Origin and differentiation of dendritic cells

Carlos Ardavín, Gloria Martínez del Hoyo, Pilar Martín, Fabienne Anjuère, Cristina F. Arias, Alvaro R. Marín, Sara Ruiz, Verónica Parrillas and Héctor Hernández

Despite extensive, recent research on the development of dendritic cells (DCs), their origin is a controversial issue in immunology, with important implications regarding their use in cancer immunotherapy. Although, under defined experimental conditions, DCs can be generated from myeloid or lymphoid precursors, the differentiation pathways that generate DCs *in vivo* remain unknown largely. Indeed, experimental results suggest that the *in vivo* differentiation of a particular DC subpopulation could be unrelated to its possible experimental generation. Nevertheless, the analysis of DC differentiation by *in vivo* and *in vitro* experimental systems could provide important insights into the control of the physiological development of DCs and constitutes the basis of a model of common DC differentiation that we propose.

Carlos Ardavín*
Gloria Martínez del Hoyo
Pilar Martín
Cristina F. Arias
Alvaro R. Marín
Sara Ruiz
Verónica Parrillas
Héctor Hernández
Dept of Cell Biology, Faculty of Biology, Complutense University, 28040 Madrid, Spain.
*e-mail: ardavin@bio.ucm.es

Fabienne Anjuère
Faculté de Médecine Pasteur, Laboratoire d'Immunologie Cellulaire et Moléculaire, INSERM U364, Avenue de Valombrose, 06107 Nice Cedex 2, France.

T-cell immunity against tumors and bacterial or viral infections relies essentially on the recognition of antigenic peptides processed and presented to T cells by antigen (Ag)-presenting cells (APCs). During *in vivo* immune responses, the role of APC is played primarily by dendritic cells (DCs) acting as initiators, stimulators and regulators of Ag-specific T lymphocytes. Despite the extensive research on DC biology over the past decade, driven by the possibility of exploiting the potential of DCs as APCs in vaccination and immunotherapeutic anticancer trials[1], their origin remains unknown largely and represents a particularly controversial issue in current immunology. However, searching for the origin of DCs must take into account the fact that different subpopulations of DCs, with unique phenotypic characteristics and functional potential, are found in different locations[2]. We discuss whether this diversity could be correlated with different developmental origins of DCs and multiple DC differentiation pathways.

Owing to certain similarities with monocytes and/or macrophages in their distribution within lymphoid tissues, morphology, phenotype, enzymatic activities, and endocytic and/or phagocytic capacity, originally, DCs were considered to be myeloid-derived[3]. Indeed, evidence generated by different experimental approaches supports, as proposed originally, the fact that certain subsets of DCs are of myeloid origin. However, a series of results generated on the basis of *in vivo* and *in vitro* systems, both in humans and mice, has led to the concept that DCs can be generated from lymphoid-committed precursors. Therefore, current information dealing with the derivation of DCs is controversial, and no definitive conclusions can be drawn about the origin of the different DC subpopulations. In this review, we will present the most relevant aspects of the available literature in this field to define theoretical models of DC differentiation, both in the human and mouse systems, which could contribute to a better understanding of the origin of DCs.

Experimental support for a dual myeloid–lymphoid model of DC origin

Myeloid-derived DCs

Evidence for a myeloid DC lineage derives, in part, from human and mouse *in vitro* DC differentiation assays, in which DCs were generated from monocytes or intermediate myeloid precursors that retained the capacity to generate macrophages. Bipotential macrophage–DC precursors have been characterized in mouse bone marrow (BM)[4], and *in vivo* differentiation of dermal $CD11b^{+}F4/80^{-}$ phagocytic cells, considered to be monocytes, into DCs upon migration to the lymph nodes has been reported[5].

Lymphoid-derived DCs

The first evidence of the generation of DCs from lymphoid-committed precursors was obtained as a result of searching for an intrathymic differentiation pathway for thymic DCs. This research was undertaken based on the hypothesis that thymic DCs, being involved in T-cell negative selection[6], should develop intrathymically. Therefore, the earliest murine thymic precursors, namely $CD4^{low}$ precursors, which generate T, B and natural killer (NK) cells, but not myeloid cells[7], were assayed for their capacity to generate DCs upon intrathymic transfer into irradiated mice. These experiments showed that $CD4^{low}$ precursors could reconstitute fully the thymic population of DCs expressing CD8α in mice, and led to the concept of lymphoid DCs (Ref. 8). Equivalent results were obtained subsequently with human early thymic progenitors[9–12] or BM lymphoid-committed precursors[13], supporting the existence of human lymphoid DCs.

Plasmacytoid cells

Human plasmacytoid cells, which represent an immature $CD11c^{-}$ DC population with a strong capacity for secretion of type I interferon (IFN) after viral stimulation[14,15] and differentiate into DC-like cells upon culture with interleukin-3 (IL-3) plus CD40

http://immunology.trends.com 1471-4906/01/$ – see front matter © 2001 Elsevier Science Ltd. All rights reserved. PII: S1471-4906(01)02059-2

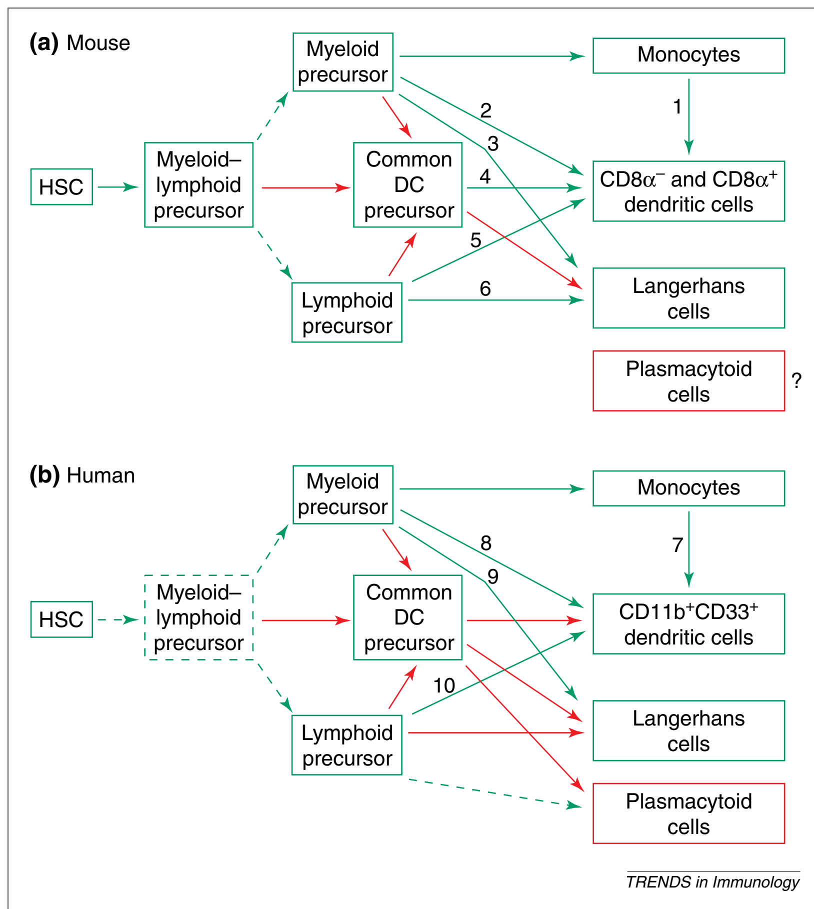

Fig. 1. Correlations between the development of dendritic cells (DCs) and myeloid versus lymphoid lineages in (a) mouse and (b) human. The multiple and controversial differentiation pathways leading to DCs that can be established on the basis of experimental evidence are shown. Solid green boxes and solid green arrows indicate the experimentally supported existence of precursor populations and differentiation pathways, respectively. Dashed green boxes and dashed green arrows indicate the nonformally proven, but indirectly supported, existence of precursor populations and differentiation pathways, respectively. Red boxes and arrows indicate the possible existence of precursor populations and differentiation pathways, respectively. Evidence for numbered pathways is supported by the following references: 1, Ref. 5; 2, Ref. 22; 3, Ref. 24; 4, C. Ardavin, unpublished; 5, Ref. 21; 6, Ref. 25; 7, Ref. 26; 8 and 9, Ref. 27; 10, Ref. 13. Abbreviation: HSC, hematopoietic stem cell.

ligand (CD40L)[16], were proposed to be lymphoid-derived owing to (1) their dependence on IL-3 but not granulocyte–macrophage colony-stimulating factor (GM-CSF)[16]; (2) their expression of pTα, a molecule which upon assembly with a T-cell receptor (TCR) β chain forms a pre-TCR (Ref. 17); and (3) the block in the differentiation of T cells, B cells and plasmacytoid cells, but not myeloid cells, from CD34$^+$CD38$^-$ fetal liver precursors transfected with inhibitors of DNA binding (Id)-2 and Id-3 (Ref. 18). However, in contrast to these reports, a myeloid origin for plasmacytoid cells was proposed on the basis of the derivation of IL-3 receptor (IL-3R)^{hi} DCs from CD34$^+$M-CSFR$^+$ progenitors[19]. Interestingly, evidence for a murine counterpart of human plasmacytoid cells has been reported recently [C. Asselin-Paturel *et al.* (2001) The IFN-α-producing cells in mice are immature CD11c^{low}, CD8α$^-$, CD11b$^-$ dendritic cells. *Keystone Symposia, Dendritic Cells: Interfaces with Immunology and Medicine*, 12–18 March 2001, Abstr. #304].

