$ <sup>+</sup> DCs in the spleen have been shown to suppress T cell responses. Further, lymph node-resident CD8 $\alpha$ <sup>+</sup> DCs were shown to be critical in inducing tolerance to endogenous antigens at steady state (Belz et al., 2002; Scheinecker et al., 2002). Therefore, CD8 $\alpha$ <sup>+</sup> DCs play a conducting role in triggering immunity and tolerance in various environments.

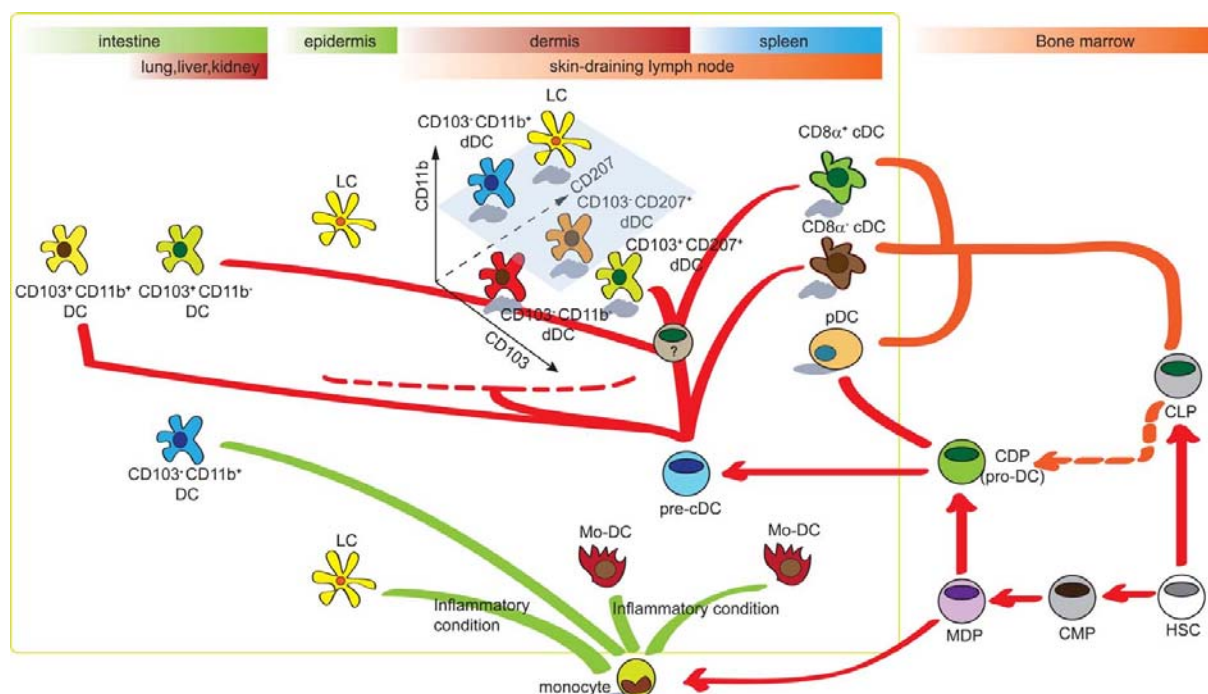
## DEVELOPMENT AND HOMEOSTASIS OF DC SUBSETS

Hematopoietic cells are derived from hematopoietic stem cells (HSCs), which diverge into common myeloid progenitors (CMPs) and common lymphoid progenitors (CLPs). CMPs serve as precursors of granulocytes, monocytes, red blood cells and platelets, whereas CLPs give rise to B, T, and NK cells. The

