ORIGIN, PRECURSORS AND DIFFERENTIATION OF MOUSE DENDRITIC CELLS

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Functional specialization allows defined dendritic-cell (DC) subsets to induce efficient defence mechanisms against pathogens and tumour cells, and maintain T-cell tolerance by inducing the inactivation of autoreactive T cells. A crucial question, which has important implications for both our understanding of the induction and control of immunity by DCs, as well as the use of DCs for immunotherapy, is whether the functional diversity of DCs results from the existence of developmentally independent DC subpopulations, or whether DC subsets that share a common differentiation origin acquire specific functions in response to environmental signals. This review discusses recent findings on mouse DC development.

DC RECONSTITUTION ASSAYS Assays that allow the *in vivo* analysis of the differentiation potential of different precursor populations after transfer into irradiated recipients; the precursor progeny is subsequently studied by using a genetic marker that allows the detection of cells from donor or recipient origin.

BROMODEOXYURIDINE INCORPORATION ASSAY (BrdU). An assay in which BrdU, a thymidine analogue, is incorporated into DNA during DNA replication. Cells that have incorporated BrdU, and presumably have divided, are then visualized using fluorescence-labelled BrdU-specific antibodies followed by flow cytometry.

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doi:10.1038/nri1127

Dendritic cells (DCs) are crucial components of the immune system owing to their essential role in the induction and control of T-cell immunity, as well as in the modulation of responses by B cells and natural killer (NK) cells. The potency of DCs in inducing T-cell activation depends on their capacity to capture, internalize and process antigens, leading to the presentation of antigenic peptides associated with MHC molecules to antigen-specific T cells. Research on DCs over the past decade has led to two important concepts. First, it has been established that the DC system comprises a large collection of subpopulations with different functions. Functional diversity of DC populations is related to their differentiation state, as well as their specific location, and is a consequence of differential interactions with antigens and effector cells of the immune system[1]. Second, DCs are promising alternative tools for vaccination and immunotherapy of cancer, autoimmunity and allergy[2,3].

Although a definitive model of DC development remains to be established, recent data that are derived mostly from *in vivo* DC-RECONSTITUTION ASSAYS and the analysis of genetically modified mice have substantially contributed to our understanding of the origin of DCs by allowing the characterization of DC precursors. These data have indicated the plasticity of myeloid- and lymphoid-developmental pathways in the generation of DCs.

Mouse dendritic-cell subpopulations

A large variety of DC subsets have been described in lymphoid and non-lymphoid organs. Although some of these subsets seem to be specific to defined tissue environments, both phenotypic and functional criteria have allowed the classification of mouse DCs into six main subpopulations (TABLE 1). DCs in lymphoid tissues can be divided into $CD8^-$ and $CD8^+$ subpopulations, which have been extensively studied over the past few years[4]. $CD8^-$ DCs can be further subdivided in $CD4^-CD8^-$ and $CD4^+$ $CD8^-$ subsets[5,6], although the functional relevance of the differential expression of CD4 by $CD8^-$ DCs remains controversial. On the basis of analysis of their cytokine secretion potential[6] and BROMODEOXYURIDINE (BRDU) INCORPORATION ASSAYS[7], $CD4^-CD8^-$ and $CD4^+CD8^-$ DCs are thought to be two developmentally and functionally independent DC subsets. By contrast, $CD8^-$ DC-reconstitution experiments and *in vitro* assays indicated that the $CD4^-CD8^-$ subset might constitute an activated or more differentiated form of the $CD4^+CD8^-$ DCs[5]. In addition, DCs that express intermediate levels of CD8 ($CD8^{int}$ DCs) constitute a lymph-node-specific subset of DCs, which gain access to the lymph nodes through the lymphatics. In peripheral lymph nodes, $CD8^{int}$ DCs seem to derive from epidermal Langerhans cells[8], and perhaps from dermal DCs, whereas those found in the mesenteric

PLASMACYTOID DCS
A subset of immature dendritic cells (DCs), originally described in humans, that are characterized by their capacity to produce high levels of type I interferon after stimulation with virus. They can differentiate into potent antigen-presenting cells after activation.

COLONY-FORMING UNIT EXPERIMENTS
These experiments address the differentiation capacity of haematopoietic precursors, by analysing the cell colonies that result from the proliferation and differentiation of single precursor cells, using *in vivo* transfer techniques or cell-culture systems.

COMMON LYMPHOID PROGENITORS
Bone-marrow clonogenic precursors that are committed to the lymphoid lineage, generating T cells, B cells and natural killer cells, but are devoid of myeloid differentiation potential.

COMMON MYELOID PROGENITORS
Bone-marrow clonogenic precursors that are committed to the myeloid lineage and give rise to megakaryocyte–erythrocyte or granulocyte–macrophage progenitors, but are devoid of lymphoid differentiation potential.

lymph nodes, seem to originate in the intestinal lamina propria (F. Anjuère, personal communication). Finally, the expression of the CD45 isoform B220 defines the mouse functional counterpart of human PLASMACYTOID DCS. Plasmacytoid B220$^+$ DCs are found in all lymphoid organs of the mouse and are characterized by their potential to produce large amounts of type I interferon (IFN) in response to virus infections[9]. In addition, it has been proposed that plasmacytoid B220$^+$ DCs are involved in the maintenance of T-cell tolerance by inducing the differentiation of T regulatory cells[10], which can block the activation of naive T cells by an interleukin-10 (IL-10)-dependent mechanism, similar to T regulatory 1 (T_R1) cells[11]. Although plasmacytoid B220$^+$ DCs, which can express CD8 at various levels depending on their location, the term CD8$^+$ DCs, as used in this article, does not include these cells. In addition to these main DC subpopulations described earlier, other DC subsets have been described in specific organs of the mouse, including the lung[12,13], heart and kidney[14], although the available phenotypic and functional data regarding these DCs have not yet allowed them to be ascribed to any of the DC subpopulations that are listed in TABLE 1.

