Origin, maturation and antigen presenting function of dendritic cells

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Dendritic cells are cells specialized for antigen capture, migration and T cell stimulation. Recent advances have been made in understanding their origin, their heterogeneity, the mechanism of antigen uptake, and the signals that induce their migration and maturation into immunostimulatory antigen-presenting cells. Dendritic cells represent the natural adjuvants for T cell responses and their therapeutic exploitation in the near future is foreseen.

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Abbreviations

APC	antigen presenting cell
BrDU	5-bromo-2′-deoxyuridine
CD40L	CD40 ligand
CTL	cytotoxic T lymphocyte
DC	dendritic cell
FcR	Fc receptor
GM-CSF	granulocyte-macrophage colony-stimulating factor
IL	interleukin
L	ligand
LPS	lipopolysaccharide
M-CSF	macrophage colony-stimulating factor
TAP	transporter associated with antigen processing
TCR	T-cell receptor
Th	T helper
TNF	tumor necrosis factor

Introduction

Dendritic cells (DCs) are bone marrow derived cells that function as professional antigen presenting cells. DC progenitors are seeded through the blood into nonlymphoid tissues, where they develop to a stage referred to as immature DCs. These immature DCs are characterized by a high capability for antigen capture and processing, but low T cell stimulatory capability. Inflammatory mediators promote DC maturation and migration out of nonlymphoid tissues into the blood or afferent lymph. These migratory cells reach secondary lymphoid organs where they home to the T cell areas. At this stage the cells (referred to as mature DCs) have undergone a dramatic change in their properties: they have lost the ability to capture antigen and have acquired an increased capacity to stimulate T cells. Mature DCs therefore present to naive T cells antigen that has been captured at the level of peripheral tissues and so can be viewed as the sentinels of the immune system [1]. We will review recent progress in understanding the origin and heterogeneity, the mechanisms of antigen uptake, the nature of the maturation process and, finally, possible therapeutic exploitation of DCs.