pathway from HSCs to DCs is less clear. Because CD8 $\alpha^+$  DCs express the lymphoid cell marker, CD8, they were thought to originate from CLPs, whereas CD8 $\alpha^+$ CD11b $^+$  DCs were thought to originate from CMPs. CD4 $^b$  precursors and CD44 $^+$ CD25 $^+$  double negative cells in the thymus were shown to generate T cells, as well as CD8 $\alpha^+$  thymic and splenic DCs, whereas transplantation of bone marrow cells through a vein generated both CD8 $\alpha^+$  and CD8 $\alpha^-$  DCs in the spleen (Ardavin et al., 1993; Wu et al., 1996), suggesting that T cells and CD8 $\alpha^+$  DCs share a common precursor. However, the lack of CD8 $\alpha^-$  DCs in the spleen given the thymic precursor was due to the experimental procedure to deplete CD4 $^+$  cells, which include many CD8 $\alpha^-$  DCs, and in a subsequent study, both CD4 $^b$  precursors and CD44 $^+$ CD25 $^+$  double negative thymic cells were shown to generate CD8 $\alpha^-$  DCs (Martín et al., 2000). These findings suggest that DCs may derive from CLPs, but it was not clear whether DCs could originate from myeloid precursors. Indeed, it was demonstrated that not only CLPs but also CMPs could produce both CD8 $\alpha^+$  and CD8 $\alpha^-$  DCs (Manz et al., 2001; Traver et al., 2000; Wu et al., 2001). The question arose whether CLP- and CMP-derived DCs are functionally equivalent or if they just look similar. CLP- and CMP-derived DCs were found to have equivalent potency in stimulating allogeneic antigens *in vitro* (Manz et al., 2001). Since both CLPs and CMPs can generate both types of DCs, the step at which DC precursors assume the program to develop into bona-fide DCs and whether the requirement for CLP- and CMP-derived DCs differs remained unclear. *In vitro*, IL-7, a critical cytokine for T and B cell development, was found necessary for DC generation from CLPs, but not from CMPs, suggesting that the requirements for development may differ. A DC precursor, characterized as CD11c $^+$  class II $^+$  cells, was reported to be an immediate progenitor of CD8 $\alpha^+$ , CD8 $\alpha^-$ , and pDCs (del Hoyo et al., 2002). These precursors migrate to lymph nodes by using CD62L and expand under the condition of infection. However, the relationship among these DC precursors was not clear and it was not possible to exclude the possibility that all DCs derive from a single precursor, which looks different due to the different makeup of surface markers used to classify the early precursors. The clue to the answer came from several observations: Flt3 ligand (Flt3L, Fms-like tyrosine kinase 3 ligand) was used to generate DCs *in vitro* (Brasel et al., 2000; Saunders et al., 1996), DCs expanded when mice were injected with Flt3L (Drakes et al., 1997; Maraskovsky et al., 1996), and DCs were deficient in mice lacking Flt3L (McKenna et al., 2000). These findings suggested that DC precursors may express Flt3, a receptor for Flt3L. Indeed, CD11c $^+$  class II $^+$  DC precursors in bone marrow were found to express Flt3. Flt3 expression was also observed at high levels in some CLPs and at low but distinct levels in some CMPs (D'Amico and Wu, 2003; Karsunky et al., 2003). The Flt3-expressing precursors and mature DCs expand in response to Flt3L, suggesting that they share a developmental pathway or homeostatic program. Further, all the above DC precursor populations, i.e. CLPs, CMPs, CD4 $^b$  thymic cells, can be divided into Flt3 $^+$  and Flt3 $^-$  expressing cells, with the former group having increased ability to produce CD8 $\alpha^+$  and CD8 $\alpha^-$  DCs. pDCs were derived from CLPs and CMPs, but few were derived from thymic precursors. When bone marrow CD11c $^+$  class II $^+$  DC precursors were divided based on their expression of B220, a marker expressed on pDCs, B220 $^-$  cells were found not to express Flt3 distinctly but were able to produce conventional CD11b $^+$  DCs, whereas B220 $^+$  cells can act as precursors for pDCs and cDCs when adoptively transferred (Diao et al., 2004).

A population of precursor cells, defined as Ly6C $^+$ CD31 $^+$ CD11c $^+$ , was found to be present in murine bone marrow and blood (Bruno et al., 2001). This population could be differentiated into macrophages and DCs with M-CSF and GM-CSF, respectively, suggesting that this 'pre-immunocyte' subset contains a common precursor or distinct precursors of macrophages and DCs. This supports the hypothesis that macrophages and DCs share a common precursor and that the local environment governs their differentiation. A bone marrow cell subset was recently defined in CX3CR1-GFP mice, in which GFP is knocked in the CX3CR1 locus (Fogg et al., 2006). Among the myeloid precursor cells positive for CD117 (c-kit) but negative for other lineage markers and IL-7R $\alpha$  which is normally expressed on CLPs, CX3CR1-positive cells could generate macrophages and DCs both *in vitro* and *in vivo*. These cells, called macrophage and dendritic cell progenitors (MDPs), express both CD34 and CD16/32 and can therefore be categorized as a subset of granulocyte/macrophage progenitors (GMPs). Of note, GMPs were shown to be less efficient than CMPs in generating DCs (D'Amico and Wu, 2003; Manz et al., 2001). A later study, however, showed that MDPs were present in both CMPs and GMPs (Waskow et al., 2008). Moreover, MDPs express M-CSFR (CD115) (Varol et al., 2007), providing another link to DC precursors. An immediate DC precursor in spleen, CD11c $^+$  class II $^+$  cells, were reported to be able to generate CD8 $\alpha^+$  and CD8 $\alpha^-$  cDCs, but not pDCs, and were therefore called 'pre-cDCs' (Naik et al., 2006). Upstream DC precursors were found to produce cDCs and pDCs. CD11c-negative precursors, called pro-DCs, located upstream of CD11c $^+$  class II $^+$  'pre-DCs', could also produce cDCs and pDCs by *in vitro* culture (Naik et al., 2007). Pro-DCs have a surface phenotype of lineage(lin) $^-$ CD117(c-kit) $^{int}$ CD115(M-CSFR) $^+$ CD135(Flt3) $^+$  and IL-7R $^-$  and thus are not CLPs. Notably, pro-DCs express low levels of CD16/32 and CD34. Only a minor fraction of pro-DCs expressing CD16/32 and CD34 at high levels have macrophage precursor activity. A similar population, common dendritic progenitor (CDPs) (Onai et al., 2007), was found to give rise to CD8 $\alpha^+$  and CD8 $\alpha^-$  cDCs and pDCs, but not to other cells including monocytes, suggesting that CDPs are probably identical to pro-DCs. Interestingly, Flt3 $^+$ IL-7R $^+$  precursors, which should be a fraction of CLPs, showed gene expression profiles similar to those of CDPs. However, it remains unclear whether CDPs can derive from CLPs. The relationship between MDPs, CDPs (pro-DCs) and pre-cDCs has been elucidated (Liu et al., 2009), with adoptive transfer showing that MDPs are precursors of CDPs and CDPs are precursors of pre-cDCs and pDCs. MDPs were shown to be lin $^-$ Sca1 $^-$ c-kit $^{int}$ CD135(Flt3) $^+$ CD115 $^+$ CX3CR1 $^+$  cells, with their higher expression of c-kit distinguishing them from CDPs, which express the same markers except that they are c-kit $^{int/lo}$ . Pre-cDCs express CD11c but not yet class II. Similar to MDPs and CDPs, pre-cDCs express Flt3 and CX3CR1 but their expression of CD115 is heterogeneous. The nomenclature of pre-DCs and pre-cDCs has been used interchangeably, and it remains to be determined whether pre-DCs, as precursors of both cDCs and pDCs downstream of CDPs (pro-DCs), are present *in vivo*. Since Flt3 expression is low in monocytes but is maintained in DCs, the commitment of Flt3 $^+$  precursors (MDPs) to a DC lineage may be marked by DC restriction to Flt3.