The origin of mouse dendritic cells

DCs were originally thought to be derived from myeloid precursors due to their functional, phenotypic and morphological similarities with macrophages, the myeloid origin of which was firmly established by classic COLONY-FORMING UNIT EXPERIMENTS[15]. In addition, Inaba *et al.*[16] reported that granulocytes, macrophages and DCs can develop from a common MHC class-II-negative progenitor in the presence of granulocyte–macrophage colony-stimulating factor (GM-CSF). The concept that DCs can be derived from myeloid precursors was definitively shown by Sallusto and Lanzavecchia[17] in a report indicating that human DCs can be differentiated *in vitro* from monocytes, and has been strengthened more recently by Randolph *et al.*[18] using an *in vivo* approach in mice. However, recent findings that are derived mainly from *in vivo* studies of DC development have shown that differentiation of DCs is more complex.

Evidence from dendritic-cell reconstitution assays. A chronology of the crucial findings that deal with the origin of mouse DCs is summarized in BOX 1. An inital study of DC reconstitution in bone-marrow irradiated chimaeras showed that thymic DCs can be derived from CD4^{low} early thymic precursors, which are devoid of myeloid reconstitution potential[19], and led to the concept that some DCs could be of lymphoid origin. Subsequent studies[20–22] contributed to the idea that CD8$^-$ and CD8$^+$ DCs (the most extensively studied mouse DC subsets) were of myeloid and lymphoid origin, respectively. However, this concept has proven to be incorrect by experiments showing that both CD8$^-$ and CD8$^+$ DCs can be derived from CD4^{low} early thymic precursors[5]. At the time, this result was interpreted as an indication of a lymphoid origin for both DC subpopulations. However, this does not seem to be correct because, in a subsequent report, Weissman and colleagues[23] showed that not only COMMON LYMPHOID PROGENITORS (CLPs), but also COMMON MYELOID PROGENITORS (CMPs), could differentiate into both CD8$^-$ and CD8$^+$ DCs. Moreover, these authors showed, by co-injecting CLPs and CMPs into irradiated recipients, that the generation of CD8$^-$ and CD8$^+$ DCs in the thymus and spleen resulted from both types of progenitor, simultaneously. These data indicated that CD8$^-$ and CD8$^+$ DCs could be derived from two distinct differentiation pathways — myeloid or lymphoid.

Several important questions arise regarding the concept that DCs are generated by both myeloid and lymphoid developmental processes. Do these experiments actually reflect the physiological situation and, if so, what is the relative contribution of the myeloid and lymphoid lineages to DC differentiation *in vivo*? Does a DC subpopulation that is exclusively derived from lymphoid precursors — that is, a lymphoid DC lineage — exist? And, as CD8$^-$ and CD8$^+$ DCs do not belong to different lineages, do they represent independent DC subsets or distinct phases of differentiation of a unique DC subset?

With regard to the contribution of myeloid versus lymphoid precursors to the generation of DCs, on the basis of the DC differentiation capacity of CMPs and CLPs, and taking into account the relative proportion of these progenitor populations in the mouse, Manz *et al.*[24] proposed that CMPs and CLPs contribute equally to the generation of thymic DCs, but that peripheral DCs are derived mainly from CMPs. Similar conclusions were reached by Wu *et al.*[25], although minor differences in the DC differention potential of these precursors were reported. Interestingly, in a recent article[26] these authors have proposed that the DC differentiation activity of bone-marrow precursors is correlated with the expression of fms-related tyrosine kinase 3 (FLT3). In addition, they reported that CLPs (which are mostly FLT3$^+$) are more efficient in the generation of DCs, especially plasmacytoid DCs, than the FLT3$^+$ fraction of CMPs.

However, it is important to take into account that, in DC reconstitution assays, the irradiation might allow DC precursors to acquire an altered non-physiological homing and/or differentiation potential. Therefore, it is possible that under physiological conditions differentiation of DCs does not always result from the activity of myeloid and lymphoid precursors simultaneously. Alternatively, DCs could differentiate from either lymphoid or myeloid precursors depending on environmental conditions, such as the location and the presence of inflammation or infection.

Table 1 | **Organ distribution of mouse dendritic-cell subpopulations**

Dendritic-cell subpopulation	Thymus	Spleen	Lymph node	Peyer's patch	Skin	Liver
CD8$^-$ DCs	*	+	+	+	–	+
CD8$^+$ DCs	+	+	+	+	–	+
CD8^{int} DCs	–	–	+	–	–	–
Langerhans cells	–	–	–	–	+	–
Dermal DCs	–	–	–	–	+	–
B220$^+$DCs	+	+	+	+	–	N.D.

*CD8$^-$ DCs can be detected in the thymus, although they constitute a minute proportion in thymic DCs[73]. +, present; –, absent; CD8^{int}, intermediate level of expression of CD8; DC, dendritic cell; N.D., not determined.

