

REVIEW

Dendritic cell immunobiology and potential roles in immunotherapy

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Abstract

Owing to the importance of dendritic cells (DC) in the induction and control of immunity, an understanding of their biology is central to the development of potent immunotherapies for cancer, chronic infections, autoimmune disease, and induction of transplantation tolerance. This review surveys the heterogeneity of DC with regards to their phenotype and developmental origin, and how they initiate, modify and regulate the immune response, with emphasis on their maturation, migration, antigen-presentation and interaction with T cells and other immune cells. Much of this knowledge is obtained through research on murine DC. Research on human DC has been hampered by limitations associated with *in vitro* assays and limited access to human tissues. New approaches on human DC research are required in order to develop novel strategies for the treatment of microbial infections, the control of graft rejection, and the improvement of DC-based immunotherapeutic protocols for autoimmunity, allergy, and cancer.

Key words: dendritic cell, immunobiology, tolerance, immunotherapy, immunity, antigen presentation.

INTRODUCTION

The past decade has been marked by spectacular progress towards understanding how dendritic cells (DC) control the immune system, arising from an intensive network dealing with a large diversity of DC research fields. These include (i) the differentiation pathways of DC, (ii) the discovery of C-type lectin receptors for antigen capture, (iii) the control of DC activation by toll like receptors (TLR), (iv) the description of the mechanisms underlying the processing and presentation of antigens derived from pathogens, apoptotic cells and tumour cells, (v) the process of DC maturation that involves regulation of cytokine, chemokine, homing, and adhesion receptor expression, cytokine/chemokine secretion and costimulatory signals, (vi) the organisation of the DC-T cell immune synapse, (vii) the role of DC in polarising T_h cell responses, (viii) the control of immunity vs tolerance by DC, (ix) the *in vivo* analysis of DC traffic/migration, (x) the regulation of B and NK cell responses, and finally, (xi) the role of DC in microbial infections, autoimmune disorders, allergic reactions, graft

rejection, and anti-tumour immune response.¹ These advances in DC immunobiology, together with the improvement of DC transfection and *in vitro* differentiation techniques, have allowed the development of promising DC-based vaccination and cancer immunotherapy protocols.

DENDRITIC CELL IMMUNOBIOLOGY

Antigen-presenting cells (APC) are critical for antigen-specific priming of T cells. DC are the most efficient APC in the immune system and are often termed “professional” APC because they are the only APC capable of stimulating resting naïve T cells *in vitro* and *in vivo*. The other “non-professional” APC (B cells, monocytes) can only stimulate experienced (activated or memory) T cells.²

DC are generated from either myeloid or lymphoid bone marrow (BM) precursors into immature DC with high phagocytic capacity.² After antigen capture in the presence of activation signals with inflammation or infection, immature DC are activated through TLR, and/or members of the tumour necrosis factor-receptor (TNF-

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R) family and undergo a complex maturation process. *In vivo* this process is paralleled by migration of DC to T cell-rich areas of lymphoid organs, where DC present the processed antigens to naïve T cells and induce antigen-specific immune responses. In contrast, antigen capture in the absence of activation stimuli may lead to the induction of T cell tolerance, as a result of antigen presentation by immature DC. In addition, mature DC can induce NK cell activation and B cell differentiation into antibody (Ab) forming cells. Therefore, DC serve as an essential link between innate and adaptive immune responses. Targeting the translation of innate to adaptive information by DC offers a new perspective for manipulating the immune system for clinical benefit.¹

The role of DC in immune regulation ranges from tolerance induction and the prevention of autoimmunity to the induction of anti-tumour immunity and the protection against infectious diseases.^{1,3} Therefore, a better understanding of DC immunobiology will provide essential insights into the manipulation of the immune system and consequently, the development of novel strategies for the treatment of microbial infections, the control of graft rejection, and the improvement of DC-based immunotherapeutic protocols for autoimmunity, allergy and cancer.

DEFINITION OF DENDRITIC CELLS

The definition, identification and characterisation of DC have been difficult because of considerable morphological and phenotypic heterogeneity.

Morphologically, DC are large cells with elongated, stellate processes (dendrites) that can give a veiled appearance. Mature DC extend long processes, up to 10 μm in length, in many directions from the cell body. In the living state, these processes are sheet-like “veils” or lamellipodia which are actively motile.² The characteristic morphology of different forms of human DC is illustrated in Fig. 1.