*In vitro* culture of bone marrow cells with Flt3L produced all lymph node-resident DC subsets, CD8 $\alpha^+$ , CD8 $\alpha^-$ , and pDCs (Naik et al., 2005). Bone marrow-derived DC subsets do not express CD8 $\alpha$ , allowing CD8 $\alpha^+$  and CD8 $\alpha^-$  DCs to be distinguished by their expression of CD24 and Sirp $\alpha$ , respectively.



**Fig. 1.** Integrated lineage map of dendritic cell (DC) subsets. The DC network is formed by the successive differentiation of DC precursors from hematopoietic stem cells (HSCs). HSCs give rise to common myeloid progenitors (CMPs) and common lymphoid progenitors (CLPs), both of which produce lymphoid organ-resident CD8 $\alpha$ <sup>+</sup>, CD8 $\alpha$ <sup>-</sup>, and pDCs. CMPs subsequently develop into macrophage and dendritic cell progenitors (MDPs), probably *via* granulocyte/macrophage progenitors (GMPs), with MDPs giving rise to monocytes and DCs. MDPs give rise to DC-restricted common dendritic progenitors (CDP), followed by the bifurcation of the pathways leading to pDCs and pre-cDCs, the immediate precursors of cDCs. Pre-cDCs seed cDCs and some peripheral tissue-derived DC subsets that can be further subdivided by differential expression of CD103, CD207, and CD11b. It remains unclear whether there is a common precursor cell in the lineage of CD8 $\alpha$ <sup>+</sup> DCs. Under inflammatory conditions, monocytes differentiate into Langerhans cells (LCs) or monocyte-derived DCs (Mo-DCs). The location of the progenitor cells and DC subsets are summarized here and the lineage relationship is shown.

GM-CSF has been widely used to generate DCs, including the generation of Mo-DCs from monocyte precursors (Robbins et al., 2008). The transcription factors required for CD8 $\alpha$ <sup>+</sup> and CD8 $\alpha$ <sup>-</sup> DCs differ. CD8 $\alpha$ <sup>+</sup> DCs are absent without interferon regulatory factor 8 (IRF8, ICSBP) (Aliberti et al., 2003; Schiavoni et al., 2002), inhibitor of DNA binding 2 (Id2) (Hacker et al., 2003) and basic leucine zipper transcription factor, ATF-like 3 (Batf3) (Hildner et al., 2008), whereas CD8 $\alpha$ <sup>-</sup> DCs require RelB (Burkly et al., 1995; Wu et al., 1998) and IRF4 (Tamura et al., 2005).

### CD103<sup>+</sup> DC IN THE PERIPHERY

CD103 is an integrin  $\alpha$ E that forms a heterodimer with the  $\beta$ 7 chain and interacts with E-cadherin. CD103 expressed on T lymphocytes in skin, gut, and lung binds to E-cadherin on epithelial cells, allowing T lymphocytes to reside within epithelial layers. CD103 is also present on a DC subset in the gut and CD103<sup>+</sup> DCs program T cells to return to the mucosal area (Annacker et al., 2005; Johansson-Lindbom et al., 2005) and to become Foxp3<sup>+</sup> regulatory T cells (Tregs), which maintain tolerance in the gut (Coombes et al., 2007). The enzyme retinaldehyde dehydrogenase, which generates retinoic acid (RA) from retinal, is expressed highly in CD103<sup>+</sup> DCs residing in the lamina propria and gut-draining mesenteric lymph nodes. RA is critical in inducing the expression of CCR9 and  $\alpha$ 4 $\beta$ 7 on T cells,

thereby calling them back to the mucosal area (Iwata et al., 2004). Further, RA together with TGF- $\beta$  drives Treg differentiation (Coombes et al., 2007). These findings indicate that CD103<sup>+</sup> DCs foster a unique immune environment in the gut in two ways.

Detailed analysis has shown that gut-associated lymphoid tissue contains at least three DC subsets: CD103<sup>+</sup>CD11b<sup>+</sup> CX3CR1<sup>+</sup>, CD103<sup>+</sup>CD11b<sup>+</sup>CX3CR1<sup>-</sup>, and CD103<sup>+</sup>CD11b<sup>-</sup>CX3CR1<sup>-</sup> DCs (Bogunovic et al., 2009; Schulz et al., 2009; Varol et al., 2009). The first two types reside in the lamina propria and the third in mesenteric lymph nodes, Peyer's patches, and possibly in isolated lymphoid follicles. However, CD103<sup>+</sup>CD11b<sup>+</sup> and CD103<sup>+</sup>CD11b<sup>-</sup>DCs are also found in mesenteric lymph nodes, although the CD11b<sup>+</sup> DCs seem to be composed of multiple types of DCs, with lamina propria-derived CD11b<sup>+</sup> DCs being a minor constituent. CD103<sup>+</sup>CD11b<sup>+</sup> and CD103<sup>+</sup>CD11b<sup>-</sup> DCs in the lamina propria of the gut seem to be distinct in their precursors and growth factors. CD103<sup>+</sup>CD11b<sup>+</sup> DCs and CD103<sup>+</sup>CD11b<sup>-</sup> Peyer's patch DCs could be derived from CDPs and pre-cDCs whereas CD103<sup>+</sup>CD11b<sup>+</sup>DCs were derived from Ly6C<sup>hi</sup> monocytes. Both populations could be derived from MDPs. The differentiation and maintenance of both populations require different growth factors, with CD103<sup>+</sup>CD11b<sup>+</sup> DCs requiring Flt3L and CD103<sup>+</sup>CD11b<sup>-</sup> DCs requiring M-CSFR and to a lesser degree Flt3L. The requirement for GM-CSF is unclear, in that one group reported that GM-CSF is necessary to