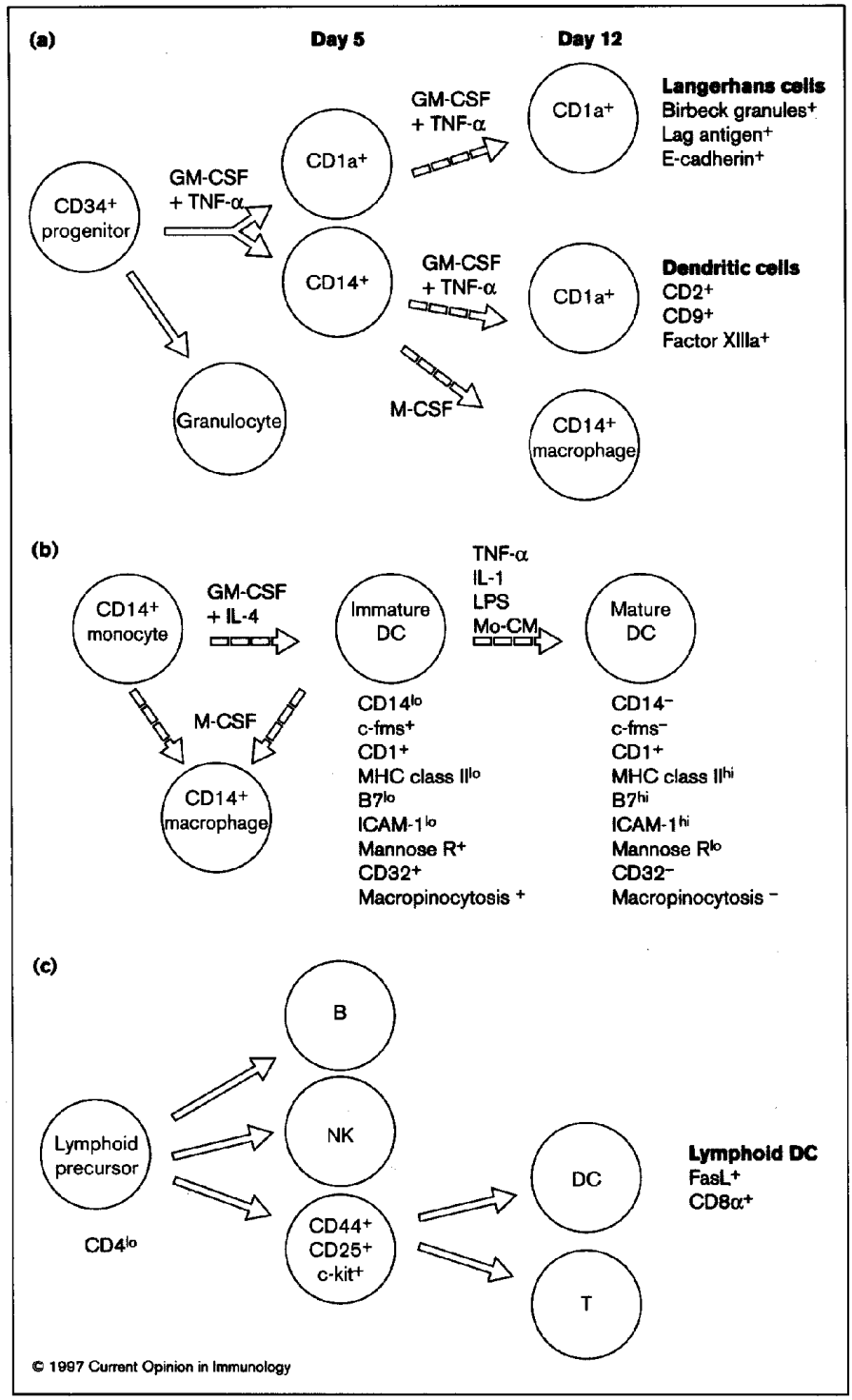
Origin and differentiation of DCs

There is evidence for a common progenitor for DCs and myeloid cells that can be expanded in cultures supplemented with GM-CSF. In the mouse, proliferating precursors are present in the bone marrow and peripheral blood and generate DCs, granulocytes and macrophages [2–4]. Human cord blood CD34+ hematopoietic progenitors cultured with GM-CSF and TNF-α generate a mixed cell population containing different types of DCs (Fig. 1a) [5,6•]. At early time points (day five of culture) two subsets of DC precursors can be identified by the mutually exclusive expression of CD1a and CD14. Both subsets mature at day 12 into typical DCs. CD1a+ precursors generate cells that express Birbeck granules, the Lag antigen and E-cadherin, markers that are characteristic of epidermal Langerhans cells. In contrast, the CD14+ progenitors mature into CD14−, CD1a+ DCs lacking Birbeck granules, E-cadherin, and Lag antigen but expressing CD2, CD9, CD68, and factor XIIIa; markers characteristic of dermal dendritic cells. Interestingly, the CD14+ precursors, but not the CD1a+ precursors, are still bipotent cells because they can differentiate into macrophages in response to M-CSF [6•,7].

DCs can also develop from CD14+ peripheral blood monocytes cultured with GM-CSF and IL-4 (Fig. 1b) [8]. Under these culture conditions monocytes develop into a homogeneous population of DCs without dividing, as shown by the lack of BrDU incorporation, and the efficiency of this process is close to 100% (M Cella, unpublished data). These cells have the characteristics of immature DCs and can be further induced to mature by inflammatory stimuli such as TNF-α, IL-1 or LPS [8] or by monocyte conditioned medium [9•]. This notion has now been supported by several studies [9•,10,11,12•,13•]. It is interesting that immature DCs generated from monocytes still retain the M-CSF receptor, although they lose it following induction of maturation [9•,13•]. Thus the emerging concept is that monocytes represent an abundant source of precursors that can polarize towards DCs or macrophages, depending on the external stimuli. This polarization can be driven *in vitro* by the addition of appropriate cytokines (GM-CSF plus IL-4 or M-CSF).