The combination of several antigens is generally used to define DC immunophenotype. DC lack cell surface antigens associated with hematopoietic lineages including B cells (CD19, CD20), T cells (CD3), NK cells (CD56), monocytes (CD14), granulocytes (CD15) and stem cells (CD34) but express high levels of MHC class I and class II antigens.⁴ Thus, immunophenotypically DC are defined as lineage-negative (lin^-) DR^+ cells. Among $\text{lin}^- \text{DR}^+$

cells, phenotypically different DC subsets are defined. Fig. 2 shows a gating strategy for identifying myeloid and plasmacytoid dendritic cells (MDC and PDC) subsets among $\text{lin}^- \text{DR}^+$ cells in adult peripheral blood (PB). Antigens widely used to define PB MDC and PDC are considered below.

HETEROGENEITY OF DC SUBSETS

Although DC are broadly defined as $\text{lin}^- \text{DR}^+$ cells, significant heterogeneity in this cell population is apparent. This heterogeneity is due to the existence of distinct lineages, and/or distinct maturation and functional stages. However, lineage origin, maturation and functional stages of the various DC populations have not been fully elucidated. For identification purposes, DC subpopulations can be classified according to their anatomical location and phenotype. In mice, so far, five distinct subtypes have been identified in lymph nodes (LN)⁵ and 3 subsets in the spleen (SP).⁶ In humans, there are relatively few studies describing DC from tissues,⁷ however, distinct DC subsets have been identified in PB.

Defining the cellular heterogeneity of DC subsets will provide a basis for the comparison of their preparations, methodology and results from different laboratories. Importantly, this effort will enable standardisation of the human peripheral blood DC subpopulations used for future molecular, functional and possibly therapeutic purposes.

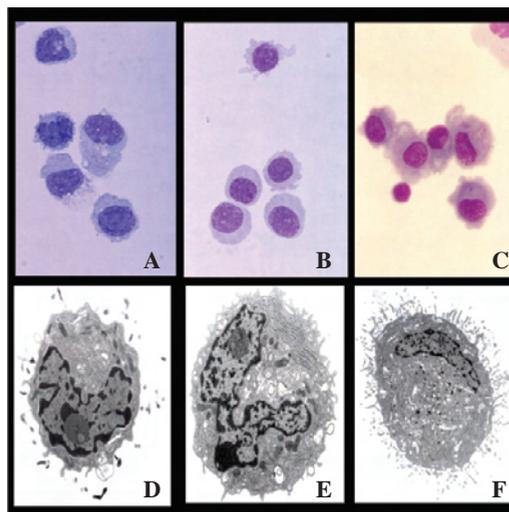


FIG. 1: Human DC morphology. PB-CD11c⁺ MDC (A, D), PB-CD123⁺ PDC (B, E) and Monocyte-derived DC (Mo-DC) generated in GM-CSF/IL-4 (C, F). Original magnifications, x 1433 (A – C; May-Grünwald-Giemsa), x 17000 (D – F; EM).

Human DC subsets

1. Peripheral Blood DC (PB-DC)

PB-DC is at present the main readily available source of DC in humans, representing 0.5–1.5% of PBMC.² So far, two main PB-DC subsets have been characterised, based on their reciprocal expression of CD11c and CD123 antigens (Fig. 2).⁸

CD11c⁺CD123^{low/+} DC, also referred to as MDC are characterised by monocytoïd morphology, express myeloid antigens (CD13, CD33) and CD45RO, and possess antigen uptake and allogeneic mixed lymphocyte reaction (MLR) capacities. They are able to secrete IL-12 in response to bacterial stimuli.⁹ These CD11c⁺ MDC include the CD16⁺, BDCA-1⁺ and BDCA-3⁺ subpopulations.¹⁰ The CD16⁺ cells comprise a large but variable (40–80%) proportion of the PB-MDC populations, express low levels of CD33, heterogeneous levels of HLA-DR and high levels of CD86 and CD40 antigen. CD16⁺ MDC survive poorly in tissue culture, with only 50% of cells remaining viable after 12 to 18 hours even in the presence of granulocyte macrophage-colony stimulating factor (GM-CSF) and IL-3.¹⁰ BDCA-1⁺ cells represent 20–50% of the PB-MDC populations, and express high levels of CD11c, HLA-DR and CD33 antigen. The BDCA-3 subpopulation is much smaller and accounts for 2–3% of PB-MDC.