generate CD103 $^+$ CD11b $^+$  DCs (Bogunovic et al., 2009) and the other group reported that GM-CSF is necessary to generate CD103 $^+$ CD11b $^+$  DCs (Varol et al., 2009). CD103 $^+$ CD11b $^+$  DCs in the lamina propria have been observed to squeeze out dendrites through the tight junction of epithelial cells, a process that depends on signals from CX3CR1 and Toll-like receptors (TLRs) (Chieppa et al., 2006; Niess et al., 2005; Rescigno et al., 2001). It was thought that during the periscopic probing of gut lumen, DC dendrites may take up some of bacteria in the gut and deliver them to the draining lymph nodes. However, while orally-infected *Salmonella* was taken up by both CD103 $^+$  and CD11b $^+$  DCs, only the former subset carried the bacteria to the mesenteric lymph nodes, with this transport severely impaired in Flt3 knockout (KO) and CCR7 KO mice (Bogunovic et al., 2009). CD103 $^+$ CD11b $^+$ , but not CD103 $^+$ CD11b $^-$ , DCs express CCR7, migrate to mesenteric lymph nodes and can stimulate T cells (Bogunovic et al., 2009; Schulz et al., 2009). In addition, a mutant strain of *Salmonella*, which cannot penetrate the intestinal epithelium, can be transported through the epithelial layer by grafting monocytes, indicating that monocyte-derived CD103 $^+$ CD11b $^+$  DCs sample the bacteria by extending their dendrites (Varol et al., 2009). These findings suggest a division of labor in the gut mucosa, with CX3CR1 $^+$ CD103 $^+$ CD11b $^+$  DCs taking up luminal antigens and delivering them to CD103 $^+$ CD11b $^+$  DCs, which transport these antigens to mesenteric lymph nodes to stimulate T cells. It was unclear, however, whether CD103 $^+$ CD11b $^+$  or CD103 $^+$ CD11b $^-$  DCs are responsible for inducing iTreg (induced Treg) cells, the expression of the gut homing receptors CCR9 and  $\alpha 4\beta 7$  on T cells, and for maintaining gut homeostasis. CD103 $^+$ CD11b $^+$ , but not CD103 $^+$ CD11b $^-$ , DCs have the ability to generate RA and induce CCR9 expression on T cells (Schulz et al., 2009). CD103 $^+$ CD11b $^+$  and CD103 $^+$ CD11b $^-$  DCs may be of different lineages, since mice deficient in Id2 and IRF8 lack the latter, but not the former, cells (Bogunovic et al., 2009). Recently, CD103 $^+$ CD11b $^-$  DCs were shown not to be required for any of the above activities, since none is impaired in Batf3 KO mice, which lack CD103 $^+$ CD11b $^-$  DCs and CD8 $\alpha^+$  cDCs, but not CD103 $^+$ CD11b $^+$  DCs, and there was no indication of gut inflammation (Edelson et al., 2010). Thus, it would be of great interest to elucidate the development process of CD103 $^+$ CD11b $^+$  DCs, their precursor cells, and their transcription factors. Of note, very recently, macrophages and dendritic cells present in colonic lamina propria were carefully examined and subdivided into five populations: CD11c $^+$ CD103 $^+$ CD11b $^+$  CX3CR1 $^{hi}$ F4/80 $^{hi}$  macrophages, CD11c $^+$ CD103 $^+$ CD11b $^+$ CX3CR1 $^{hi}$ F4/80 $^{hi}$  macrophage-like cells, CD11c $^+$ CD103 $^+$ CD11b $^+$ CX3CR1 $^{int}$ F4/80 $^{int}$  DCs, CD11c $^+$ CD103 $^+$ CD11b $^+$ CX3CR1 $^{hi}$ F4/80 $^{int}$  DCs and CD11c $^+$ CD103 $^+$ CD11b $^+$ CX3CR1 $^{hi}$ F4/80 $^{int}$  DCs. Previously described CD103 $^+$ CD11b $^+$ CX3CR1 $^+$ CD11c $^+$  cells were shown to contain CX3CR1 $^{hi}$ F4/80 $^{hi}$  and CX3CR1 $^{int}$ F4/80 $^{int}$  populations, the former of which looks more similar to CD11c-negative macrophages rather than dendritic cells, based on analysis of transcriptome, morphology and function (Rivollier et al., 2012). Interestingly, monocytes give rise mainly to CX3CR1 $^{hi}$ F4/80 $^{hi}$  macrophage-like cells and partly to CX3CR1 $^{int}$ F4/80 $^{int}$  DCs in non-inflammatory condition, but differentiate to pro-inflammatory CD103 $^+$ CD11b $^+$ CX3CR1 $^{int}$  DCs in an inflammatory setting.