Box 1 | **Essential findings dealing with the origin of DCs**

- *1973* First description of dendritic cells (DCs) as myeloid-derived antigen-presenting cells (APCs)[72].
- *1993* Thymic DCs derived from $CD4^{low}$ early thymic precursors[19].
- *1996* $CD8^-$ DCs and $CD8^+$ DCs proposed to be myeloid and lymphoid DCs, respectively[20].
- *1999* First evidence of *in vivo* differentiation of DCs from monocytes[18].
- *2000* Generation of both $CD8^-$ and $CD8^+$ DCs from $CD4^{low}$ early thymic precursors[5].
- *2000* Generation of both $CD8^-$ and $CD8^+$ DCs from common lymphoid progenitors (CLPs) or common myeloid progenitors (CMPs)[23].
- *2002* Generation of $CD8^-$, $CD8^+$ and plasmacytoid $B220^+$ DCs from common DC precursors[51].
- *2003* Generation of plasmacytoid DCs from CLPs and CMPs[26].

In addition, an unresolved issue, with important implications regarding this problem, is whether DCs are generated from CMPs and CLPs in the bone marrow, from where they migrate to lymphoid tissues or from circulating DC precursors that home to the peripheral lymphoid organs where they differentiate. Whether or not thymic DCs are generated from DC precursors that are derived from thymic lymphoid precursors remains to be shown conclusively. Nevertheless, it has been proposed that thymic DCs are derived from the lymphoid lineage on the basis of recent data that show the existence of D–J rearrangements at the immunoglobulin H gene locus in these cells[27]. In this regard, Zenke and colleagues[28] have suggested the existence of a common DC/B-cell developmental pathway in which the DC-lineage decision would be under the control of the helix–loop–helix (HLH) family transcription factor inhibitor of DNA binding 2 (Id2), whereas the activating HLH protein E2A would regulate B-cell development. On the basis of their findings, these authors have suggested that upregulation of expression of inhibitory HLH proteins, such as Id2, Dec1 and activated B-cell factor 1 (Abf1), and downregulation of expression of HLH activator proteins, such as E2A, Scl and Ly11, might be essential components of the DC differentiation programme.

On the basis of these considerations, a theoretical model of the developmental origin of mouse DCs, which takes into account present uncertainties, is proposed in FIG. 1.

Evidence from the analysis of knockout mice. The analysis of mice that are deficient for molecules known to be required for the development of defined haematopoietic lineages (TABLE 2) have not, so far, allowed definitive conclusions with regard to the origin of DCs to be drawn; on the contrary, they have contributed somewhat to the controversy concerning the origin of DCs.

In mice homozygous for a dominant-negative mutation in the *Ikaros* gene (*Ikaros* $DN^{-/-}$) — which encodes a family of zinc-finger transcription factors that are expressed by early haematopoietic and lymphoid lineage cells — differentiation of myeloid cells is normal but there is a severe defect in the development of lymphoid cells and DCs that cannot be rescued by adoptive transfer of bone-marrow cells from *Ikaros* $DN^{-/-}$ mice into normal recipient mice[21,29]. These data indicate an essential role for the lymphoid lineage in DC development and argues against the dual contribution model that was proposed by Traver *et al.*[23], as in *Ikaros* $DN^{-/-}$ mice, myeloid progenitors were not sufficient for the differentiation of DCs. Alternatively, Ikaros might have an essential role in the development of DCs, irrespective of whether DCs are generated from the myeloid or lymphoid differentiation pathway.

By contrast, mice deficient in the cytokine receptor common γ chain (γc)[30] or Notch1[31] have a marked deficiency in T-cell development and a lack of $CD4^{low}$ early thymic precursors, although differentiation of thymic DCs is normal. In these reports, the developmental dissociation of T cells and DCs was interpreted as evidence that thymic DCs were not derived from early T-cell precursors, arguing against a lymphoid origin of DCs. These data would suggest that, in Notch1- and γc-knockout mice, thymic DCs were generated from myeloid progenitors, but, taking into account the data that show the dual contribution of CMPs and CLPs in the generation of thymic DCs in irradiation chimaeras[24], the possibility that thymic DCs can be derived from lymphoid precursors in normal mice cannot be ruled out.

A defect in the differentiation of $CD8^-$ DCs, in presence of normal $CD8^+$ DC development and alterations in B-cell and myeloid differentiation, has been described in mice with an *Ikaros*-null mutation (*Ikaros* $C^{-/-}$)[21] — *Ikaros* $C^{-/-}$ mice have less severe defects in lymphoid development than *Ikaros* $DN^{-/-}$ — mice deficient for the nuclear factor-κB (NF-κB) subunit Relb[22] and mice deficient for the transcription factor PU.1 (REFS 32,33), indicating independent differentiation pathways for $CD8^+$ and $CD8^-$ DCs.

By contrast, mice deficient in the IFN consensus sequence binding protein (ICSBP)[34,35] — a transcription factor that has a crucial role in the regulation of lineage commitment — and mice deficient in Id2 (REF. 28) have a marked defect in the differentiation of $CD8^+$ DCs, accompanied by a defective DC MATURATION process for $CD8^-$ and $CD8^+$ DCs. Together, these data support a role for Relb and PU.1 in the development of $CD8^-$ DCs, and for ICSBP and Id2 in the differentiation of $CD8^+$ DCs. Although the relevance of these results with regard to DC origin is not clear, they do have important implications concerning the developmental relationship between $CD8^-$ and $CD8^+$ DCs, as discussed later.

Evidence from in vitro differentiation assays. Most experimental systems using mouse DCs that are differentiated *in vitro* depend on DCs that are generated in bone-marrow cultures in the presence of GM-CSF with or without IL-4. This method leads to the differentiation of DCs with phenotypic and functional characteristics that are similar to those described for human monocyte-derived DCs — that is, cells that are positive for CD11c, MHC molecules and co-stimulatory molecules, negative for T- and B-cell markers, and have a high T-cell stimulation capacity — but, so far,

DC MATURATION
A process resulting from the engagement of activation receptors on dendritic cells (DCs), such as Fc receptors, Toll-like receptors, cytokine receptors or CD40, which involves the upregulation of MHC molecules and co-stimulatory molecules and the acquisition of specific functions that enable DCs to activate T cells efficiently.