BDCA-3⁺ cells express low levels of CD86 but high levels of CD40 antigen.¹⁰

CD123⁺CD11c⁻ DC also referred to as PDC or lymphoid DC resemble plasma cells morphologically, lack myeloid antigens (CD13, CD33), and express high levels of IL-3R α , BDCA-2, BDCA-4, CD4, L-selectin (CD62L), pre-T cell receptor α chain and immunoglobulin (Ig)- λ -like transcript.¹¹ They are characterised by modest antigen presenting potentials, and an extraordinary capacity for IFN- α production in response to CpG,¹² certain viruses and CD40L.¹³ PDC depend on IL-3 for their survival and differentiation into mature DC with typical dendritic morphology and potent T cell stimulatory function.¹⁴ Costimulatory, C-type lectins (CD205 and CD209), and TLR are also expressed differentially on these distinct PB-DC subsets (Table 1).

2. Epithelial and interstitial DC

In peripheral tissues, Langerhans' cell (LC) and interstitial DC are well-established DC subtypes. LC, also called epidermal DC, are present only in epithelial tissues (epidermis, mucosa), whereas interstitial DC have been identified in the interstitium of most tissues including the dermis of the skin. LC and interstitial DC can activate CD4⁺ and CD8⁺ naïve T cells and secrete IL-12.¹⁵

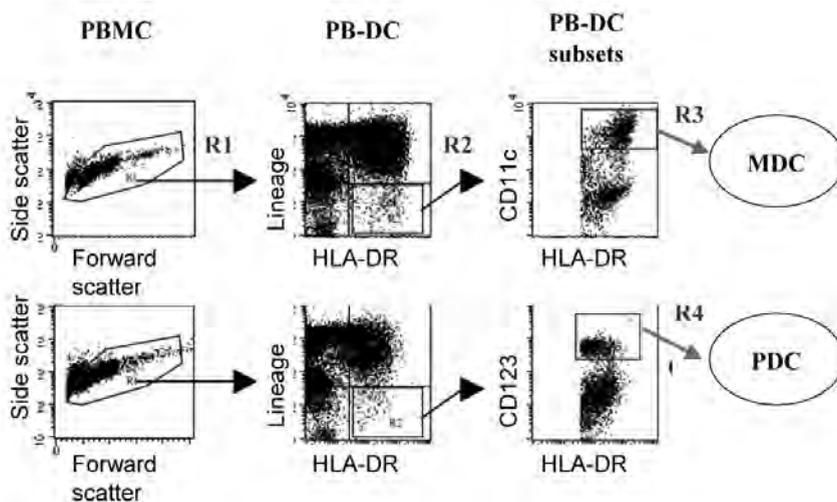


FIG. 2: Identification of PB-DC subsets by flow cytometry.

Peripheral blood mononuclear cells (PBMC) are isolated from healthy donors by Ficoll density gradient centrifugation. Region R1 is created in a dot plot of forward scatter (FSC) vs side scatter (SSC) to include viable mononuclear cells (MNC) and to exclude cell debris. Events from R1 are displayed in a dot plot of lineage (CD3, CD14, CD15, CD19, CD20, CD34, CD56) vs HLA-DR. Region R2 is created to include all lin-DR⁺ cells. Lin-DR⁺ cells are displayed in a dot plot of HLA-DR vs CD11c or in a dot plot of HLA-DR vs CD123. Region R3 is created to include all CD11c⁺ cells (MDC) (upper row) and region R4 is created to include all CD123⁺ cells (PDC) (bottom row).

TABLE 1: Phenotypic and functional properties of human PB-MDC and PB-PDC.