The lineage relationship between CD8 $\alpha^+$  and CD103 $^+$ CD11b $^-$  DCs was also observed in the spleen and the skin. CD103 was expressed by splenic CD8 $\alpha^+$  DCs and a subset of dermal DCs. In contrast to splenic CD8 $\alpha^+$  DCs, CD103 is expressed minimally in CD8 $\alpha^+$  DCs in skin-draining lymph nodes (Bedoui et al., 2009). Batf3 KO mice were deficient of lymphoid organ CD8 $\alpha^+$  cDCs and CD103 $^+$ CD11b $^-$  DCs in the lung, the gut and mesen-

teric lymph nodes, and the dermis and cutaneous lymph nodes (Hildner et al., 2008), supporting the notion that lymph node-resident CD8 $\alpha^+$  DCs share the developmental process with peripheral tissue CD103 $^+$ CD11b $^-$  DCs. Further, CD103 $^+$ CD11b $^-$  dermal DCs were shown to be critical cross-presenters to CD8 $^+$  T cells in the recrudescence model of herpes simplex virus (Bedoui et al., 2009), providing further evidence that these DC subsets share the same functionality. Subsequently, the function of peripheral CD103 $^+$  DCs in an infectious condition was tested in Batf3 KO mice (Edelson et al., 2010). Batf3 KO mice were susceptible to virus infection and had lower numbers of virus-specific CD8 $^+$  T cells than wild type mice, in accordance with the role of lung CD103 $^+$  DCs in inducing influenza virus-specific CD8 $^+$  T cells (Geurtsvankessel et al., 2008; Kim and Braciale, 2009).

### LANGERIN (CD207) $^+$ DC

LCs are the only subset of DCs located in the epidermis, a layer separated from the dermis by a basement membrane. LCs migrate to draining lymph nodes at steady state and during inflammatory conditions; during the migration, LCs upregulate class II and costimulatory molecules. LCs were distinguished from DCs in the dermis in that LCs were reported to express Langerin (CD207), a C-type lectin localized in an LC-specific subcellular organelle, Birbeck granule. The function of Langerin is not clear, but it is thought to mediate antigen uptake and form Birbeck granules (Valladeau et al., 2000). LC development requires M-CSFR and TGF- $\beta$ . Importantly, under steady state conditions, LCs maintain homeostasis by self-renewal or by the generation from their precursors, which are resistant to irradiation (Merad et al., 2002). However, upon skin inflammation, radiosensitive precursors, in particular monocytes, participate in replenishing LCs (Ginhoux et al., 2006).

Use of an anti-Langerin antibody showed that LCs are not the only DCs that express Langerin. Langerin is expressed by splenic CD8 $\alpha^+$  DCs (Idoyaga et al., 2009; McLellan et al., 2002), which localize in the marginal zone, mainly in the T cell zone. At steady state, Langerin $^+$ CD8 $\alpha^+$  DCs are mainly detected in marginal zones, with some in T cell zones, but, upon microbial stimulation, Langerin was transiently upregulated and detected only in T cell zones. The role of this dynamic regulation remains unclear. Langerin $^+$  DCs have been detected in cutaneous lymph nodes (CLNs), but these were considered to be LCs originating and migrating from the epidermis (Stoitzner et al., 2003). Later, CD8 $\alpha^+$  DCs in CLNs were also shown to express Langerin (Douillard et al., 2005), consistent with the expression of Langerin mRNA in CD8 $\alpha^+$  DCs, in both spleen and LNs (Takahara et al., 2002). About 60% of lymph node-resident CD8 $\alpha^+$  DCs express Langerin, although its expression level was lower than in LCs and dermal DCs (Henri et al., 2010). In addition, Langerin $^+$  DCs have been reported in mucosal areas. DCs positive for Langerin have been observed in the intestinal mucosa (Flores-Langarica et al., 2005) and in airways of mice (Sung et al., 2006). CD11c $^+$ CD11b $^{hi}$ class II $^{hi}$  and CD11c $^+$ CD11b $^{lo}$ class II $^{hi}$  DCs and CD11c $^{int}$ CD11b $^{lo}$ class II $^{lo}$  macrophages have been identified in the lungs. CD11b $^{lo}$  DCs, but not CD11b $^{hi}$  DCs, express CD103, similar to lamina propria DC subsets. CD103 $^+$ CD11b $^{lo}$  DCs also express Langerin. This cell subset was tightly associated with lung epithelial cells, probably by expressing tight junction proteins, and was found to be competent in phagocytosing particulate antigens, in stimulating T cells, and in producing IL-12 upon TLR ligation, particularly the ligation of TLR3. Importantly, the expression of Langerin and the

responsiveness to TLR3 ligand suggest that, although these cells do not express CD8 $\alpha$ , they are related to CD8 $\alpha$ <sup>+</sup> DCs ontologically. Interestingly, although monocytes are not precursors of CD8 $\alpha$ <sup>+</sup> cDCs in spleen and lymph nodes, they have been shown to be precursors of lung and intestinal DCs (Landsman et al., 2007; Varol et al., 2007). Subsequently, Ly6C<sup>hi</sup> monocytes were found to generate CD103<sup>+</sup> DCs, whereas Ly6C<sup>lo</sup> monocytes generated CD11b<sup>+</sup> DCs in the lungs (Jakubczik et al., 2008). CD103<sup>+</sup> and CD103<sup>-</sup> DCs move to the lung-draining mediastinal lymph nodes following CCR7 ligands and activate CD8<sup>+</sup> T cells and CD4<sup>+</sup> T cells, respectively (del Rio et al., 2007). Interestingly, CD103<sup>+</sup> DCs cross-present antigens to CD8<sup>+</sup> T cells, which provides a clue for a functional link between CD103<sup>+</sup> and CD8 $\alpha$ <sup>+</sup> DCs. This dichotomy of function of CD103<sup>+</sup> and CD11b<sup>+</sup> DCs is analogous to the previous findings that CD8 $\alpha$ <sup>+</sup> DCs present antigens to CD4<sup>+</sup> T cells whereas CD8 $\alpha$ <sup>+</sup> DCs cross-present antigens to CD8<sup>+</sup> T cells (Pooley et al., 2001). In contrast to lung mucosal DCs, Langerin is not expressed by lamina propria DCs and is expressed by only a subset of CD8 $\alpha$ <sup>+</sup> cDCs in mesenteric lymph nodes (Edelson et al., 2010; Ginhoux et al., 2009).