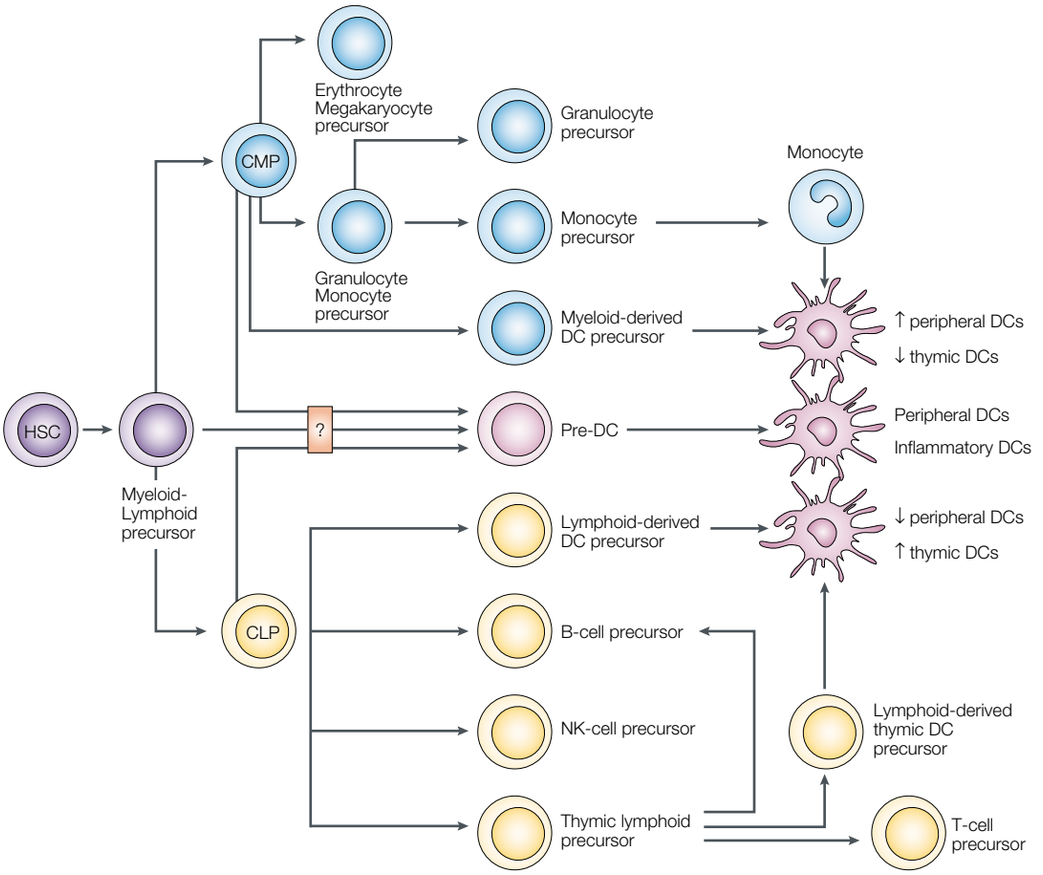

Figure 1 | **Theoretical model of the developmental origin of mouse dendritic cells.** The differentiation of dendritic cells (DCs) — including $CD8^-$, $CD8^+$, plasmacytoid $B220^+$ DCs and Langerhans cells — has been proposed to proceed directly through myeloid- and lymphoid-derived DC precursors[23], and through circulating common DC precursors (pre-DCs)[51]. On the basis of the relative DC differentiation potential and the absolute number of common lymphoid progenitors (CLPs) and common myeloid progenitors (CMPs), it has been established that thymic DC differentiation results from an equal contribution of both precursors, whereas peripheral DCs are derived mainly from CMPs[24,25]. Myeloid-derived DC precursors have been shown to be derived from the fraction of CMPs that express fms-related tyrosine kinase 3 ($FLT3^+$)[26]. Lymphoid-derived DC precursors could derive from CLPs that are located in the bone marrow or from thymic lymphoid precursors, which give rise to T cells, thymic DCs and B cells. Pre-DCs and monocytes have been proposed to be involved in the generation of DCs after recruitment to reactive sites[51]. The origin of pre-DCs from myeloid and/or lymphoid progenitors, and their relative contribution to the generation of DCs in relation to CMPs and CLPs under steady-state conditions have also to be determined. HSC, haematopoietic stem cell; NK, natural killer.

this system has not contributed markedly to our knowledge of DC differentiation. An alternative differentiation system of DCs that is based on the culture of lineage-negative, c-kit$^+$ bone-marrow cells with GM-CSF and tumour-necrosis factor (TNF), has allowed the *in vitro* characterization of an intermediate precursor of DCs and macrophages[36], as well as the generation of transforming growth factor-β (TGF-β)-dependent Langerhans-cell-like cells in culture[37]. Interestingly, these Langerhans-cell-like cells upregulated the expression of CD8 and migrated efficiently to the lymph nodes after subcutaneous injection[38], as described for epidermal Langerhans cells using an *in vivo* assay[8]. More recently, bone-marrow cultures, in the presence of the cytokine ligand (FLT3L), have been shown to generate DCs that are susceptible to upregulating the expression of CD8 after *in vitro* activation[39], as well as plasmacytoid-like DCs[40–42], and so this system might mimic differentiation of DCs *in vivo* more closely than previous systems. The analysis of such cultures could provide important insights into the identification of specific DC precursors and the cytokines that control their differentiation. But the DC culture systems described earlier have not allowed any definitive conclusions to be drawn regarding the cytokines that influence DC differentiation *in vivo*, as the analyses of mice deficient for GM-CSF, IL-4 or TNF have shown that these cytokines are dispensable for the development of DCs[43]. By contrast, mice that lack FLT3L have defective development of DCs, but they also have defects in haematopoiesis involving a reduced number of haematopoietic progenitor cells and defective myeloid and B-cell differentiation[44]. These data showed the essential role of FLT3L in the development of DCs. In further support of this, D'Amico and Wu[26] have recently shown that the DC-precursor activity of bone-marrow cells resides in the $FLT3^+$ fraction of