Properties	PB-MDC	PB-PDC
<i>Defining antigens</i>	HLA DR ^{high}	HLA DR ⁺
	CD11c⁺	CD11c⁻
	CD123 ^{low}	CD123^{high}
	CD33⁺	CD33⁻
	CD4 ^{low}	CD4 ^{high}
	CD1a ⁻	CD1a ⁻
	CD45RO⁺	CD45RO ⁻
	CD45RA ⁻	CD45RA^{high}
	CD16 ^{+*}	CD16 ⁻
	BDCA-1 ^{+*}	BDCA-2⁺
BDCA-3 ^{+*}	BDCA-4⁺	
<i>Costimulatory antigens</i>	CD40 ⁺	CD40 ⁻
	CD80 ⁻	CD80 ⁻
	CD86 ⁺	CD86 ⁻
<i>C-type lectins</i>	CD205 ⁺	CD205 ^{low}
	DC-SIGN/CD209 ⁺	DC-SIGN/CD209 ⁻
<i>TLR</i>	TLR 1, 2, 3, 4	TLR 7, 9
<i>Function</i>	Stimulate strong allogenic MLR	No or weak allogeneic MLR
	Stimulate strong primary T response	Stimulate specific primary T response
	Produce IL-12 upon microbial stimulation	Produce IFN- α upon microbial stimulation
<i>Viability</i>	Modest <i>in vitro</i> viability	Poor <i>in vitro</i> viability

* Subset positive.

Characteristic surface antigens that help to identify distinct PB-MDC and PB-PDC are listed, with the most relevant antigens for each subset highlighted (in bold).

Adapted with modification from ^{1,2,3,4,10}

3. Thymic DC

Thymic PDC represent the most abundant subsets and share common features with PDC found in PB and secondary lymphoid organs.¹⁶ The CD11c⁺ immature DC share similar properties with CD11c⁺ DC isolated from PB or from tonsillar germinal centres (GC). GC-DC present antigen to both T and B cells and may directly contribute to GC formation. CD11c⁺ mature interdigitating DC represent < 7% of thymic DC, and they exhibit all the properties of mature MDC, including the expression of DC-LAMP and CCR7, and production of IL-12.

4. Splenic, nodal and tonsillar DC

Cells with DC-like qualities have been identified

in the B cell-rich follicles, follicular mantle zone and T cell-rich zones of lymphoid organs. They represent 0.5 – 1% of the total germinal cell population in human tonsils, SP and LN.¹⁴ Most (about 80%) SP-DC express CD11c, while a minority of activated CD86⁺ DC are found in the T cell zone.¹⁷ Five phenotypically distinct DC subsets have been identified within human tonsils and notably only a small proportion were activated DC. The relationship of these DC subsets to human PB-DC subsets is unclear. However, DC resembling PB-MDC have been found in the GC.¹⁸ DC resembling PB-PDC have been detected in the T cell-rich zones of the lymphoid tissues.¹⁴ This indicates that PB-MDC and PB-PDC are in the traffic on their way to lymphoid organs.

5. Bone marrow-DC (BM-DC)

About 1% of the cells in freshly isolated human bone marrow mononuclear cells have qualities similar to freshly isolated PB-DC.¹⁹ Recent reports^{20,21} showed for the first time that murine BM harbours DC that capture, process and present blood-borne antigen to naïve CD4⁺ and CD8⁺ T cells, resulting in primary immune responses. The antigen specific responses generated in the BM occur in the absence of secondary lymphoid organs (SP, LN and Peyer's patches). The responses are not tolerogenic and result in generation of cytotoxic T cells, protective anti-tumour immunity and immunological memory. These findings suggest that BM, similar to secondary lymphoid organs contain a microenvironment that allows cognate interactions between DC and circulating antigen-specific T cells, leading to induction of primary and memory CD8⁺ T cell responses.

DC HEMATOPOIESIS

1. Origin and differentiation of DC

Despite extensive research on DC biology over the past decade, driven by the possibility of exploiting the potential of DC as APC in vaccination and immunotherapeutic cancer trials, their origin remains largely unknown and represents a particularly controversial issue in current immunology. Because various populations of DC in mice or humans are able to induce distinct types of immune responses, it is important to determine the role of their origin in determining functional heterogeneity. An important question is how the heterogeneity arises at the developmental level.

The expression of antigens considered to be lymphoid or myeloid might not be the decisive evidence of DC lineage derivation, because murine CD8 α ⁻ DC, expressing the myeloid antigens CD11b and F4/80, could be derived from lymphoid precursors after intravenous transfer.²² Moreover, recent studies showed that all murine DC subsets could be generated along both myeloid and lymphoid pathways.²³ In humans, DC that express myeloid antigens could be generated *in vitro* from both myeloid²⁴ or lymphoid precursors.²⁵ Although under defined experimental conditions, DC can be generated from myeloid and lymphoid precursors, human DC differentiation pathway(s) *in vivo* remain unknown. Defining the physiological mechanisms governing the development of DC

could provide new insights for the optimisation of *in vitro* DC production protocols.