The recent development of genetically-modified mice, in which a certain cell type is tagged with traceable reporter proteins and diphtheria toxin receptors, enabling specific killing of these cells with diphtheria toxin, has increased the ability to determine cell lineages. In 2005, three groups generated mice to trace the origin of Langerin<sup>+</sup> cells. Two of these groups placed a reporter protein, eGFP, under the control of endogenous Langerin promoter (Bennett et al., 2005; Kissenpfennig et al., 2005), whereas the third generated a transgenic line with a bacterial artificial chromosome (BAC) spanning the human Langerin loci (Kaplan et al., 2005). Experiments using the Langerin-reporter strain showed that Langerin<sup>+</sup> DCs are composed of CD8 $\alpha$ <sup>+</sup> resident DCs and migratory DCs and are located in T cell areas of lymph nodes. It appears that Langerin<sup>+</sup> dermal DCs take up antigen, migrate first to LNs and occupy the outer paracortex underneath B cell follicles. Langerin<sup>+</sup> DCs with antigen arrive at the LNs later and are localized deep inside T cell areas (Kissenpfennig et al., 2005). Although the Langerin BAC transgenics specifically targeted Langerhans cells, the Langerin knock-in approaches revealed a previously unappreciated dermal DC subset. These Langerin<sup>+</sup> DCs are found in the dermis of the skin (Bursch et al., 2007; Ginhoux et al., 2007; Poulin et al., 2007), as well as in other tissues such as the lungs and liver (Ginhoux et al., 2009). Irradiation and adoptive transfer of bone marrow cells showed that these cells were susceptible to ablation by irradiation, unlike LCs which are resistant to irradiation. Moreover, these Langerin<sup>+</sup> DCs are replenished by blood-derived precursors, which are located in the dermis under the control of CCR2. All migratory DCs migrate to LNs in a CCR7-dependent manner, and Langerin<sup>+</sup> DCs are absent from the LNs of CCR7 KO mice. In contrast, Langerin<sup>+</sup>CD8 $\alpha$ <sup>+</sup> cDCs are present in CCR7 KO mice and are replenished by pre-cDCs which home to LNs through CD62L. Although CD11b is expressed by LCs, it is not expressed by the Langerin-positive migratory subset of DCs in the periphery. Dermal DCs in the skin were initially thought to be composed of two subsets, CD103<sup>+</sup>CD11b<sup>-</sup> and CD103<sup>+</sup>CD11b<sup>+</sup> dDCs (Bursch et al., 2007). Similarly, pulmonary DCs were thought to consist of two subsets, which can be divided based on their mutually exclusive expression of CD11b and CD103. This gating scheme was used to characterize DCs residing in non-lymphoid tissues (Ginhoux et al., 2009). The dermis, lungs, liver, pancreatic islets and kidneys contain two populations of DCs, CD103<sup>+</sup>CD11b<sup>-</sup>