There is also evidence for a lymphoid DC precursor (Fig. 1c); a cell capable of generating both lymphocytes as well as DCs has been identified in the mouse thymus

Figure 1



Origin and maturation of DCs. Solid arrows indicate differentiation with cell proliferation. Dashed arrows indicate differentiation without proliferation. A minus sign (−) indicates negative, and a + sign indicates positive. **(a)** Hematopoietic progenitors cultured in GM-CSF and TNF-α proliferate and generate two distinct precursors at day five. The first is CD1a+ and differentiates into Langerhans cells at day 12. The second is CD14+ and differentiates into either DCs or macrophages, depending upon the influence of different cytokines. The original myeloid precursors can also generate granulocytes. **(b)** Peripheral blood monocytes cultured in GM-CSF and IL-4 develop into immature DCs without proliferating. TNF-α, IL-1, LPS or monocyte-conditioned medium (Mo-CM) induces maturation with loss of antigen capturing capacity and increased T cell stimulatory capacity. Monocytes and immature DCs express c-fms (M-CSF receptor) and can develop into macrophages under the influence of M-CSF. Peripheral blood monocytes may therefore correspond to the CD14+ cells in (a) that can generate DCs and macrophages. ICAM, intercellular adhesion molecule; R, receptor. **(c)** A common lymphoid precursor can generate DCs, T, B and natural killer (NK) cells. Lymphoid DCs may have different functional properties compared with those of myeloid DCs; for instance, they express FasL.

and human thymus [14,15] and in human bone marrow [16]. The murine lymphoid DCs express CD8α and other lymphoid markers. Interestingly, the lymphoid DCs develop in the absence of GM-CSF [17•].

An intriguing possibility that is now being actively investigated is that the different lineages of DCs may perform different functions. It has been shown that CD8+ lymphoid DCs, but not CD8− DCs, may exert a suppressive effect on T cells as they express FasL and can induce apoptosis in responding T cells [18•]. In addition, among the DCs generated from CD34+ progenitors using GM-CSF and TNF-α, only the monocyte derived DCs can support B cell proliferation and differentiation into

IgM secreting cells ([6•]; C Caux, J Banchereau, personal communication).

An important question is which cytokines or signals are responsible for DC development *in vivo*. The cytokines that support the generation of DCs *in vitro* are GM-CSF, IL-4 and TNF-α. Addition of the cytokines c-KitL or Flt3L to the culture medium can increase the yield of DCs generated from bone marrow precursors [19,20] or from progenitors mobilized in peripheral blood [21]. Surprisingly, GM-CSF does not appear to be a major growth factor for DCs *in vivo* because mice that overexpress this cytokine do not have increased numbers of DCs [22]. Interestingly, injection of Flt3L into mice results in a dramatic increase in both lymphoid and myeloid DCs, suggesting that this cytokine can be used to expand DC populations *in vivo* [23•].

Antigen capture by immature DCs

DCs can efficiently internalize a diverse array of antigens for processing and loading onto MHC class II molecules. Immature DCs are characterized by high level of endocytic activity which is lost upon maturation (reviewed in [24]). The mechanisms of antigen uptake have been studied on monocyte derived DCs generated in culture with GM-CSF and IL-4 [25]. These cells have a very high level of constitutive macropinocytosis that allows them to take up large volumes of fluid and then to concentrate the macrosolutes. In addition, they express the low-affinity receptor for Fcγ, FcγRII, and the mannose receptor. These receptors allow efficient capture of IgG immune complexes [8] and mannosylated antigens [25] respectively. The mannose receptor contains multiple carbohydrate-binding domains and mediates endocytosis or phagocytosis of a variety of antigens that expose mannose or fucose residues [26]. Unlike the Fc receptors and membrane Ig, which are degraded together with the bound antigen, the mannose receptor releases its cargo at endosomal pH and recycles to the cell surface. It therefore allows internalization of ligands in successive rounds, providing a sustained capacity for antigen capture. In DCs the mannose receptor does not colocalize with MHC class II molecules, but efficiently delivers antigens for processing and class II restricted presentation, and recycles back to the plasma membrane. Indeed, mannosylated proteins are presented with 100-fold higher efficiency than unglycosylated proteins by dendritic cells (AJ Engering *et al.*, personal communication; A Tan *et al.*, personal communication). Mannosylation thus represents an effective way to target antigens to DCs *in vivo*.