DC PRECURSORS (Pre-DCs). A population of dendritic cell (DC)-committed precursors present in mouse blood that can fully reconstitute the $CD8^-$, $CD8^+$ and plasmacytoid $B220^+$ DC subpopulations after transfer into irradiated recipients[51]. Precursor DCs are $CD11c^+$ $B220^+CD11b^+$ $CD43^+CD44^+$ $CD62L^+$ $FcR\gamma^+$MHC class $II^-CD19^-CD40^-CD86^-$c-kit^- $IL\text{-}7R\alpha^-IL\text{-}3R\alpha^-DEC205^-$ $F4/80^-Gr1^-$.

CMPs and CLPs. With regard to *in vitro* DC differentiation from bone-marrow precursors, Manz *et al.*[24] reported that IL-7 was an essential factor for the generation of DCs from CLPs, whereas GM-CSF and stem-cell factor (SCF) were the essential cytokines for inducing the differentiation of CMPs into DCs.

Finally, the data showing the requirement for TGF-β in the generation of Langerhans cells *in vitro* confirmed previous results from TGF-β-deficient mice, which have a severe defect in the differentiation of Langerhans cells, but not DCs[45]. Interestingly, a recent study by Merad *et al.*[46] indicated that, in contrast to DCs that are located in peripheral lymphoid organs, which are renewed from circulating bone-marrow-derived precursors, Langerhans cells are derived from proliferating precursors that are present in the skin, although inflammatory changes in the skin induced the recruitment of blood-borne progenitors with Langerhans-cell differentiation potential.

To conclude, although the experiments on the generation of DCs *in vitro* that were discussed earlier allow the study of different precursor populations and their differentiation potential using cytokine combinations[43], so far, they have provided only limited information on the origin and differentiation of DCs in the mouse.

Does a lymphoid dendritic-cell lineage exist?

The reports described earlier support the concept that both $CD8^-$ and $CD8^+$ DCs can be generated from lymphoid precursors or myeloid precursors. Therefore, these experiments do not show that either the $CD8^-$ or $CD8^+$ DC subpopulation is derived specifically from lymphoid precursors and, consequently, they question the existence of a lymphoid DC lineage. Similarly, there is no experimental evidence, so far, of a specific lymphoid origin for $CD8^{int}$ DCs, Langerhans cells or dermal DCs. However, plasmacytoid $B220^+$ DCs have been proposed to be of lymphoid origin, on the basis of the study of plasmacytoid DCs, their putative human counterparts. Human plasmacytoid DCs have been reported to depend on IL-3, but not on GM-CSF to differentiate into DCs[47] and to express, in the thymus, pTα — a molecule that after assembly with the T-cell receptor (TCR) β-chain forms a pre-TCR[48]. In addition, transfection of $CD34^+CD38^-$ fetal liver precursors with Id2 and Id3 blocks their differentiation into T cells, B cells and plasmacytoid DCs, but not NK cells or myeloid cells[49]. However, in this experimental model, not all lymphoid development was defective, as NK-cell differentiation remained unaffected. Finally, the transcription factor SPI-B, expressed by human plasmacytoid DCs and lymphoid cells, but not by myeloid-derived cells, has been proposed to be involved in the control of plasmacytoid DC development by limiting the capacity of lymphoid precursors to generate T, B and NK cells[50]. These data have led to the proposal that plasmacytoid DCs are of lymphoid origin, but this has not been shown directly. Moreover, this hypothesis has been challenged by a report showing that a $CD11c^+$ MHC class II^- common DC precursor found in mouse blood (PRE-DC) can generate $CD8^-$, $CD8^+$, as well as plasmacytoid $B220^+$ DCs after transfer into irradiated mice[51], indicating that plasmacytoid $B220^+$ DCs share a common developmental origin with $CD8^-$ and $CD8^+$ DCs, which can be generated by both myeloid and lymphoid precursors, as discussed earlier[23]. In support of this concept, D'Amico and Wu[26] have recently reported that mouse plasmacytoid $B220^+$ DCs can be generated from both CLPs and CMPs after transfer into irradiated recipients. Therefore, as previously stated for conventional DCs, plasmacytoid DCs are most probably generated by a dual contribution of lymphoid and myeloid precursors. Consequently, the data dealing with the involvement of ID2 of ID3 and SPI-B in the differentiation of human plasmacytoid DCs[49,50] might not support the lymphoid origin of plasmacytoid