Dual myeloid–lymphoid DC differentiation

The fact that CD8α$^+$, but not CD8α$^-$, DCs were generated from CD4^{low} precursors when transferred intravenously[20] led to the hypothesis that mouse CD8α$^-$ DCs were myeloid-derived, and to a dual-origin model for DCs, involving the existence of myeloid and lymphoid DCs. However, although CD8α$^-$ and CD8α$^+$ DCs have been considered since then as myeloid- and lymphoid-derived, respectively[2], this concept has been challenged by a recent report by our group demonstrating that both subpopulations can be generated from CD4^{low} lymphoid-committed precursors[21]. These findings were confirmed subsequently and extended by showing that CD8α$^-$ and CD8α$^+$ DCs could both be derived from either lymphoid- or myeloid-committed progenitors[22,23]. In conclusion, experimental evidence no longer supports the concept that CD8α$^-$ and CD8α$^+$ DCs correspond to myeloid and lymphoid DCs, respectively, or represent different DC lineages.

Moreover, although, under defined experimental conditions, DCs can be generated from both myeloid and lymphoid precursors, the available information in either the murine or human system does not allow one to define conclusively the DC differentiation pathways generating DCs *in vivo*. On the basis of these considerations and additional data discussed previously, the possible correlations between the development of DCs and myeloid or lymphoid lineages are shown in Figure 1, illustrating the multiple and controversial differentiation pathways leading to DCs that can be proposed on the basis of experimental evidence. For example, CD8α$^-$ and CD8α$^+$ DCs could be derived from either myeloid or lymphoid precursors. Similarly, murine Langerhans cells (LCs) can be generated *in vitro* from myeloid precursors[24,28] or from CD4^{low} lymphoid precursors after intravenous transfer[25]. In human, DCs that express some myeloid markers were generated from both myeloid[27] and lymphoid[13] precursors. Therefore, whether a particular DC subpopulation is differentiated under physiological conditions from a myeloid or lymphoid progenitor could be unrelated to its possible derivation under experimental conditions, making the issue of *in vivo* DC derivation an unresolved and open question. As a consequence, although DC diversity implies functional specialization, at this stage, it remains to be defined whether the derivation of a DC lineage determines its specific functions. Nevertheless, the analysis of DC differentiation in *in vivo* and *in vitro* experimental models could provide important insights into the mechanisms controlling the physiological generation of DCs.

Mouse DC differentiation

Most of the relevant information relating to the *in vivo* development of murine DCs has been reported in the previous section. Different experimental approaches have been designed to analyze the

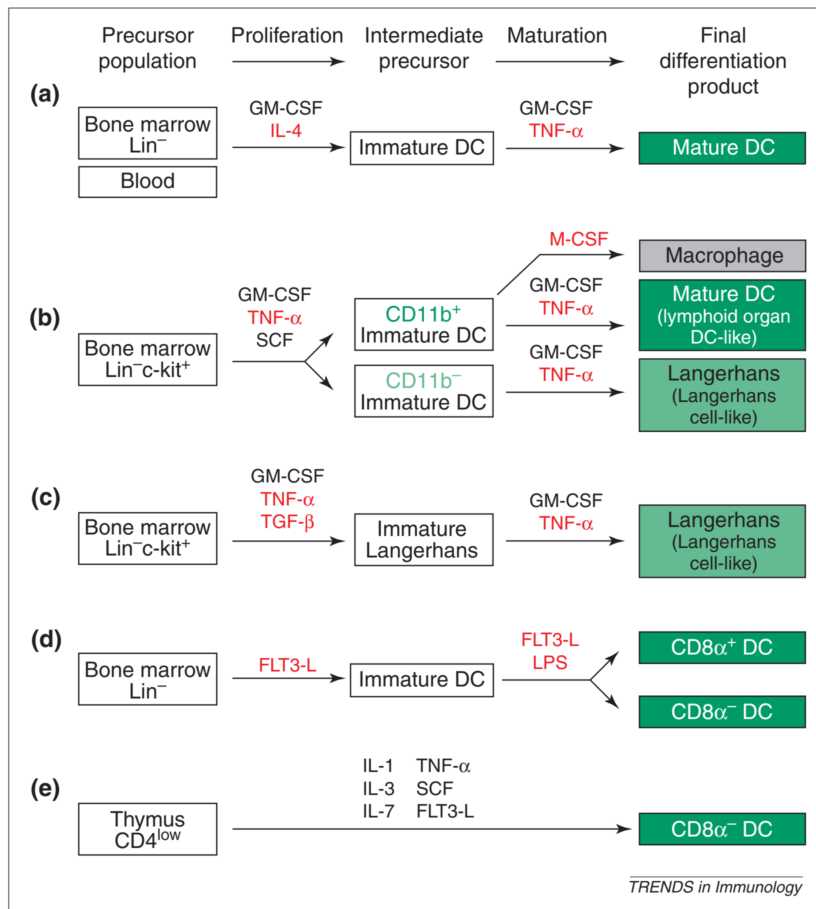

Fig. 2. Main *in vitro* differentiation pathways of dendritic cells (DCs) in mice. For each differentiation pathway, precursor populations are defined in the left-hand column. The central column corresponds to the intermediate precursors or to immature DCs obtained after the first proliferative phase of differentiation [with the exception of pathway (e), which describes a single-step culture method]. The final differentiation products, after the second nonproliferative maturation phase, are defined in the right-hand column. The cytokines required for each pathway are indicated; those claimed to have a decisive role in a specific step are marked in red. Evidence for pathways is supported by the following references: (a), Refs 29,30; (b), Ref. 28; (c), Ref. 24; (d), Ref. 31; (e), Ref. 32. Abbreviations: FLT3-L, fms-like tyrosine kinase 3 ligand; GM-CSF, granulocyte–macrophage colony-stimulating factor; IL, interleukin; Lin, lineage; LPS, lipopolysaccharide; M-CSF, macrophage colony-stimulating factor; SCF, stem cell factor; TGF-β, transforming growth factor β; TNF-α, tumor necrosis factor α.

development of murine DCs *in vitro*, which involve different hematopoietic precursors, the use of diverse DC differentiation- and/or maturation-inducing mediators and different culture protocols. Most of the relevant data on this topic is summarized in Figure 2. Most methods developed for generating DCs *in vitro* rely on two-step culture protocols, which generate immature DCs after a first culture period, followed by a second incubation phase leading to mature DCs. The first culture phase involves cell proliferation, whereas no expansion takes place during maturation.