Other receptors may contribute to the ability of DCs to capture exogenous antigens. These receptors include lectins such as DEC-205 [27], and CD23 [28], the FcεRI [29,30] and possibly two new receptors homologous to FcαR that are selectively expressed on monocytes and DCs (Marco Colonna, personal communication).

It is known that professional APCs can capture exogenous antigens for presentation on MHC class I molecules [31]. This function is particularly important for *in vivo* priming of CTL responses to antigens that are not synthesized by professional APCs [32]. Immature DCs are phagocytic, although less than macrophages [33], and have a high level of constitutive macropinocytosis [25]. Interestingly, phagocytosis and macropinocytosis have been shown to deliver exogenous antigens into the cytosol for processing and presentation on class I molecules by the classical proteasome and TAP-dependent pathway [34–36]. When compared to macrophages, DCs were found to be much more efficient in presenting a soluble protein antigen on class I molecules (C Watts, personal communication) possibly because of the high constitutive level of macropinocytosis.

The influence of DC maturation on antigen capture, processing and presentation

Work in the early 1990s established that fresh Langerhans cells upon *in vitro* culture lose antigen capturing and processing ability, MHC class II molecule synthesis and acidic organelles and mature into immunostimulatory DCs [37–39]. These changes parallel those occurring *in vivo* when Langerhans cells migrate from the epidermis to the draining lymph nodes. There is growing evidence that inflammatory cytokines and bacterial products can stimulate DC maturation and migration. Indeed, systemic administration of TNF-α, IL-1 or LPS induces depletion of DCs from nonlymphoid organs and migration into lymph nodes [40,41•], suggesting that these stimuli might be responsible for DC maturation *in vivo*.

The induction of DC maturation and its effect on antigen capture and presentation have been studied *in vitro*. DCs generated from monocytes using GM-CSF and IL-4 have the properties of immature DCs, since they have high endocytic activity but low T cell stimulatory capacity [8,25]. These cells have relatively low levels of surface MHC class II molecules and these are rapidly internalized and recycle through a large intracellular pool. The high level of class II internalization and recycling may be relevant for the function of immature DCs, as it has been shown that recycling class II molecules can bind peptides generated in the early endosomal compartment [42]. TNF-α, IL-1 and LPS added to immature DCs induce a coordinate series of changes resulting in the loss of endocytic activity, upregulation of adhesion and costimulatory molecules and redistribution of class II molecules [25]. In maturing DCs the level of surface class II molecules increases up to fourfold within 24 hours as a consequence of reduced internalization and increased biosynthesis. In this way DCs can load many antigenic peptides rapidly following exposure to the inflammatory stimulus, thereby favoring presentation of infectious antigens (M Cella, A Engering, V Pinet, J Pieters, A Lanzavecchia, unpublished data).

The signal transduction pathways that control DC maturation are still poorly characterized but we anticipate that this will become an important area of investigation. Ceramide is produced by DCs stimulated by TNF-α, IL-1 and CD40L, and can transiently inhibit endocytosis [43]. What role this pathway may play in the induction of DC maturation remains to be established.