Table 2 | **Essential data on dendritic-cell differentiation in knockout mice**

Knockout mice	$CD8^-$ DCs Spleen	$CD8^+$ DCs Spleen	$CD8^+$ DCs Thymus	LCs Skin	Comments	References
Ikaros DN	NO	NO	NO	Yes	Marked deficiency in lymphoid development, normal myeloid development	29
Notch1	Yes	Yes	Yes	Yes	Marked deficiency in T-cell development, normal B-cell and myeloid development	31
Common γ-chain	Yes	Yes	Yes	N.D.	Marked deficiency in T-cell development, defects in B-cell and NK-cell development	30
Ikaros C	NO	Yes	Yes	N.D.	Marked deficiency in B-cell and NK-cell development, defects in T-cell development, disrupted splenic marginal-zone organization	21
Relb	NO	Yes	Yes	Yes	Myeloid hyperplasia, normal lymphoid development, reduced splenic white pulp and splenomegaly	22
PU.1	NO	Yes	Yes	N.D.	Marked deficiency in B-cell and myeloid development	32,33
ICSBP	Yes	NO*	NO*	Yes	Defects in plasmacytoid DC and myeloid development; defective DC maturation	34,35
Id2	Yes	NO*	NO*	NO	Marked deficiency in NK-cell development, normal T- and B-cell development	28
TGF-β	Yes	Yes	Yes	NO	Normal lymphoid and myeloid development	45

* Low numbers of $CD8^+$ DCs can be detected in ICSPB and Id2 knockout mice. DC, dendritic cell; ICSBP, interferon consensus sequence binding protein; Id2, inhibitor of DNA binding 2; Ikaros C, Ikaros null mutation; Ikaros DN, dominant-negative Ikaros mutation; LC, Langerhans cell; N.D., not determined; NK, natural killer; TGF-β, transforming-growth factor-β.

DCs, but rather indicate the factors that control the T-cell–B-cell–NK-cell–plasmacytoid DC lineage decision during the differentiation of lymphoid-committed precursors.

In conclusion, although lymphoid precursors have been shown to generate DCs and plasmacytoid B220$^+$ DCs, so far, there is no experimental evidence that shows the existence of a specific lymphoid DC lineage in mice.

Dendritic-cell precursors

The definition of DC-committed precursors has remained elusive both in humans and mice. Several reports on DC precursors have been published previously[4]. These articles described either precursor populations that can generate DCs, but the differentiation potential of which was not restricted to the DC lineage, or immediate precursors of defined DC subsets, such as plasmacytoid cells or monocytes, with limited DC differentiation potential. However, a CD11c$^+$ MHC class-II$^-$ DC-restricted precursor population, which can fully reconstitute splenic CD8$^-$, CD8$^+$ and plasmacytoid B220$^+$ DC subpopulations, and is devoid of lymphoid- or myeloid-differentiation potential, has been recently described in mouse blood[51]. Whether these pre-DCs are a common precursor for all DC subpopulations or an environmentally regulated DC precursor that is involved in the generation of only certain DC subpopulations, remains to be addressed. As mentioned earlier, an important and unresolved issue regarding the development of DCs is whether DCs are essentially generated from CMPs and CLPs in the bone marrow from where they migrate to the periphery, or alternatively, from the differentiation of DC precursors that are located in the peripheral lymphoid organs. In the latter case, the correlation between these putative local precursors with pre-DCs, and how and when these precursors are recruited to the lymphoid organs, remain to be determined.

Interestingly, during infection by the mouse mammary tumour virus (MMTV), CD11c$^+$ pre-DCs seem to be recruited to the lymph nodes in which they differentiate into DCs[51]. These results support the hypothesis that DC-mediated induction of T-cell responses against pathogens might involve the recruitment of pre-DCs and the subsequent differentiation into DCs. This concept is also supported by recent data indicating that differentiation of DCs in mice with a granulomatous liver disease resulted from the recruitment of blood-borne precursors, which probably correspond to pre-DCs[52]. In addition, recruitment of blood DC precursors to the spleen, similar to pre-DCs, occurred during infection by *Streptococcus pneumoniae*[53]. In addition, recent findings from my laboratory have indicated that mouse monocytes differentiate into CD8$^-$ and CD8$^+$ splenic DCs, as well as plasmacytoid B220$^+$ DCs, after transfer into irradiated mice and can be recruited to the lymph nodes during infection with MMTV (C.A., unpublished observations). Therefore, the differential role of pre-DCs and other DC precursors, such as monocytes, in the development of DCs remains to be addressed, and could be of special relevance in relation to our understanding of DC differentiation and function during immune responses.

CROSS-PRIMING
A mechanism by which an antigen-presenting cell processes exogenous cell-associated antigens and presents them in the context of MHC class I molecules, leading to the activation of antigen-specific CD8$^+$ T cells.

The relationship between CD8$^+$ and CD8$^-$ DCs

Are CD8$^+$ and CD8$^-$ DCs functionally distinct? Numerous reports published over the past 10 years have studied the phenotypic and functional characteristics of CD8$^-$ and CD8$^+$ DCs, which are generally considered to be functionally distinct DC subpopulations[4]. In the spleen[54] and Peyer's patches[55], CD8$^-$ DCs are located mainly in zones of antigen uptake — that is, the marginal zone and the subepithelial dome, respectively — whereas CD8$^+$ DCs are found mostly in the T-cell areas. In addition, CD8$^-$ DCs, but not CD8$^+$ DCs, have been reported to have the ability to induce B-cell activation and plasmablast differentiation[53,56].

Both subsets contain immature or partially mature DCs that can be induced to undergo DC maturation after activation[57]. And although CD8$^-$ DCs seem to have a higher endocytic and phagocytic capacity than CD8$^+$ DCs[7,54], recent data indicate that CD8$^+$, but not CD8$^-$, DCs can internalize apoptotic cells[58].