In vitro *differentiation of DCs driven by GM-CSF plus TNF-α*

BM lineage marker (Lin)⁻ cells or blood adherent cells cultured with GM-CSF and IL-4, followed by maturation induced by tumor necrosis factor α (TNF-α) (Fig. 2a), generated a DC population that was phenotypically homogeneous and did not express CD8α (Refs 29,30). No significant differences were found when other maturation inducers, such as lipopolysaccharide (LPS) or CD40L, were employed[29]. However, when BM Lin⁻ cells were incubated with GM-CSF, stem cell factor (SCF) and TNF-α, followed by maturation with GM-CSF and TNF-α (Fig. 2b), two mature DC subsets with different phenotypes were generated[28]. Both were CD11c⁺ MHC class II (MHCII)^{hi}CD86^{hi}CD40⁺, and negative for CD8α and DEC-205 [an endocytic receptor expressed by CD8α⁺ DCs and LCs (Ref. 33)], but differed in their expression of E-cadherin (E-cadh), M-CSFR and nonspecific esterase (NSE). E-cadh⁻M-CSFR⁺NSE⁺ DCs derived from CD11c⁺CD11b⁺ immature DCs, which also generated macrophages when cultured with M-CSF. This result indicates that E-cadh⁻ M-CSFR⁺NSE⁺ DCs are derived from a CD11c⁺CD11b⁺ myeloid intermediate. By contrast, E-cadh⁺M-CSFR⁻NSE⁻ DCs, displaying characteristics of epidermal LCs, were derived from CD11c⁺CD11b⁻ immature DCs without the capacity to differentiate into macrophages. Therefore, TNF-α drives DC precursors along two independent differentiation pathways, generating lymphoid-organ DC-like and LC-like DCs.

In vitro *differentiation of LC-like DCs*

The generation of LCs from CD11c⁺CD11b⁻ immature cells is not dependent on the presence of exogenous transforming growth factor β1 (TGF-β1), which is required for the *in vivo* differentiation of LCs (Ref. 34). *In vitro* generation of LC-like DCs without TGF-β might reflect an incomplete differentiation and/or maturation of LCs, or the acquisition of certain LC markers by *in-vitro*-derived DCs unrelated to physiological LCs. An alternative differentiation protocol for LCs from BM Lin⁻ cells requires the addition of GM-CSF and TGF-β, followed by maturation with GM-CSF and TNF-α (Fig. 2c), and generates E-cadh⁺DEC-205⁺ LC-like cells[24]. The expression of DEC-205 by these LC-like cells could correspond to a more mature and/or physiological phenotype, and might reflect the requirement for TGF-β for the *in vivo* differentiation of LCs. Indeed, LC-like DCs generated by this culture method migrated efficiently to the lymph nodes after subcutaneous injection[35], and up-regulated their expression of CD8α upon migration, as described for epidermal LCs using an *in vivo* assay[33]. These data on the *in vitro* generation of LCs do not reveal whether LCs could derive physiologically from a myeloid progenitor. By contrast, the generation of LCs from CD4^{low} precursors[25] demonstrates that LCs can differentiate from a lymphoid precursor under experimental conditions.

FLT3L-driven differentiation of DCs in vitro

Interestingly, culture of BM Lin⁻ cells with fms-like tyrosine kinase 3 ligand (FLT3L) alone, followed by maturation with either LPS or IFN-α (Fig. 2d), generated both CD8α⁻CD11b^{hi} and CD8α⁺CD11b^{low}

DCs, suggesting that FLT3L is a key cytokine to drive the *in vitro* differentiation of DCs with a similar phenotype to that described for their physiological counterparts. However, when defining the cytokine requirements of a particular DC differentiation pathway, it is important to take into account that certain cytokines can be produced endogenously during DC differentiation assays. In this sense, it has been demonstrated that antibodies against IL-6, but not IL-2, IL-3, IL-4, IL-7, IL-11, IL-15, G-CSF, CSF-1 or TGF-β1, could block FLT3L-driven differentiation of DCs, revealing an essential role for IL-6 in the FLT3L-mediated generation of CD8α$^-$ and CD8α$^+$ DCs (Ref. 31). Anyhow, these results do not allow one to define whether FLT3L-driven DCs were generated from myeloid or lymphoid precursors in this assay.

DC differentiation from multipotent thymic lymphoid precursors in vitro

The generation of DCs from CD4^{low} lymphoid precursors can be achieved in cultures with IL-1, IL-3, IL-7, TNF-α, SCF and FLT3L (Ref. 32) (Fig. 2e), without GM-CSF, which is required for the differentiation of myeloid precursors. CD4^{low}-derived DCs did not express CD8α, in contrast to thymic DCs generated *in vivo* from these precursors[8], suggesting that additional cytokines are required to achieve a more physiological *in vitro* differentiation of DCs from CD4^{low} precursors.

Model of mouse DC development

In conclusion, data from *in vitro* studies in the mouse have proven to be particularly relevant to define the cytokine requirements that drive the differentiation of DCs, but have not allowed us to draw conclusions about the *in vivo* origin of mouse DC subsets. This controversial issue could be addressed conclusively by defining the DC precursor populations responsible for the *in vivo* development of DCs. Importantly, in reports dealing with the differentiation of DCs, immature DCs are often considered to be DC precursors. This is incorrect, because immature DCs express specific differentiation markers and are nonproliferating cells[2]. However, a Lin$^-$MHCII$^-$CD11c$^+$ DC precursor population from mouse blood has been characterized recently (C. Ardavín, unpublished). These DC precursors were devoid of lymphoid or myeloid differentiation potential, and had the capacity to reconstitute fully all splenic DC subpopulations, including CD8α$^-$ and CD8α$^+$ DCs, as well as a new CD11c$^+$MHCII$^+$B220$^+$CD40$^-$ subpopulation of DCs (also found in mouse thymus, lymph nodes and BM). This novel subpopulation had tolerogenic potential and was characterized by a very low stimulatory capacity, but acquired a strong stimulatory ability upon microbial stimulation (C. Ardavín, unpublished). Interestingly, a CD11c$^-$B220$^+$ DC subset with tolerogenic capacity has been isolated recently from mouse liver[36]. Whether these DC-committed precursors are derived *in vivo* from a myeloid or lymphoid progenitor is currently under investigation. However, the characterization of this common DC precursor provides the basis for integrating the results dealing with the generation of CD8α$^-$ and CD8α$^+$ DC subsets from either myeloid or lymphoid precursors. Interestingly, it has been demonstrated recently that splenic CD8α$^+$ DCs can be generated from the CD8α$^-$ DC subset upon intravenous transfer, by a maturation process involving the up-regulation of expression of CD8α, DEC-205 and CD24 (Ref. 67). These data support the hypothesis that CD8α$^-$ and CD8α$^+$ DCs correspond to different maturation and/or differentiation stages of the same DC population. On the basis of these data, an integrated model of mouse DC development is presented in Figure 3.

Differentiation of human DCs

In vitro studies of the differentiation of human DCs have been influenced greatly by the aim of optimizing culture systems to allow an efficient production of DCs for use in cancer immunotherapy. Additional studies have been conducted simultaneously to explore the mechanisms controlling different generation pathways for human DCs. The essential information derived from these studies is summarized in Figure 4. Two main protocols to generate DCs, from either monocytes or CD34$^+$ precursors, have been described, and generally involve, as mentioned for the *in vitro* differentiation of mouse DCs, a first differentiation phase followed by a maturation step.

Monocyte-derived DCs

Peripheral blood monocytes cultured with GM-CSF and IL-4 (Fig. 4a) generate immature DCs that can be driven into a mature state by TNF-α (Ref. 26), following a pathway similar to that described for mice in Figure 2a. Other maturation mediators, such as LPS, IFN-γ or CD40L, had similar effects, although slight phenotypical or functional differences have been reported (reviewed in Ref. 1). Importantly, a number of experiments support the idea that GM-CSF- and IL-4-driven differentiation of monocytes generates nonstable DCs, which can revert to a monocytic stage upon the withdrawal of cytokines[41]. Moreover, the reversal of TNF-α-mediated maturation of monocyte-derived DCs occurred when TNF-α was removed[42]. These data might have important implications regarding the use of monocyte-derived DCs with GM-CSF and IL-4 in cancer immunotherapy, and suggest that monocyte-derived DCs might not have a physiological counterpart.

Differentiation of DCs from CD34$^+$ precursors in vitro

CD34$^+$ precursors obtained from cord blood[43] or BM (Ref. 27) differentiated into DCs when cultured with GM-CSF and TNF-α (Fig. 4b). In this culture system, differentiation proceeded through two independent, immature DC intermediates, defined by their mutually exclusive expression of CD14 and CD1a.