CX3CR1<sup>-</sup> and CD103<sup>+</sup>CD11b<sup>+</sup>CX3CR1<sup>+</sup> DCs. CD103<sup>+</sup> DC subsets in all these tissues, except for pancreatic islets and lamina propria, express Langerin. The development or homeostasis of peripheral CD103<sup>+</sup> DCs was dependent on Flt3, Id2 and IRF8. CD11b<sup>+</sup> DCs were dependent on Flt3L and, to a varying degree, M-CSFR, probably due to the heterogeneity of this cell population. Notably, Flt3L KO mice showed a more severe defect than Flt3 KO mice, indicating that there may be another receptor for Flt3L. Adoptively transferred CDPs and pre-cDCs gave rise to CD103<sup>+</sup> DCs and to a lesser degree CD11b<sup>+</sup> DCs. The latter population can also be derived from adoptively transferred monocytes, providing further support for the heterogeneity of CD11b<sup>+</sup> DCs. Subsequently, the division of dermal DCs by their expression of CD103 and CD11b was found insufficient to describe all dermal DCs. The dermis contains two CD11b<sup>+</sup> subsets, CD207<sup>+</sup>CD103<sup>+</sup>CD11b<sup>+</sup> LCs and CD207<sup>+</sup>CD103<sup>+</sup>CD11b<sup>+</sup> dDCs, and three CD11b<sup>lo</sup> subsets, CD207<sup>+</sup>CD103<sup>+</sup>CD11b<sup>lo</sup>, CD207<sup>+</sup>CD103<sup>+</sup>CD11b<sup>lo</sup>, and CD207<sup>-</sup>CD103<sup>+</sup>CD11b<sup>lo</sup> dDCs (Henri et al., 2010). Therefore, the skin migratory DCs, which were previously categorized as LCs and dermal DCs, have been further subdivided into five subsets. LCs in the dermis were thought to be in transit to the draining lymph nodes. Langerin<sup>+</sup> dDCs and LCs prepared from skin, which express similar levels of Langerin, could be clearly distinguished by their relative expression of CD11b and Langerin. CD103<sup>+</sup> DCs expressed Langerin, whereas only some Langerin<sup>+</sup> DCs expressed CD103. Separation of migratory DCs in the cutaneous lymph nodes from resident DCs, based on the higher expression of class II molecules in the former showed DCs that resemble those in the dermis, but with different surface molecule profiles. LCs in the lymph node have lower expression of CD11b than epidermal LCs, being merged with Langerin<sup>+</sup> dDCs in the separation plot. Both Langerin<sup>+</sup> and Langerin<sup>-</sup> DCs expressed CD103, irrespective of their expression of CD11b. In addition, CD8 $\alpha$ <sup>+</sup> resident cDCs expressed low levels of CD103 (Bedoui et al., 2009; Edelson et al., 2010) and two thirds of CD8 $\alpha$ <sup>+</sup> resident cDCs expressed a lower level of Langerin than Langerin<sup>+</sup> dDCs and LCs (Henri et al., 2010). These results suggest that CD103 and Langerin are not reliable markers by themselves to define DC subsets and that many additional markers should be included. This was the case in characterizing Batf3 KO mice and Langerin<sup>+</sup> cell deletion mice. In Batf3 KO mice, Langerin<sup>+</sup>CD103<sup>+</sup> LCs and CD103<sup>+</sup>CD11b<sup>+</sup> dDCs, but not Langerin<sup>+</sup>CD103<sup>+</sup> dDCs, remain intact, indicating that Langerin<sup>+</sup>CD103<sup>+</sup> dDCs are developmentally distinct from the other subsets. This phenotype was also observed in IRF8 mutant and Id2 KO mice (Ginhoux et al., 2009), providing further evidence that lymphoid organ-resident CD8 $\alpha$ <sup>+</sup> cDCs, skin-derived Langerin<sup>+</sup>CD103<sup>+</sup> DCs and CD103<sup>+</sup>CD11b<sup>-</sup> DCs in other peripheral tissues are all related. It is necessary to determine, however, whether DC precursors in these mutant mice are defective. Pre-cDCs, the immediate precursors of CD103<sup>+</sup> DCs, are normally maintained in Batf3 KO mice. Therefore, the stages of differentiation or homeostasis at which Batf3, IRF8 and Id2 act remain to be determined. Following transfer of CDPs and pre-cDCs, their DC progeny were not detected in the lungs. However, since CD103<sup>+</sup>CD11b<sup>-</sup> DCs derived from those precursors were present in the kidney and liver of these mice (Ginhoux et al., 2009), it is conceivable that lung CD103<sup>+</sup> DCs may originate from CDP/pre-cDCs, as well as from Ly6C<sup>hi</sup> monocytes.

Comparative analysis of Batf3 KO and Langerin<sup>+</sup> cell deletion mice revealed that CD103<sup>+</sup> dermal DCs were not equivalent to Langerin<sup>+</sup> dDCs. GM-CSF KO mice were previously shown to

be defective in the induction of experimental autoimmune encephalomyelitis (EAE), which was thought to be due to their lack of CD103<sup>+</sup> dDCs, the cell type that induces pathogenic Th cells. Further, when the Langerin<sup>+</sup> dDCs were ablated, the expression of IFN- $\gamma$  and, to a lesser degree, IL-17 was decreased (King et al., 2010). Langerin<sup>+</sup>CD103<sup>+</sup> dDCs, however, may not be responsible for EAE because Batf3 KO mice showed normal susceptibility to EAE (Edelson et al., 2011). These results can be reconciled if Langerin<sup>+</sup>CD103<sup>+</sup> dDCs are indeed the culprit, which requires further study. Following the ablation of Langerin<sup>+</sup> cells, Langerin<sup>+</sup>CD103<sup>+</sup> dDCs were found to cross-present keratinocyte-associated antigen, making LCs dispensable (Henri et al., 2010). Thus, cross-presenting CD103<sup>+</sup> DCs described previously (Bedoui et al., 2009) can be better described as Langerin<sup>+</sup>CD103<sup>+</sup> DCs. It will require to test whether Batf3 KO mice are defective in the cross-presentation of skin-derived antigens.

### THE HUMAN EQUIVALENTS OF CD8 $\alpha$ <sup>+</sup> DC

The ability of DCs to regulate immunity and tolerance has received considerable attention during the development of immunotherapy. Recently, targeting antigens to DCs by conjugating the antigen to an antibody specific for a certain subset of DCs is suggested as a promising therapy for patients with cancer and for boosting or dampening immune responses. Although the unique ability of CD8 $\alpha$ <sup>+</sup> DCs to cross-present antigens to CD8<sup>+</sup> cytotoxic T cells has drawn considerable interest, the relatively poor availability of human lymphoid tissues, the lack of CD8 $\alpha$  expression by human DCs (Winkel et al., 1994), and the lack of distinct surface markers to define human DC subsets has hampered direct application of murine results to humans. Under steady state conditions, human DCs in blood can be classified into three groups, based on their reaction with monoclonal antibodies generated against blood DCs: BDCA1<sup>+</sup> (blood dendritic cell antigen 1, CD11c) DCs, BDCA2 (LILRA4)<sup>+</sup>BDCA4<sup>+</sup> pDCs which express high levels of CD123 (IL-3R), and BDCA3 (CD141)<sup>+</sup> DCs (Dzionek et al., 2000). Except for pDCs, little was known about the relationship between mouse DC subsets and human BDCA1<sup>+</sup> and BDCA3<sup>+</sup> DCs. Recently, a great leap has been made in identifying human equivalents of murine CD8 $\alpha$ <sup>+</sup> DCs. The first clue came with a report showing that Nectin-like protein 2 (Nect2), also called cell adhesion molecule 1 (CADM1) was found to be expressed on mouse CD8 $\alpha$ <sup>+</sup> and human BDCA3<sup>+</sup> DCs in blood and spleen (Galibert et al., 2005), suggesting their ontological link. Nect2 activates NK cells and activated CD8<sup>+</sup> T cells by binding to its receptor, CRTAM (IGSF4), on lymphocytes (Arase et al., 2005; Boles et al., 2005). Moreover, the expression of Clec9A (or DNGR-1), a C-type lectin-like molecule, was found to be restricted to mouse CD8 $\alpha$ <sup>+</sup> DCs and pDCs, but not skin-derived DCs. This molecule was also found to be specifically expressed on human BDCA3<sup>+</sup> DCs, but not on pDCs (Caminschi et al., 2008; Huysamen et al., 2008; Sancho et al., 2008). Clec9A associates with Syk kinase, inducing the production of inflammatory cytokines (Huysamen et al., 2008), and mediates cross-presentation of antigens derived from necrotizing cells (Sancho et al., 2009). Clec9A does not mediate dead cell uptake but rather enhances cross-presentation through a Syk-dependent pathway. It remains unclear whether Syk-dependent signaling modifies cross-presentation machineries or accessory molecules involved in activating T cells.