Recruitment and migration

The ability of DCs to be recruited to sites of antigen challenge and travel to secondary lymphoid organs is an essential function of this professional APC [44]. Recruitment of DCs from blood to tissues can be observed in the lung and the liver but not in other districts and several pathways of recruitment are known depending on the method of antigen challenge. Following inhalation of bacteria, DC precursors are rapidly recruited into the airway epithelium where they develop into typical DCs that subsequently migrate to the regional lymph nodes [45]. After intravenous injection of inert particles, particle-laden cells can be detected in the hepatic lymph [46•]. These cells represent recently produced immature DCs, most likely monocytes, that are recruited to the hepatic sinusoids by phagocytosing Kupffer cells and manifest a temporary phagocytic activity for intravascular particles. Such phagocytic activity is subsequently downregulated when these cells translocate from the sinusoidal area to the hepatic lymph. After intratracheal injection of a protein antigen, antigen-loaded DCs are found in the draining lymph nodes [47]. Antigens present in the intestinal lumen are taken up by specialized epithelial cells (M cells) present in the epithelium overlying the dome region of Peyer's patches [48]. Immature DCs are strategically located below the M cells and have been shown to capture incoming antigens *in vivo* [49•,50•]. It has been suggested that these immature DCs migrate to the T cell areas of the same Peyer's patches or mesenteric lymph nodes where they present antigen to naive T cells. Recently it has been shown that a subset of DCs distinct from follicular DCs can be found in germinal centers. These cells are $CD4^+$, $CD11c^+$, $CD1a^-$, $CD3^-$, have low endocytic and phagocytic capacity and are highly stimulatory for T but not for B cells. It has been suggested that they may be derived from blood $CD4^+$ $CD11c^+$ DCs and may play an important role in activation of germinal center T cell [51•].

While the pathways of DC migration are relatively well characterized, the molecular mechanisms that control recruitment and migration of DCs are far less defined. It is possible that certain combinations of chemokines and cytokines may effectively recruit DC precursors (possibly monocytes) from the blood stream; DCs, macrophages and monocytes respond to different sets of chemokines [52]. In addition to chemokines, adhesion molecules are likely to control DC migration from peripheral tissues to lymph nodes. Firstly, Langerhans cells that migrate out of the skin downregulate E-cadherin and so lose their interaction with the surrounding keratinocytes [53]. Secondly, CD44 expression on DCs can be modulated by external stimuli that induce migration, resulting in increased expression of CD44 splicing variants that have been implicated in metastatic dissemination [8,54]. Interestingly, the maturation process results in a dramatic rearrangement of the cytoskeleton and increased motility that may promote cell migration [55•].

Interaction between DCs and T cells

The high capacity of mature DCs to stimulate T cells has been attributed to a variety of factors. Low DC surface levels of sialic acid may decrease repulsive forces and facilitate clustering with T cells [56] and high DC levels of adhesion molecules may favor TCR engagement [57]. In addition, high expression levels of costimulatory molecules on DCs facilitate T cell activation by lowering the activation threshold [58].