During *in vivo* T-cell responses, CD8$^-$ DCs mainly induce T helper 2 (T_H2)-cell responses, whereas CD8$^+$ DCs, elicit strong T_H1-cell responses due to their high capacity to produce IL-12 (REF. 59). However, importantly, *in vitro* studies indicate that this functional division could be altered by factors that modulate the function of DCs, in particular the ability to produce IL-12. These factors include the activation state of the DC[42], the nature of the antigen[60], the concentration of antigen[42,60], the type of receptor that is responsible for antigen uptake and specific cytokines, such as IL-10 and IFN-γ[61,62]. For example, it has been reported that under defined experimental conditions, such as in the absence of IL-10 or after CD40L-mediated activation, CD8$^-$ DCs produced IL-12 (REFS 61,62) and induced the production of T_H1 cytokines[42], and that at low antigen doses CD8$^+$ DCs induced the production of T_H2 cytokines[42].

Interestingly, under *in vivo* conditions, CD8$^+$ DCs have specialized functions related to their highly efficient T-cell stimulatory potential, which are absent in the CD8$^-$ DC subset. These include the capacity of CD8$^+$ DCs to capture apoptotic-cell antigens, to produce high amounts of IFN-γ[63] and IL-12 (REF. 64), and to activate T cells by CROSS-PRIMING[65]. But it has been shown that after *in vitro* activation with microbial stimuli, CD8$^-$ DCs can be induced to produce IFN-γ and IL-12 (REF. 59), and to acquire cross-priming capacity after Fc receptor (FcR)-mediated activation[66], indicating that after engagement of a specific activation programme, CD8$^-$ DCs could develop the ability to stimulate T cells, similar to that described for CD8$^+$ DCs. However, whether this functional plasticity exists *in vivo* remains to be elucidated, and is an essential issue regarding the physiological relevance of CD8$^+$ and CD8$^-$ DC subsets.

Are CD8$^+$ DCs derived from CD8$^-$ DCs? Some experimental data support the hypothesis that CD8$^+$ DCs are derived from CD8$^-$ DCs. Fluorescein-induced migration of Langerhans cells to the lymph nodes, which involves their activation, is paralleled by the upregulation of expression of CD8 and leukocyte function-associated antigen 1 (LFA1)[8]. It has been reported that splenic

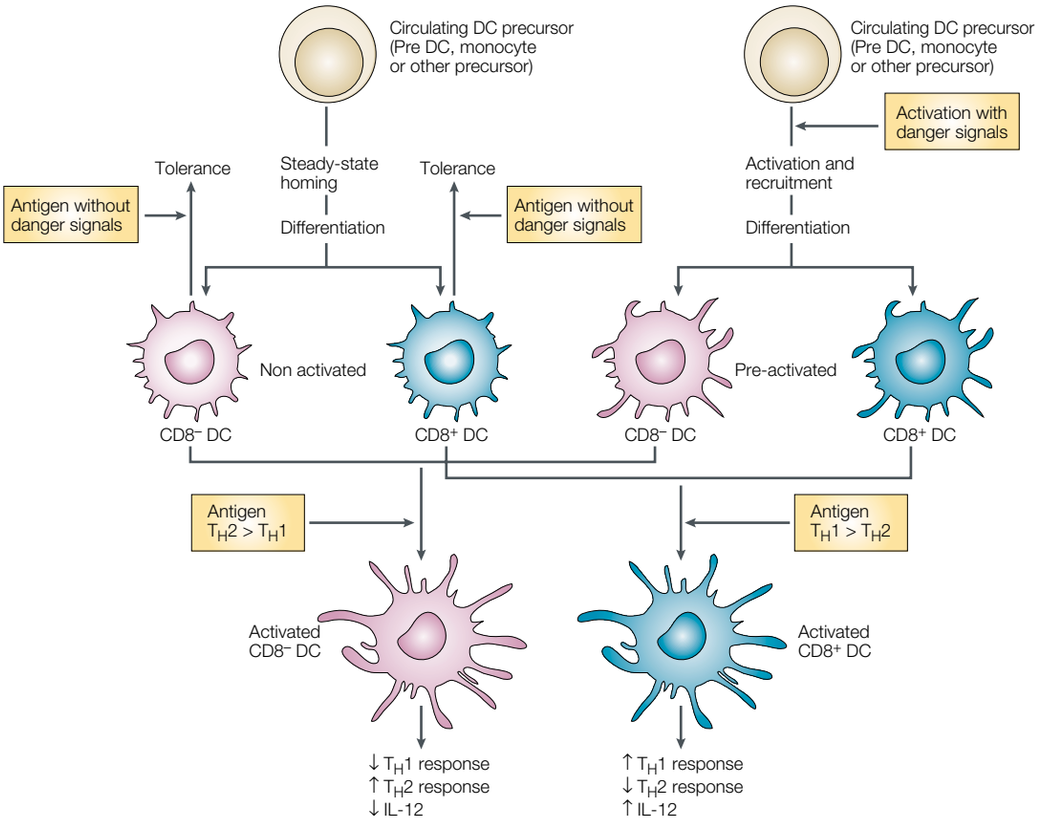

Figure 2 | **Theoretical model of CD8⁻ and CD8⁺ dendritic-cell differentiation, activation and function.** Induction of T-cell immunity might depend on CD8⁻ and CD8⁺ dendritic cells (DCs) that are already present in the lymphoid organs at the time of antigen entry (left), and/or on DCs that are newly generated after antigen-induced recruitment and differentiation of circulating DC precursors (right). In the absence of DANGER SIGNALS, non-activated DCs might induce peripheral T-cell tolerance. Differentiation of DCs from DC precursors that are activated and recruited by a danger-signal-mediated process in the presence of antigen would lead to the generation of pre-activated CD8⁻ and CD8⁺ DCs. Whether or not differentiation of antigen-recruited DC precursors into CD8⁻ or CD8⁺ DCs depends on the type of antigen is unknown. After uptake of antigen and engagement of activation receptors, in the presence of danger signals, CD8⁻ and CD8⁺ DCs enter an activation programme that leads to the activation of antigen-specific T cells. CD8⁻ DCs would mainly respond to T helper 2 (T_H2) antigens and consequently induce mainly T_H2-cell responses and plasmablast differentiation. By contrast, CD8⁺ DCs would be mainly activated by T_H1 antigens and, therefore, involved in the induction of T_H1-cell responses and the differentiation of precursors of cytotoxic T lymphocytes (CTL), by the production of high levels of interleukin-12 (IL-12).