The current view on human DC hematopoiesis is summarised in Fig. 3.

2. Myeloid-derived DC

Evidence for a myeloid DC lineage derives from human *in vitro* DC differentiation assays in which DC were generated from monocytes or intermediate myeloid precursors that retained the capacity to generate macrophages.²⁴ Murine studies have shown the presence of a common myeloid precursor, which can give rise to DC and granulocyte-macrophage precursors.²³ It has been proposed that myeloid interstitial DC may derive from circulating DC or monocytic precursors. Human monocytes cultured in the presence of GM-CSF and IL-4 differentiate into monocyte-derived DC (Mo-DC).²⁶

3. Lymphoid-derived DC

A lymphoid DC lineage was proposed based on the evidence that DC could be derived from early T cell precursors within the murine thymus²⁷ or from mouse CD19⁺ pro-B cells in cultures.²⁸ The existence of lymphoid-related DC development in humans was primarily obtained from *in vitro* studies (Fig. 4). DC can be generated *in vitro* from lymphoid committed precursors using a complex combination of cytokines. CD34⁺CD1a⁻ lymphoid-committed thymic precursors generated CD1a⁺ DC after culture with IL-7, TNF- α , Stem Cell Factor (SCF) and Flt3L, in the absence of GM-CSF, although this cytokine improved the yield of DC produced (Fig. 4c).²⁹ BM CD34⁺CD38⁻CD10⁺lin⁻ precursors endowed with T-, B- and NK-cells, but not myeloid, differentiation capacity, produced DC after culture with IL-1, IL-7, GM-CSF, SCF and Flt3L in another study (Fig. 4d).²⁵ Clonal analysis demonstrated that single CB CD34⁺CD38⁻CD7⁺ cells could generate DC, B, and NK but were devoid of myeloid or erythroid potential.²⁵ Collectively, these data suggest that the DC lineage is developmentally more closely related to lymphoid lineage than to myeloid lineage. Humans PDC have been proposed to be lymphoid-derived owing to their (i) dependence on IL-3 but not GM-CSF,¹⁴ (ii) expression of pT α , a molecule which upon assembly with a T cell receptor (TCR) β chain forms a pre-TCR³⁰ and the (iii) block in the differentiation of T cells, B cells and PDC, but not myeloid cells, from CD34 foetal liver precursors transfected with inhibitors of DNA binding (Id)-2 and Id-3.¹¹