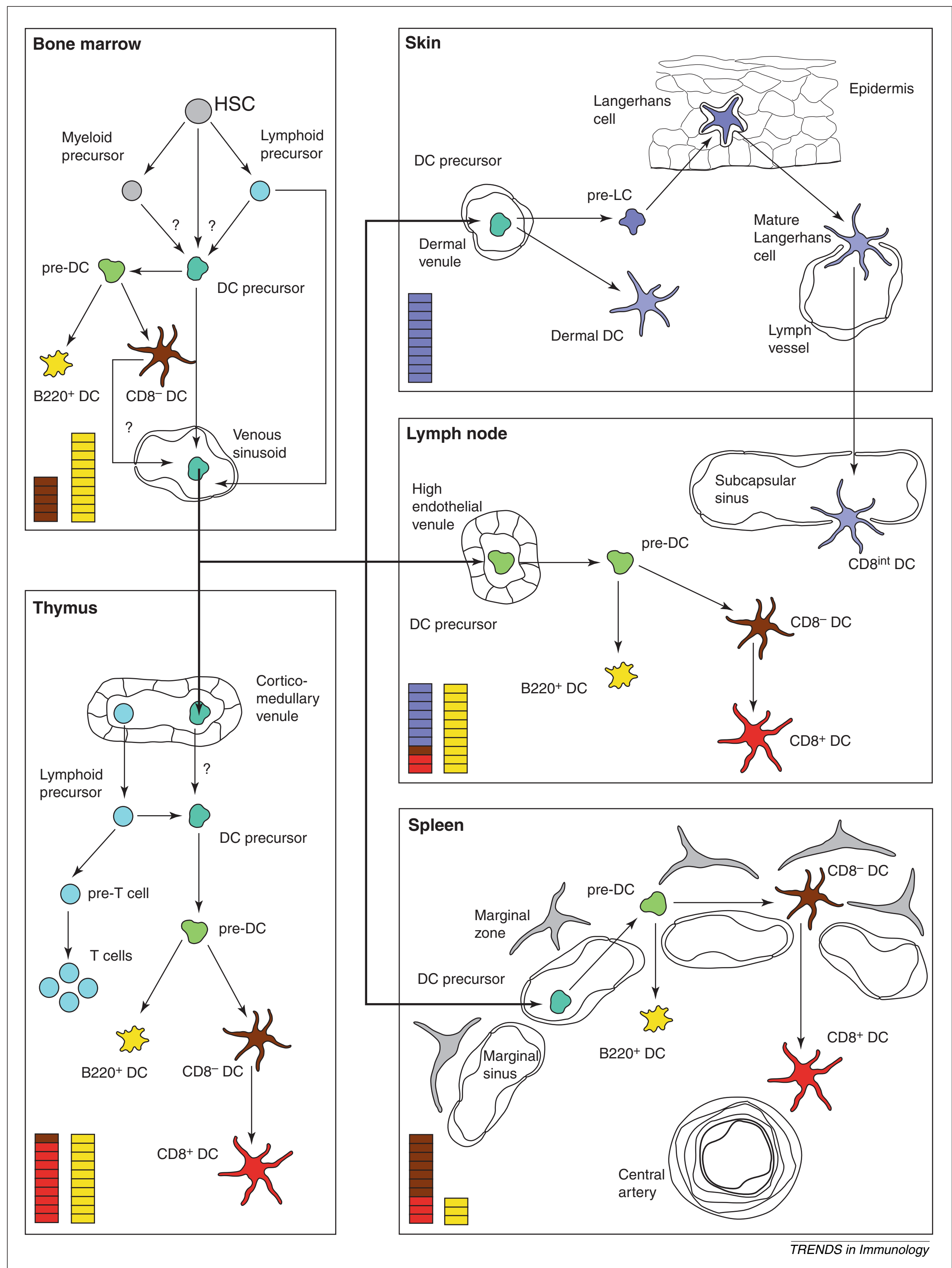

Fig. 3. A hypothetical model of mouse dendritic cell (DC) development. DC precursors, derived from either lymphoid or myeloid precursors in the bone marrow, would differentiate locally into B220$^+$ or CD8α$^-$ DCs or gain access, via venous sinusoids, to the blood stream, where both DC precursors and CD8α$^-$ DCs can be found. DC precursors then enter the skin, lymph nodes, spleen and mucosal lymphoid organs (not illustrated), via specialized blood or lymph vessels. In the skin, DC precursors generate both Langerhans cells (LCs), under the control of transforming growth factor β, and dermal DCs. Upon stimulation, LCs undergo maturation mediated by tumor necrosis factor α and migration to the peripheral lymph nodes, where they constitute the CD8^{int} DC subset. Within the lymph nodes or spleen, DC precursors differentiate into B220$^+$ and CD8α$^-$ DCs. The latter generate CD8α$^+$ DCs after completion of their differentiation program and localization in specialized T-cell areas. Thymic B220$^+$, CD8α$^-$ and CD8α$^+$ DCs differentiate following the same pathway described for lymph nodes or spleen, from either DC precursors homing to the thymus, via cortico-medullary venules, or pluripotent intrathymic lymphoid precursors. See the text for an explanation of the experimental support for this model. The relative proportion of CD8^{int} (blue), CD8α$^-$ (brown), CD8α$^+$ (red) and B220$^+$ (yellow) DC subpopulations, within each compartment, is represented in the bottom left-hand corner of each box. Abbreviation: HSC, hematopoietic stem cell.

When cultured with GM-CSF and TNF-α, CD14$^+$CD1a$^-$ intermediates generated E-cadh$^-$ mature DCs, with a dermal or lymphoid-organ DC phenotype. They could generate macrophages also when cultured with M-CSF, indicating that the CD14$^+$CD1a$^-$ pathway originated from a myeloid progenitor. By contrast, CD14$^-$CD1a$^+$ intermediates generated E-cadh$^+$langerin$^+$ LC-like DCs, by a TGF-β-independent pathway. This differentiation pathway parallels murine pathway (b) described in Figure 2. Differentiation of LCs can also be achieved from the CD11b$^-$ fraction of CD14$^+$CD1a$^-$ intermediates (Fig. 4c), by a TGF-β-dependent pathway[37], as reported for the differentiation of mouse LCs from BM