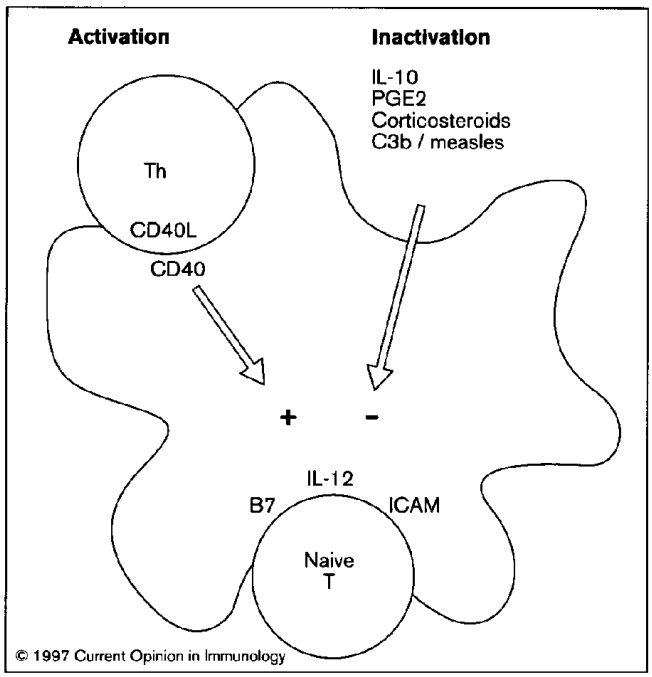
While the DC–T cell interaction has been traditionally viewed as a one way interaction, recent data suggest that T cells may play an important role in activating DCs thus further enhancing the T cell stimulatory capacity of the DCs (Fig. 2). Indeed, ligation of CD40 has been shown to increase DC viability [59,60] and to induce DC maturation [8]. DCs can produce IL-12, a key cytokine for the generation of Th1 responses [61•] and CD40L is a selective and powerful inducer of IL-12 production in DCs [62•,63•]. The fact that DCs are likely to deliver IL-12 to T cells in a cognate fashion makes this source of IL-12 particularly effective.

Several reports described suppression of DC function (see Fig. 2). IL-10 can induce apoptosis in DCs [60] and decrease T cell stimulatory capacity [64] as well as IL-12 production [63•]. Glucocorticoids can decrease DC viability as well as the expression of costimulatory molecules [65]. Prostaglandin E2 decreases IL-12 while increasing IL-10 production, thus promoting generation of Th2 responses [66]. Interestingly, cross-linking of the complement receptor CD46 by measles virus or by the natural ligand C3b inhibits IL-12 production by human monocytes and DCs, thus providing a plausible mechanism for virus induced immunosuppression and Th2 polarization ([67]; C Caux, personal communication).

Therapeutic applications

The availability of large numbers of DCs has opened up new possibilities for immunization, particularly with respect to the generation of CTL responses to tumor antigens (reviewed in [68]). Several studies in experimental animal models have shown that this approach is effective. Mice immunized with DCs pulsed with synthetic peptides [69•,70•], acid-eluted peptides from tumor cell lines [71•] and even intact soluble proteins [72•] develop protective CTL responses against tumors. Using peptide-pulsed DCs, CTL responses to tumor antigens can be induced from peripheral blood lymphocytes of healthy donors [73•,74•]. As an alternative to peptides, DCs can be

Figure 2



Priming of naive T cells by DCs is regulated by stimulatory and inhibitory signals. Effector Th cells can activate DCs via the CD40L–CD40 interaction, thus increasing the expression of intercellular adhesion molecules (ICAMs) and costimulatory (B7) molecules and inducing the production of IL-12. This activation process facilitates priming and Th1 polarization of naive T cells clustered around the same DC. The inhibitory stimuli decrease DC viability, IL-12 production and stimulatory capacity and so favor Th2 responses.

transfected with the relevant genes using expression vectors, naked DNA or RNA [75•]. In humans the use of DCs as adjuvants will be tested in pilot immunogenicity studies (R Steinman, personal communication). It will be important to establish the best source of DCs, the best conditions for pulsing, whether maturation should be induced *in vitro* before injection of the cells into the patient and, finally, whether the addition of T helper epitopes may further increase DC immunogenicity by promoting DC activation by memory Th cells [62•]. Clinical trials in melanoma patients have been started using DCs generated with GM-CSF and IL-4 from the patients' peripheral blood monocytes (F Nestle, personal communication).

Although the working principles of DCs are increasingly understood we still need more information on the molecular mechanisms of DC function in order to be able to fully exploit their therapeutic potential. At present, this area of research has entered the exciting stage where knowledge can be applied *in vivo* and, in some cases, in promising clinical trials. It truly appears that the long-term goal of using DCs for the immunotherapy of human diseases may be achieved in the not too distant future.

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