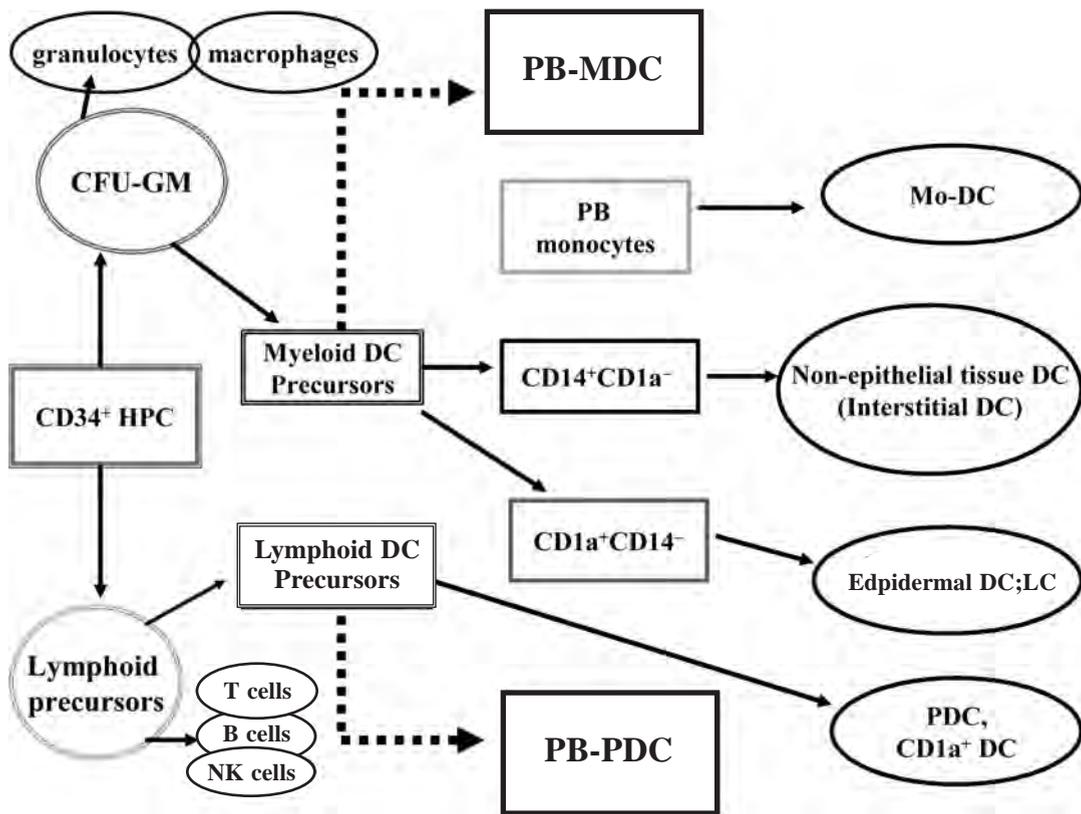


FIG. 3: Current proposal of human DC haematopoiesis.

DC are produced from precursors derived from the haematopoietic progenitor cells (CD34⁺ HPC). Recent experimental data (derived from *in vitro* studies) suggest that human DC subsets can be generated from discrete lymphoid and myeloid precursors. The myeloid DC model suggests 2 main differentiation pathways that generate human DC from myeloid precursors, leading either to the CD14⁺CD1a⁻ DC (non-epithelial tissue DC; interstitial DC) or CD1a⁺CD14⁻ DC (epidermal DC; LC)²⁴. PB monocytes could differentiate into DC like cells (immature Mo-DC) that have many of the functional features of myeloid DC. Lymphoid precursors can give rise to PDC³⁰ and CD1a⁺ DC²⁵. Dotted line indicates our proposed differentiation pathway for the development of PB-MDC and PB-PDC.

4. *In vitro* development of human DC

Methods to isolate human DC from PB are time consuming and the yield is low.³¹ Alternatively, large numbers of human DC can be generated using *in vitro* culture systems. Cultured cells are found to be as active as DC purified from other sources. *In vitro*-derived DC have provided major insights into the human DC developmental pathway (summarised in Fig. 4). In addition, the ability to generate DC *in vitro* from CD34⁺ cells obtained from BM, CB or mPB in the presence of cytokines enables large-scale generation of DC for therapeutic purposes. Loading DC with antigen *ex vivo* prior to immunisation makes it possible to control DC maturation and deliver a broad spectrum of tumour antigens to DC.

DC loaded with antigen in co-culture with T cells enables stimulation of T cells away from the tumour microenvironment, which might be immunosuppressive.

Two main protocols to generate DC, from either PB monocytes or CD34⁺ cells have been described, and generally involve a first differentiation phase followed by a maturation step. DC derived in these cultures generally have the phenotype of LC but not PB-DC. However, a recent study showed that PDC could be generated *in vitro* from BM, CB and foetal liver CD34⁺CD45RA⁻IL-3Rα⁻ cells after culture in the presence of Flt3L (Fig. 4e).³² Their maturation into antigen-presenting DC can be induced with IL-3 and CD40L¹⁴ or IL-3 and TNF-α,³³ whereas IL-4 induced apoptosis of PDC. In contrast,