CD8⁻ DCs upregulate the expression of CD8 and other CD8⁺ DC-related markers after adoptive transfer and homing to the spleen[67], although this result has been challenged by a recent report by Naik *et al.*[68], indicating that CD8⁺ DCs found after transfer of CD8⁻ DCs were, in fact, derived from a CD11c⁻ CD8⁻ precursor, presumably present as a contaminant of the transferred CD8⁻ DCs. Upregulation of expression of CD8 by splenic CD8⁻ DCs has also been described after *in situ* contact of CD8⁻ DCs with virus particles[69], and after internalization of apoptotic cells by CD8⁻ DCs in the splenic marginal zone and migration to the white pulp[70].

Moreover, it has been reported that IL-12 production induced by a soluble extract of tachyzoites (STAg) of *Toxoplasma gondii* depends on CD8⁺ DCs, and is paralleled by the migration of DCs from the spleen marginal zone to the inner white pulp[71]. In addition, it has been shown that DCs that are generated *in vitro* in the presence of FLT3L can also be induced to express CD8 after activation with lipopolysaccharide (LPS)[39]. Finally, mice deficient in ICSBP[34,35] and Id2 (REF. 28) have a blockade in the development of CD8⁺ DCs, which is paralleled by defective maturation of CD8⁻ DCs. Together, these studies support the idea that differentiation and/or activation of CD8⁻ DCs, under specific as-yet-undefined conditions, can lead to the upregulation of expression of CD8, acquisition of the capacity to produce IL-12 and migration from the antigen-capture to the T-cell areas.

By contrast, some experimental data argue against the hypothesis that CD8⁺ DCs are derived from CD8⁻ DCs. As discussed earlier, a marked defect in the development of splenic CD8⁺, but not CD8⁻, DCs, occurs in *Ikaros* $DN^{-/-}$ mice[21], mice deficient for Relb[22] and mice deficient for PU.1 (REF. 32). However, these genetically deficient mice also have important alterations in the development of other haematopoietic lineages, as well as in their splenic architecture (TABLE 2), which could explain their unbalanced CD8⁻ versus CD8⁺ DC development.

DANGER SIGNALS
Cell-wall components and other products of pathogens that alert the innate immune system to the presence of potentially harmful invaders, usually by interacting with Toll-like receptors and other pattern recognition receptors expressed by tissue cells and dendritic cells, for example.

In addition, BrdU incorporation assays did not indicate a delay in the turnover of splenic $CD8^+$ DCs compared to $CD8^-$ DCs, which should occur if $CD8^-$ DCs differentiate into $CD8^+$ DCs[7]. However, it is important to take into account that $CD8^-$ and $CD8^+$ DCs are present in the spleen at a 3:1 ratio. Therefore, although the relative percentage of BrdU-positive DCs indicated an independent generation of $CD8^-$ and $CD8^+$ DCs, the absolute number of BrdU-positive DCs of each subset was compatible with the hypothesis that a proportion of the $CD8^-$ DC subset could differentiate into $CD8^+$ DCs. Therefore, additional experiments that allow the definition of the differential involvement of $CD8^+$ versus $CD8^-$ DCs in the immunity against microbial pathogens should be carried out to determine conclusively the relationship between the two DCs subsets.

On the basis of the data discussed in this review, a theoretical model of $CD8^-$ DC and $CD8^+$ DC differentiation, activation and function is illustrated in FIG. 2.

Concluding remarks

In conclusion, recent reports on mouse DC precursors and differentiation have challenged some important concepts regarding the origin of DCs that have mostly now been accepted. No experimental evidence supports that $CD8^-$ and $CD8^+$ DC subsets correspond to the myeloid and lymphoid lineages, respectively, and therefore the terms myeloid and lymphoid should not be used to describe them. Similarly, neither the proposed lymphoid origin for plasmacytoid DCs nor the existence of a developmentally restricted lymphoid DC subset have been formally shown, so far. By contrast, different experimental approaches strongly support that DCs are generated by a dual contribution of DC precursors that are derived from both the myeloid- and lymphoid-differentiation pathways. Conversly, new data support the concept that induction of T-cell immunity against microbial infection involve the recruitment of DC precursors to lymphoid tissues and their local differentation into DCs.

The discovery of new DC-specific transcription factors and genes that are related to DC differentiation and function, as well as the study of the differential involvement of DC subpopulations in *in vivo* immune responses against pathogens and tumour cells should provide important insights into the present controversy regarding both the developmental origin of DCs and the functional correlation between DC subpopulations.

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Acknowledgements

I would like to thank G. Martínez del Hoyo, V. Parrillas, M. López-Bravo and B. León for critical reading of this manuscript and help in its preparation, and K. Shortman, M. Zenke, M. Luisa Toribio and A. Corbí for helpful discussions. Our studies were funded by the European Commission, the Community of Madrid, Spain and the Ministry of Science and Technology of Spain.

Online links

DATABASES

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