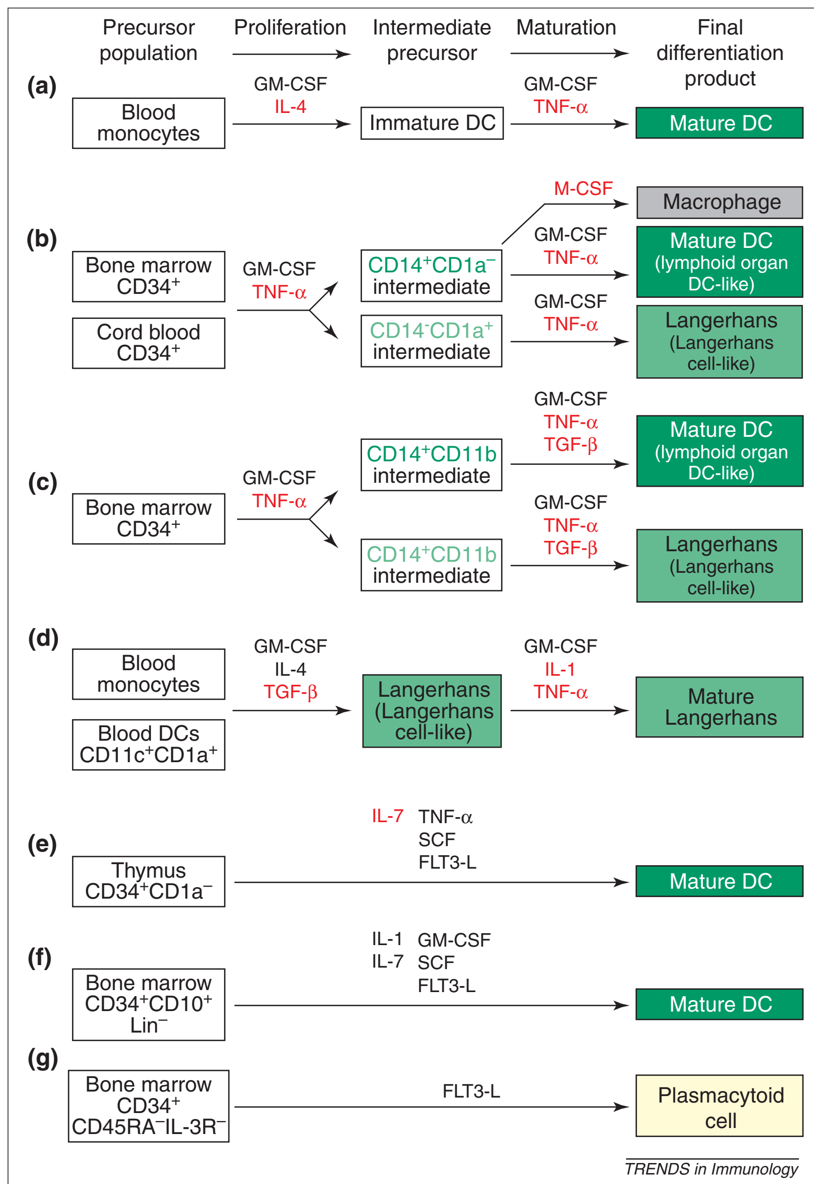

Fig. 4. Main *in vitro* differentiation pathways for dendritic cells (DCs) in human. For each differentiation pathway, precursor populations are defined in the left-hand column. The central column corresponds to the intermediate precursors or to immature DCs obtained after the first proliferative phase of differentiation [with the exception of pathways (e), (f) and (g), which describe a single-step culture method]. The final differentiation products, after the second nonproliferative maturation phase, are defined in the right-hand column. The cytokines required for each pathway are indicated; those claimed to have a decisive role in a specific step are marked in red. Evidence for pathways is supported by the following references: (a), Ref. 26; (b), Ref. 27; (c), Ref. 37; (d), Refs 38,39; (e), Ref. 11; (f), Ref. 13; (g), Ref. 40. Abbreviations: FLT3-L, fms-like tyrosine kinase 3 ligand; GM-CSF, granulocyte–macrophage colony-stimulating factor; IL, interleukin; Lin, lineage; M-CSF, macrophage colony-stimulating factor; SCF, stem cell factor; TGF-β, transforming growth factor β; TNF-α, tumor necrosis factor α.

Lin−c-kit+ precursors (Fig. 2c). Similarly, LC-like DCs can be differentiated by culturing monocytes[38] or blood CD11c+CD1a+ DCs (Ref. 39) with GM-CSF, IL-4 and TGF-β (Fig. 4d). Subsequent maturation of LCs occurred after incubation with TNF-α and IL-1. As discussed for mouse LCs, the TGF-β-independent human LC differentiation pathway might reflect a defective LC differentiation or maturation process, or the expression of LC markers by *in-vitro*-derived DCs unrelated to a physiological LC counterpart.

DC differentiation from multipotent thymic lymphoid precursors in vitro

As described for murine CD4^{low} precursors[32], DCs can be generated *in vitro* from lymphoid-committed precursors using a complex combination of cytokines. BM CD34+CD10+Lin− precursors endowed with T-, B- and NK-cell, but not myeloid, differentiation capacity, produced DCs after culture with IL-1β, IL-7, GM-CSF, SCF and FLT3L (Ref. 13) (Fig. 4f). Interestingly, CD34+CD1a− lymphoid-committed thymic precursors generated DCs after culture with IL-7, TNF-α, SCF and FLT3L (Refs 11,12) (Fig. 4e), in the absence of GM-CSF, although this cytokine improved the yield of DCs produced[11]. Therefore, mouse and human thymic precursors share a requirement for IL-7, TNF-α, SCF and FLT3L to generate DCs [pathway (e) in Figs 2,4]. GM-CSF is dispensable, in contrast with GM-CSF-dependent differentiation of DCs from BM precursors, with the exception of FLT3L-driven generation of DCs (Fig. 2d). Nevertheless, it was reported that CD34+CD1a− thymic precursors generated monocytes when cultured with M-CSF, indicating that they retained some myeloid capacity[11]. Alternatively, this result might support the theory that human thymic nonplasmacytoid, CD11c+ DCs are myeloid-derived. In this sense, a thymic CD11b+ DC subset expressing the myeloid markers M-CSFR, CD14, CD33 and CD64 was described recently[44]. Moreover, a common differentiation pathway for DCs and monocytes from thymic CD34+CD1a− precursors, branching from a CD5− intermediate, independent of the CD5+ T-cell differentiation pathway, has been identified (M.L. Toribio, pers. commun.). However, additional experiments are required to address this issue conclusively. The expression of markers considered to be lymphoid or myeloid might not be decisive evidence of DC lineage derivation, because murine CD8α− DCs, expressing the myeloid markers CD11b and F4/80, could be derived from lymphoid precursors after intravenous transfer[21,22].

Differentiation of plasmacytoid cells in vitro

Plasmacytoid cells can be generated *in vitro* from blood CD34+CD45RA−IL-3Rα− cells after culture in the presence of FLT3L (Ref. 40) (Fig. 4g). Their maturation into Ag-presenting DCs can be induced with IL-3 and CD40L (Ref. 16) or IL-3 and TNF-α (Ref. 45), whereas IL-3 and IL-4 induced apoptosis of plasmacytoid cells, indicating their differential requirements compared with DCs.

Globally, as concluded for murine DC differentiation assays, results derived from *in vitro* human DC differentiation studies have provided relevant information with regard to the cytokines controlling the development of human DCs, and could lead to the optimization of DC-mediated immunotherapy protocols, although they do not allow one to define conclusively the physiological lineage derivation of human DCs.

Control of DC differentiation

Cytokines

As mentioned previously, different cytokines are required to induce the *in vitro* differentiation of DCs from different precursors. These cytokine combinations were defined on the basis of differentiation protocols from hematopoietic precursors and complex cytokine-combination tests. Additional information concerning the involvement of cytokines in the differentiation of DCs arises from genetically deficient mice. These experimental data indicate that some cytokines appear to have a dispensable role, whereas others are strictly required for the generation of DCs. The most relevant cytokines involved in the differentiation of DCs are considered.

GM-CSF appears to be required for the *in vitro* differentiation of DCs from BM and blood progenitors, but not from thymic precursors. Nevertheless, the generation of DCs from BM Lin$^-$ cells in the absence of GM-CSF was reported[31], suggesting that its role can be fulfilled by other cytokines. In this report, the differentiation of DCs was driven only by exogenously supplied FLT3L, although it was demonstrated that endogenously produced IL-6 was required. Therefore, when ascribing a role to a defined cytokine in the differentiation of DCs, it is important to consider its involvement in driving the differentiation of DC precursors and/or inducing the production of other cytokines. Moreover, cytokines claimed to be essential *in vitro* could be dispensable *in vivo*, due to the action of replacing cytokines. In this sense, GM-CSF- or GM-CSFR-deficient mice displayed normal development of DCs (Ref. 46).

TNF-α, employed in most mouse and human DC and LC differentiation assays from BM precursors, has been proposed to be a crucial cytokine for the generation of DCs from CD34$^+$ precursors[43]. The generation of DCs from BM Lin$^-$c-kit$^+$ precursors driven by GM-CSF, SCF and TNF-α is impaired in TNFRI-deficient mice[47]. However, no defects in the differentiation of DCs have been reported in TNF-α- or TNFRI-deficient mice, suggesting that TNF-α does not play an essential role in this process. In this sense, lymphotoxin α (LTα) could achieve at least some of the activities attributed to TNF-α with regard to the development of DCs, because LTα can bind to TNFRI and TNFRII, and interestingly, LTα-deficient mice have reduced numbers of splenic DCs (Ref. 48). In addition, TNF-α could exert a regulatory role in the growth of DCs, because TNF-α has been shown to diminish granulocyte differentiation and increase the responsiveness of early progenitors to GM-CSF, by inducing expression of the GM-CSFR β chain[49]. Therefore, TNF-α appears to have a double function in the differentiation of DCs by promoting the early phases of DC differentiation and acting as a maturation stimulus in later phases.

FLT3L has been demonstrated to have a strong differentiation-promoting potential for mouse and human DCs, both *in vivo* and *in vitro*[31,50–52]. Consequently, FLT3L-deficient mice displayed important defects in the differentiation of DCs (Ref. 53). In addition, FLT3L has been claimed to increase the *in vitro* survival of DC precursors[52], selectively favor their differentiation[52] and promote the differentiation of mixed DC–macrophage colonies in combination with leukemia inhibitory factor (LIF)[4]. Interestingly, differentiation of CD8α$^+$ and CD8α$^-$ DCs *in vitro* can only be achieved in FLT3L-driven BM cultures[31], suggesting that FLT3L induces a more physiological DC differentiation process than other stimuli, such as GM-CSF and TNF-α. In conclusion, FLT3L appears to play an essential role in the differentiation of DCs under both *in vitro* and *in vivo* conditions.