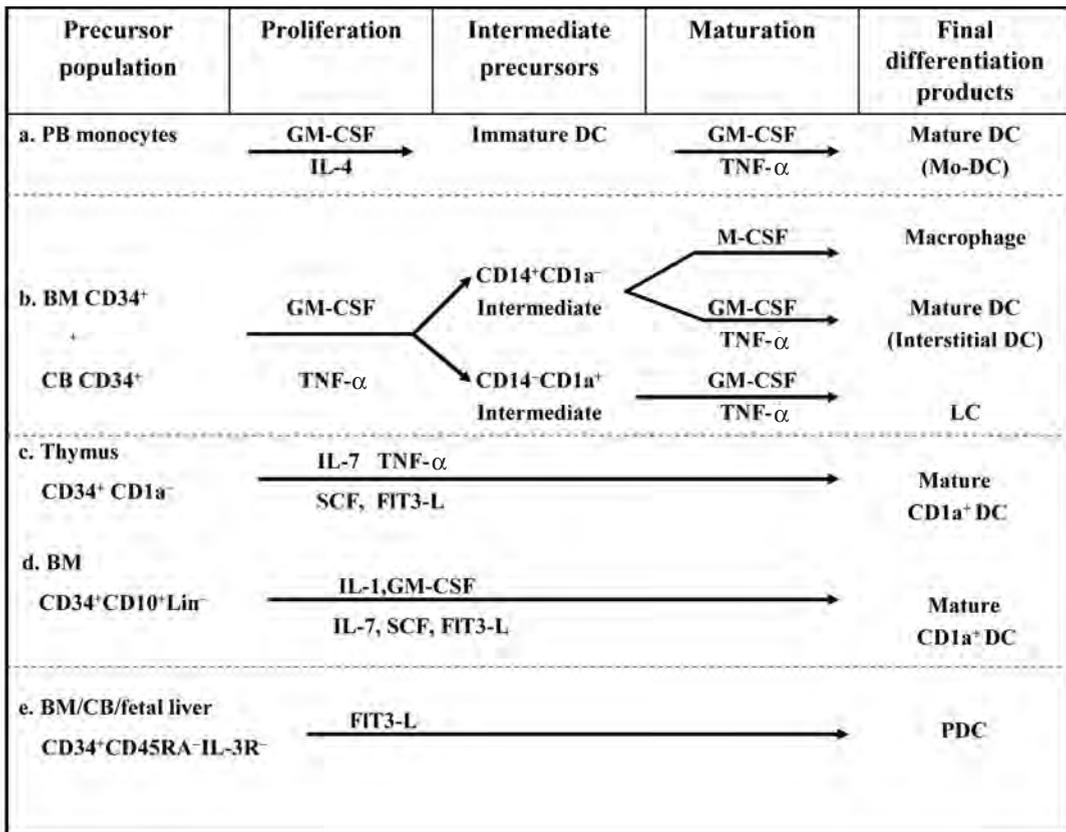


FIG 4: Main *in vitro* development of human DC.

For each differentiation pathway, precursor populations are defined in the left-hand column. The central column corresponds to the intermediate precursors or to immature DC obtained after the first proliferative phase of differentiation [with the exception of pathways (e), which describe a single-step culture method]. The final differentiation products (blue, myeloid-derived; green, lymphoid-derived), after the second non-proliferative 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)²⁶; (b)²⁴; (c)²⁹; (d)²⁵; (e)³².

generation of PB-MDC like cells from CD34⁺ cells has not been reported.

We have developed an *in vitro* model for the production of MDC from cord blood CD34⁺ stem cells. We showed for the first time that myeloid and lymphoid precursors obtained from cord blood CD34⁺ stem cells have the potential to develop progeny with the phenotype and functional features of MDC found in adult PB. Establishment of conditions that enable human BDC generation from CD34⁺ cells would provide a previously unexplored way to study the origin and differentiation of human BDC. Importantly, as myeloid BDC are promising candidates for DC vaccine, their successful production provides an alternative source of DC for immunotherapy (Fadilah SAW, Thesis, 2005).³⁴

THE LIFE CYCLE OF DC

The life cycle of DC is a complex process, consisting of antigen uptake and presentation, DC maturation and activation by TLR, and DC migration (Fig. 5). DC activation and subsequent migration regulate the types of T cell responses, notably immunity or tolerance.

Certain chemokines, adhesion molecules and costimulatory molecules orchestrate the maturation and migration of DC. Adhesion and costimulatory molecules are crucial for the cellular interactions that DC undergo during their journey from BM through blood into peripheral organs and subsequently lymphoid tissues. Chemokines produced by DC within the LN attract naïve T cells, enabling maximal presentation of MHC-peptide complexes.