A recent genomic analysis of gene expression in human and mouse DC subsets revealed the relationship among DC subsets (Robbins et al., 2008). Considerable similarity was ob-

served between human and mouse pDCs. Notably, the approach established the similarity between human CD141<sup>+</sup> and murine CD8 $\alpha$ <sup>+</sup> DCs. Among the genes preferentially expressed in both cells are Clec9A, Nect2, IRF8, and TLR3. Moreover, *in vitro* GM-CSF-mediated bone marrow-derived DCs were found to be close to monocytes, a finding that should be considered when evaluating results using GM-CSF-derived DCs. An additional link between human CD141<sup>+</sup> DCs and mouse CD8 $\alpha$ <sup>+</sup> DCs is the shared expression of XCR1, the chemokine receptor for XCL1 (ATAC, lymphotactin or SCM-1). In the mice, the expression of XCR1 is restricted to CD8 $\alpha$ <sup>+</sup> DCs and XCL1, which is secreted by activated CD8<sup>+</sup> T cells, CD4<sup>+</sup> Th1 cells, and NK cells, induces a calcium influx in CD8 $\alpha$ <sup>+</sup> DCs (Dörner et al., 2009). Therefore, XCL1 may recruit CD8 $\alpha$ <sup>+</sup> DCs to lymphocytes and potentiate their reciprocal activation. The features ascribed to murine CD8 $\alpha$ <sup>+</sup> DCs together with newly identified molecules on these cells made possible the identification of a subset of human DCs that look and function similarly to mouse CD8 $\alpha$ <sup>+</sup> DCs. Human BDCA3<sup>+</sup> DCs are likely the equivalent of mouse CD8 $\alpha$ <sup>+</sup> DCs (Bachem et al., 2010; Crozat et al., 2010; Jongbloed et al., 2010; Poulin et al., 2010), with both cell types showing expression of Nect2, CD207, Batf3, IRF8, TLR3, and XCR1. Functionally, human BDCA3<sup>+</sup> DCs were similar to murine CD8 $\alpha$ <sup>+</sup> DCs in their secretion of IL-12, resulting in efficient polarization of Th1 cells. Human BDCA3<sup>+</sup> DCs preferentially express TLR3, but not TLR9 which is expressed by mouse CD8 $\alpha$ <sup>+</sup> DCs. TLR3 has been shown to enhance cross-presentation, and in line with this, poly I:C-activated BDCA3<sup>+</sup> DCs cross-present exogenously-added antigens to CD8<sup>+</sup> T cells. Similar to murine CD8 $\alpha$ <sup>+</sup> DCs, human BDCA3<sup>+</sup> DCs were superior in taking up dead cells, expressed XCR1 and responded to XCL1, leading to calcium mobilization and chemotaxis. BDCA3<sup>+</sup> DCs also express the transcription factors Batf3 and IRF8, but not IRF4, indicating that these cells and murine CD8 $\alpha$ <sup>+</sup> DCs may differentiate through a common gene expression program. These findings provide a deeper understanding of human DC biology and novel targeting strategies to deliver antigens to or manipulate BDCA3<sup>+</sup> cells. Further, given the relationship between lymphoid organ-resident CD8 $\alpha$ <sup>+</sup> DCs and peripheral tissue-derived CD103<sup>+</sup> DCs, it will be interesting to determine whether human peripheral DCs express CD103 and play similar roles as mouse CD103<sup>+</sup> cells.

### CONCLUSIONS

Based on studies assessing various surface markers, requirements for transcription factors, functional analyses and gene expression profiling, it is becoming clear that CD8 $\alpha$ <sup>+</sup> cDCs are related to CD103<sup>+</sup> DCs residing in various peripheral tissues. The discovery of a human CD8 $\alpha$ <sup>+</sup> DC lineage may enable the development of novel therapeutic interventions in human diseases, by harnessing the dual role of CD8 $\alpha$ <sup>+</sup> DCs in regulating immunity and tolerance. Additional studies are required to dissect the precursor and progeny cells for CD8 $\alpha$ <sup>+</sup> DC subsets and to determine whether these cells have a common immediate precursor. These studies may be accomplished by developing a more sophisticated lineage imprinting method. Similarly, delineating the family tree and the development process of CD8 $\alpha$ <sup>+</sup> DCs, which look more heterogeneous, will be challenging but undoubtedly fruitful.

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