IL-4 has been shown to be a key cytokine for inducing the differentiation of DCs from human monocytes, and interestingly, it has been shown that this cytokine exerts an inhibitory function on macrophage differentiation[49]. It has also been shown that IL-4 allows the generation of murine DCs from BM cells, in combination with GM-CSF (Ref. 29). Moreover, treatment of cancer patients with GM-CSF and IL-4 has been reported to enhance the number of circulating DCs (Ref. 54). However, as discussed previously, the fact that human monocyte-derived DCs could represent an unstable form of DC, which could revert to a macrophage state upon removal of cytokines, might indicate that murine DCs derived from GM-CSF- and IL-4-driven cultures could undergo a similar phenotypic and functional reversal upon cytokine withdrawal.

Although LC-like DCs can be generated *in vitro* in the absence of TGF-β, when these are compared with LCs differentiated in the presence of TGF-β, their phenotype suggests that TGF-β is required to achieve a complete and/or more physiological differentiation of LCs. Importantly, the skin of TGF-β-deficient mice is devoid of epidermal LCs (Ref. 34).

With regard to the differentiation of DCs from either mouse or human thymic early lymphoid precursors, although complex combinations of cytokines appear to be required, several reports support an essential role for IL-7 in this process[11,12,32]. The differentiation steps, in which the cytokines considered in this section appear to have a relevant function, are indicated in Figure 5 parts (a) and (b).

Transcription factors

The analysis of DC subpopulations in mice deficient for transcription factors involved in myeloid and lymphoid development was undertaken to address the derivation of DC lineages. DCs were absent in mice homozygous for a dominant-negative mutation in the *Ikaros* gene (*Ikaros* DN$^{-/-}$), which display a profound deficiency in lymphoid, but not myeloid, development[55], although epidermal LCs were generated[56]. These data could be interpreted as evidence of a lymphoid origin for DCs, compared with

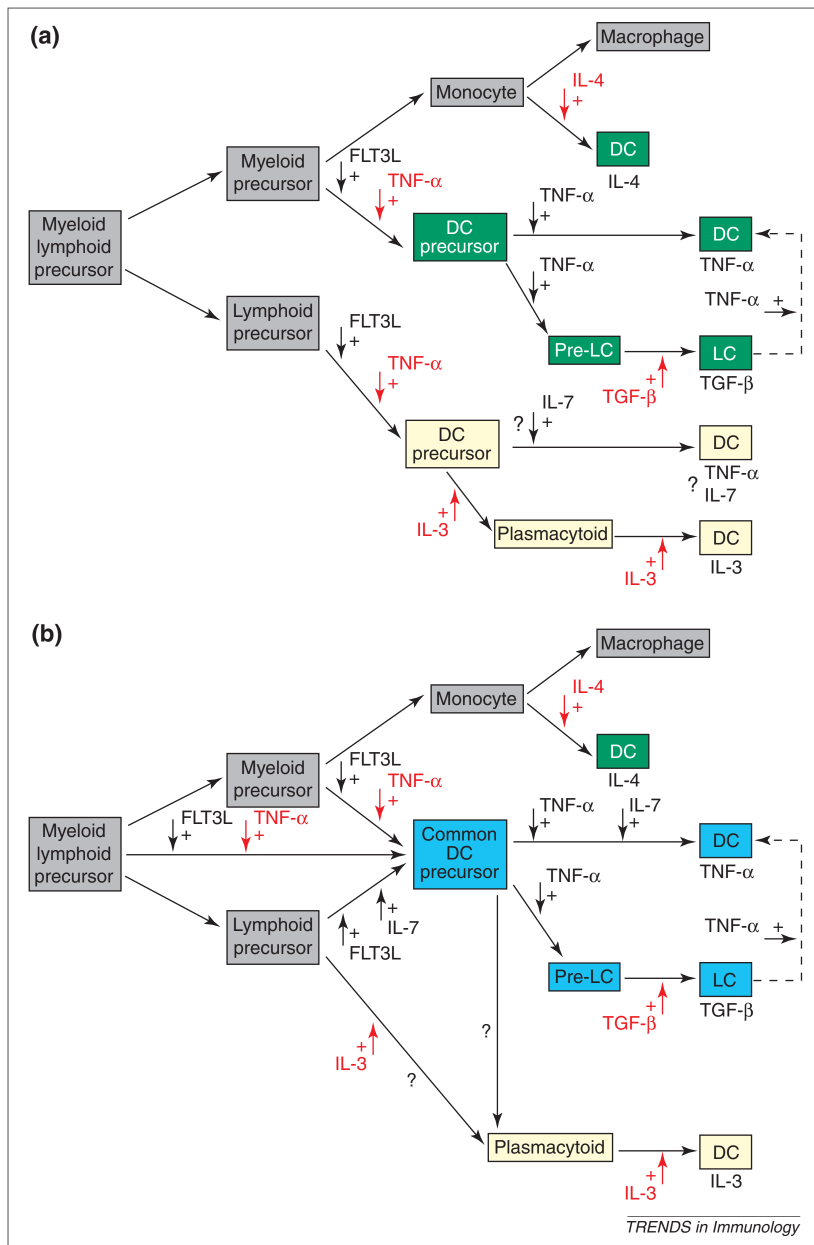

Fig. 5. Alternative models of human and mouse differentiation of dendritic cells (DCs). (a) Corresponding to the current view of DC development, this model considers the existence of two independent differentiation routes: a main myeloid DC development pathway giving rise to lymphoid organ DCs and Langerhans cells (LCs); and a secondary pathway generating lymphoid DCs, which might include thymic DCs, interstitial DCs and plasmacytoid cells. (b) This model, based on our unpublished data, proposes a common DC differentiation pathway that will generate most, if not all, DC subpopulations (with the exception of monocyte-derived DCs and, possibly, plasmacytoid cells). Below the box representing the end product of each differentiation pathway the key cytokines involved are indicated. See text for a detailed explanation. Abbreviations: FLT3-L, fms-like tyrosine kinase 3 ligand; IL, interleukin; TGF-β, transforming growth factor β; TNF-α, tumor necrosis factor α.

a myeloid origin for LCs. However, DC deficiency in these mice could reflect an altered lymphoid-organ environment, impairing the normal homing and differentiation of DC precursors. Mice with an *Ikaros* null mutation (*Ikaros* $C^{-/-}$) display less-severe defects in lymphoid development[57], having normal differentiation of CD8α$^+$ DCs, but low numbers of splenic CD8α$^-$ DCs. These data might indicate a selective defect in CD8α$^-$ DCs, as well as a developmental link between T cells and CD8α$^+$ DCs (Ref. 56). However, because B-cell differentiation is impaired severely in these mice[57], their splenic architecture, particularly of the marginal zone, is most probably disrupted; marginal-zone organization is known to be controlled by B cells[58]. Therefore, on the basis of the experimental evidence supporting the DC-differentiation model proposed in Figure 3, the low numbers of CD8α$^-$ DCs present in *Ikaros* $C^{-/-}$ mice could reflect a rapid transition of CD8α$^-$ to CD8α$^+$ DCs, owing to an altered marginal zone that does not allow the stabilization of a CD8α$^-$ DC population. A similar defect in the ratio of CD8α$^-$ to CD8α$^+$ DCs has been reported in mice deficient for PU.1 (Ref. 59) or RelB (Ref. 60), which have important defects in myelomonocytic and B-cell development (PU.1$^{-/-}$ mice), and T-cell development (RelB$^{-/-}$ mice). As discussed for *Ikaros* $C^{-/-}$ mice, the profound deficiency of B cells and macrophages in PU.1$^{-/-}$ mice and the splenomegaly of RelB$^{-/-}$ mice, with increased numbers of myeloid cells and a reduced white pulp[61], could explain the defects in the number of CD8α$^-$ DCs reported in these mice, although alternative interpretations cannot be excluded. Therefore, data derived so far from the study of DCs in mice deficient for these transcription factors do not allow us to draw definitive conclusions with regard to the lineage derivation of DCs.

Conclusions

Current information from humans and mice does not support a conclusive definition of the physiological differentiation pathways that generate DCs *in vivo*. This controversial situation originates as the consequence of experimental evidence suggesting that the *in vivo* differentiation of a particular DC subpopulation from a myeloid or lymphoid progenitor might be unrelated to its possible generation under experimental conditions.

In this sense, when analyzing the derivation of mouse DCs by transfer of precursor populations into irradiated mice, the physiological developmental potential of a defined precursor might be altered by the experimental situation, resulting in a nonphysiological differentiation behaviour owing to partial reversion of its lineage commitment. In support of this view, recent reports have demonstrated reversal of lymphoid-precursor commitment, with the acquisition of myeloid differentiation capacity[62,63]. Similarly, the *in vitro* differentiation of human DCs induced by complex combinations of cytokines could drive a defined progenitor to develop a nonphysiological differentiation potential. Moreover, this controversial situation is complicated further by the existence of bipotential lymphoid–myeloid precursors, described both in humans and mice[64–66]. Therefore, as discussed, the issue of ascribing a certain DC subset to a myeloid or lymphoid lineage remains largely unresolved. An alternative view of DC development, which would involve a common

DC differentiation pathway from either myeloid or lymphoid precursors, is supported by two important conclusions that can be drawn from the experiments described. First, in mice, a single lymphoid or myeloid progenitor population seems to be endowed with the capacity to reconstitute most, if not all, DC subpopulations, including those present in the thymus, skin and spleen, upon intravenous transfer[8,21–23,25]. Second, both in the mouse and human systems, at least certain DC subsets with a defined phenotype can be differentiated *in vitro* from either myeloid or lymphoid precursors. For example $CD1a^{+}CD11b^{+}CD33^{+}CD83^{+}$ human DCs can be generated from myeloid[27] or lymphoid[11] precursors.

Integrated human and mouse DC differentiation models

Based on the information presented, two alternative models for the differentiation of mouse and human DCs are proposed in Figure 5. Model (a), which corresponds essentially to the current view of DC development, considers the existence of two parallel, but independent, DC differentiation routes originating from myeloid or lymphoid precursors. According to this model, the main differentiation pathway, generating DCs such as those found in the dermis and lymphoid organs, as well as LCs, would be myeloid-derived. A lymphoid-derived DC precursor would generate lymphoid DCs, of which thymic DCs, interstitial DCs and plasmacytoid cells have been proposed as the most probable candidates.

An alternative model (b), which we favor, relies on the existence of a common DC differentiation pathway that generates most DC subpopulations, including lymphoid- and nonlymphoid-organ DCs and LCs, through a common DC precursor. This common DC precursor could differentiate from myeloid, lymphoid or more primitive myeloid–lymphoid progenitors, either in the BM or, alternatively, in an extramedullar location, such as the thymus. By contrast, monocytes could generate DCs by an independent route although, as discussed previously, it must be clarified whether this constitutes a pathway that occurs *in vivo*. Finally, human plasmacytoid cells would derive from a lymphoid precursor, as proposed, or from the common DC precursor. In this model, only monocyte-derived DCs and, eventually, plasmacytoid cells would genuinely represent myeloid and lymphoid DCs, respectively, with the remaining DCs and LCs not being ascribable to either the myeloid or lymphoid lineages.

Ongoing research will allow us to define conclusively the physiological mechanisms governing the development of DCs, and therefore, design alternative protocols for the use of DCs in cancer immunotherapy. In this sense, the concept that the development of DCs results essentially from the differentiation potential of a common DC progenitor, could provide new insights for the optimization of *in vitro* DC production techniques, required for DC vaccination trials to induce activation or suppression of T-cell immunity.

Acknowledgements
Our studies were funded by the European Commission (grant #QLRT-1999-00276), the Comunidad de Madrid of Spain (grant #08.1/0076/2000) and the Ministerio de Ciencia y Tecnología of Spain (grant #BOS 2000-0558).

References

1 Timmerman, J.M. and Ronald Levy, M.D. (1999) Dendritic-cell vaccines for cancer immunotherapy. *Annu. Rev. Med.* 50, 507–529
2 Banchereau, B. *et al.* (2000) Immunobiology of dendritic cells. *Annu. Rev. Immunol.* 18, 767–811
3 Steinman, R.M. and Cohn, Z.A. (1973) Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation and tissue distribution. *J. Exp. Med.* 137, 1142–1162
4 Metcalf, D. (1997) Murine hematopoietic stem cells committed to macrophage dendritic-cell formation: stimulation by Flk2 ligand with enhancement by regulators using the gp130 receptor chain. *Proc. Natl. Acad. Sci. U. S. A.* 94, 11552–11556
5 Randolph, G.J. *et al.* (1999) Differentiation of phagocytic monocytes into lymph node dendritic cells *in vivo*. *Immunity* 11, 753–761
6 Ardavín, C. (1997) Thymic dendritic cells. *Immunol. Today* 180, 350–361
7 Wu, L. *et al.* (1991) CD4 expressed on earliest T-lineage precursor cells in the adult murine thymus. *Nature* 349, 71–74
8 Ardavín, C. *et al.* (1993) Thymic dendritic cells and T cells develop simultaneously in the thymus from a common precursor population. *Nature* 362, 761–763
9 Márquez, C. *et al.* (1995) The development of T- and non-T-cell lineages from $CD34^{+}$ human thymic precursors can be traced by the differential expression of CD44. *J. Exp. Med.* 181, 475–483
10 Res, P. *et al.* (1996) $CD34^{+}CD38^{dim}$ cells in the human thymus can differentiate into T, natural killer and dendritic cells but are distinct from pluripotent stem cells. *Blood* 87, 5196–5206
11 Dalloul, A.H. *et al.* (1999) Functional and phenotypic analysis of thymic $CD34^{+}CD1a^{-}$ progenitor-derived dendritic cells: predominance of $CD1a^{+}$ differentiation pathway. *J. Immunol.* 162, 5821–5828
12 Kelly, K.A. *et al.* (2001) Development of dendritic cells in culture from human and murine thymic precursor cells. *Cell. Mol. Biol.* 47, 43–54
13 Galy, A. *et al.* (1995) Human T, B, natural killer and dendritic cells arise from a common bone marrow progenitor-cell subset. *Immunity* 3, 459–473
14 Siegal, F.P. *et al.* (1999) The nature of the principal type-1-interferon-producing cells in human blood. *Science* 284, 1835–1837
15 Cella, M. *et al.* (1999) Plasmacytoid monocytes migrate to inflamed lymph nodes and produce large amounts of type I interferon. *Nat. Med.* 5, 919–923
16 Grouard, G. *et al.* (1997) The enigmatic plasmacytoid T cells develop into dendritic cells with interleukin (IL)-3 and CD40 ligand. *J. Exp. Med.* 6, 1101–1111
17 Res, P.C.M. *et al.* (1999) Expression of pTα mRNA in a committed dendritic-cell precursor in the human thymus. *Blood* 94, 2647–2657
18 Spits, H. *et al.* (2000) Id2 and Id3 inhibit development of $CD34^{+}$ stem cells into pre-dendritic cell (pre-DC)2 but not into pre-DC1: evidence for a lymphoid origin of pre-DC2. *J. Exp. Med.* 192, 1775–1783
19 Olweus, J. *et al.* (1997) Dendritic-cell ontogeny: a human dendritic-cell lineage of myeloid origin. *Proc. Natl. Acad. Sci. U. S. A.* 94, 12551–12556
20 Wu, L. *et al.* (1996) Thymic dendritic-cell precursors: relationship to the T-lymphocyte lineage and phenotype of the dendritic-cell progeny. *J. Exp. Med.* 184, 903–911
21 Martín, P. *et al.* (2000) Concept of lymphoid versus myeloid dendritic-cell lineages revisited: both $CD8\alpha^{-}$ and $CD8\alpha^{+}$ dendritic cells are generated from $CD4^{low}$ lymphoid-committed precursors. *Blood* 96, 2511–2519
22 Traver, D. *et al.* (2000) Development of $CD8\alpha^{+}$ dendritic cells from a common myeloid progenitor. *Science* 290, 2152–2154
23 Manz, M.G. *et al.* (2001) Dendritic-cell potentials of early lymphoid and myeloid progenitors. *Blood* 97, 3333–3341
24 Zhang, Y. *et al.* (1999) Transforming growth factor β1 polarizes murine hematopoietic progenitor cells to generate Langerhans-cell-like dendritic cells through a monocyte/macrophage differentiation pathway. *Blood* 93, 1208–1220
25 Anjuère, F. *et al.* (2000) Langerhans cells develop from a lymphoid-committed precursor. *Blood* 96, 1633–1637
26 Sallusto, F. and Lanzavecchia, A. (1994) Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte–macrophage colony-stimulating factor plus interleukin-4 and down-regulated by

tumor necrosis factor α. *J. Exp. Med.* 179, 1109–1118
27 Caux, C. *et al.* (1996) CD34+ hematopoietic progenitors from human cord blood differentiate along two independent dendritic-cell pathways in response to GM-CSF and TNF-α. *J. Exp. Med.* 184, 695–706
28 Zhang, Y. *et al.* (1998) Bifurcated dendritic-cell differentiation *in vitro* from murine lineage phenotype-negative *c-kit*+ bone marrow hematopoietic progenitor cells. *Blood* 92, 118–128
29 Mayordomo, J.I. *et al.* (1995) Bone-marrow-derived dendritic cells pulsed with synthetic peptides elicit protective and therapeutic antitumour immunity. *Nat. Med.* 1, 1297–1302
30 Schreurs, M.W.J. *et al.* (1999) Generation and functional characterization of mouse monocyte-derived dendritic cells. *Eur. J. Immunol.* 29, 2835–2841
31 Brasel, K. *et al.* (2000) Generation of murine dendritic cells from flt3-ligand-supplemented bone-marrow cultures. *Blood* 96, 3029–3039
32 Saunders, D. *et al.* (1996) Dendritic-cell development in culture from thymic precursor cells in the absence of granulocyte–macrophage colony-stimulating factor. *J. Exp. Med.* 184, 2185–2196
33 Anjuère, F. *et al.* (1999) Definition of dendritic-cell subpopulations present in the spleen, Peyer's patches, lymph nodes and skin of the mouse. *Blood* 93, 590–598
34 Borkowski, T.A. *et al.* (1996) A role for endogenous transforming growth factor β1 in Langerhans-cell biology: the skin of transforming growth factor β1 null mice is devoid of epidermal Langerhans cells. *J. Exp. Med.* 184, 2417–2422
35 Merad, M. *et al.* (2000) Differentiation of myeloid dendritic cells into CD8α+ dendritic cells *in vivo*. *Blood* 96, 1865–1872
36 Lu, L. *et al.* (2001) Liver-derived DEC205+B220+CD19− dendritic cells regulate T-cell responses. *J. Immunol.* 166, 7042–7052
37 Jaksits, S. *et al.* (1999) CD34+ cell-derived CD14+ precursor cells develop into Langerhans cells in a TGF-β1-dependent manner. *J. Immunol.* 163, 4869–4877
38 Geissmann, F. *et al.* (1998) Transforming growth factor β1 in the presence of granulocyte–macrophage colony-stimulating factor and interleukin-4, induces differentiation of human peripheral blood monocytes into dendritic Langerhans cells. *J. Exp. Med.* 187, 961–966
39 Ito, T. *et al.* (1999) A CD1a+/CD11c+ subset of human blood dendritic cells is a direct precursor of Langerhans cells. *J. Immunol.* 163, 1409–1419
40 Blom, B. *et al.* (2000) Generation of interferon-α-producing pre-dendritic cell (pre-DC)2 from human CD34+ hematopoietic stem cells. *J. Exp. Med.* 192, 1785–1795
41 Romani, N. *et al.* (1996) Generation of mature dendritic cells from human blood. An improved method with special regard to clinical applicability. *J. Immunol. Methods* 196, 137–151
42 Nelson, E.L. *et al.* (1999) Cycling of human dendritic-cell effector phenotypes in response to TNF-α: modification of the current 'maturation' paradigm and implications for *in vivo* immunoregulation. *FASEB J.* 13, 2021–2030
43 Caux, C. *et al.* (1992) GM-CSF and TNF-α cooperate in the generation of dendritic Langerhans cells. *Nature* 360, 258–261
44 Vandenabeele, S. *et al.* (2001) Human thymus contains two distinct dendritic-cell populations. *Blood* 97, 1733–1741
45 Kohrgruber, N. *et al.* (1999) Survival, maturation and function of CD11c− and CD11c+ peripheral-blood dendritic cells are differentially regulated by cytokines. *J. Immunol.* 163, 3250–3259
46 Vremec, D. *et al.* (1997) The influence of granulocyte–macrophage colony-stimulating factor on dendritic-cell levels in mouse lymphoid organs. *Eur. J. Immunol.* 27, 40–44
47 Zhang, Y. *et al.* (1997) Induction of dendritic-cell differentiation by granulocyte–macrophage colony-stimulating factor, stem cell factor and tumor necrosis factor α *in vitro* from lineage-phenotypes-negative c-kit+ murine hematopoietic progenitor cells. *Blood* 90, 4842–4853
48 Wu, Q. *et al.* (1999) The requirement of membrane lymphotoxin for the presence of dendritic cells in lymphoid tissues. *J. Exp. Med.* 190, 629–638
49 Romani, N. *et al.* (1994) Proliferating dendritic-cell progenitors in human blood. *J. Exp. Med.* 180, 83–93
50 Maraskovsky, E. *et al.* (1996) Dramatic increase in the numbers of functionally mature dendritic cells in Flt3-ligand-treated mice: multiple dendritic-cell subpopulations identified. *J. Exp. Med.* 184, 1953–1962
51 Maraskovsky, E. *et al.* (2000) Generation of human dendritic-cell subsets by Flt3 ligand. *Blood* 96, 878–884
52 Curti, A. *et al.* (2001) Stem cell factor and Flt3 ligand are strictly required to sustain the long-term expansion of primitive CD34+DR− dendritic-cell precursors. *J. Immunol.* 166, 848–854
53 McKenna, H.J. *et al.* (2000) Mice lacking Flt3 ligand have deficient hematopoiesis affecting hematopoietic progenitor cells, dendritic cells and natural killer cells. *Blood* 95, 3489–3497
54 Roth, M.D. *et al.* (2001) Granulocyte–macrophage colony-stimulating factor and interleukin-4 enhance the number and antigen-presenting activity of circulating CD14+ and CD83+ cells in cancer patients. *Cancer Res.* 60, 1934–1941
55 Georgopoulos, K. *et al.* (1994) The Ikaros gene is required for the development of all lymphoid lineages. *Cell* 79, 143–156
56 Wu, L. *et al.* (1997) Cell-autonomous defects in dendritic-cell populations of Ikaros mutant mice point to a developmental relationship with the lymphoid lineage. *Immunity* 7, 483–492
57 Wang, J.H. *et al.* (1996) Selective defects in the development of the fetal and adult lymphoid system in mice with an Ikaros null mutation. *Immunity* 5, 537–549
58 Kraal, G. (1992) Cells in the marginal zone of the spleen. *Int. Rev. Cytol.* 132, 31–74
59 Guerriero, A. *et al.* (2000) PU.1 is required for myeloid-derived but not lymphoid-derived dendritic cells. *Blood* 95, 879–885
60 Wu, L. *et al.* (1998) RelB is essential for the development of myeloid-related CD8α− dendritic cells but not of lymphoid-related CD8α+ dendritic cells. *Immunity* 9, 839–847
61 Weih, F. *et al.* (1995) Multiorgan inflammation and hematopoietic abnormalities in mice with a targeted disruption of RelB, a member of the NF-κB/Rel family. *Cell* 80, 331–340
62 Rolink, A.G. *et al.* (1999) Long-term *in vivo* reconstitution of T-cell development by Pax5-deficient B-cell progenitors. *Nature* 401, 603–606
63 Kondo, M. *et al.* (2000) Cell-fate conversion of lymphoid-committed progenitors by instructive actions of cytokines. *Nature* 407, 383–386
64 Hao, Q.L. *et al.* (1998) *In vitro* identification of single CD34+CD38− cells with both lymphoid and myeloid potential. *Blood* 91, 4145–4151
65 Mebius, R.E. *et al.* (2001) The fetal liver counterpart of adult common lymphoid progenitors gives rise to all lymphoid lineages, CD45+CD4+CD3− cells, as well as macrophages. *J. Immunol.* 166, 6593–6601
66 Montecino-Rodríguez, E. *et al.* (2001) Bipotential B-macrophage progenitors are present in adult bone marrow. *Nat. Immunol.* 2, 83–88
67 Martinez del Hoyo, G. *et al.* CD8α+ dendritic cells originate from the CD8α− dendritic cell subset by a maturation process involving CD8α, DEC-205 and CD24 upregulation. *Blood* (in press)

Searching *Trends in Immunology*

We have not printed author, subject or book indexes for this volume. *Trends in Immunology* is hosted online by **BioMedNet** and the full text of all issues can be accessed at **http://immunology.trends.com**, which provides an extensive browsing and searching facility.

Search facilities allow you to:

- **view the latest issue**
- **browse previous issues by volume and issue number**
- **search the journal for words found anywhere in the article, and by authors or publication date**
- **jump quickly to your required volume or page number**