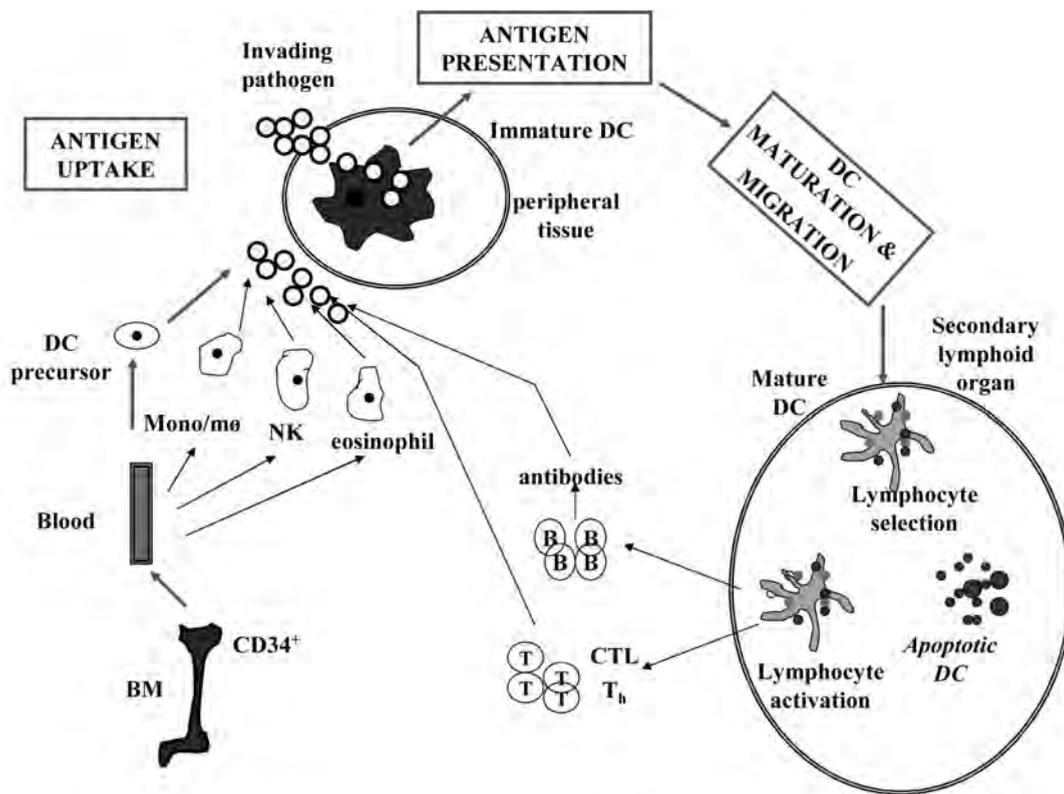


FIG. 5: The life cycle of human DC.

The life cycle of DC is a complex process, consisting of antigen uptake and presentation, DC maturation and activation by TLR, and DC migration. DC activation and subsequent migration regulate the types of T cell responses. DC can contribute to the expansion and differentiation of most classes of lymphocytes; not just T cells, but also B cells and NK cells.

Understanding the life cycle of DC is critical to their appropriate clinical use.

1. Antigen uptake

DC reside as sentinels in peripheral tissues in an immature state, characterised by a high ability of antigen uptake and processing. They possess several mechanisms that enable them to capture antigen including (i) fluid-phase macropinocytosis; (ii) receptor-mediated endocytosis (Fcγ-receptors, for antigen-antibody complexes,⁴ C-type lectin receptors,³⁵ mannose receptors,³⁶ DC-SIGN (CD209),³⁷ for glycoproteins and TLR for microbial antigen,³⁸ and (iii) phagocytosis of apoptotic cell fragments, viruses, bacteria or intracellular parasites.

DC have an array of cell surface receptors for antigen uptake CD205,³⁹ CD209,³⁷ Fcγ receptor,⁴ αVβ5 integrin⁴⁰ and CD36⁴⁰. Many of them also function in signalling or cell-cell interactions. For example, CD209 (DC-SIGN) also binds adhesion molecules (ICAM-2 and

ICAM-3) that are important in DC trafficking and cell-cell interactions.³⁷ Antigens targeted to CD205 and mannose receptors are processed by DC for presentation by both MHC class I and II molecule.⁴¹

In vivo targeting of ovalbumin conjugated with CD205-specific antibodies to CD205 induces tolerance, whereas co-injection of agonistic anti-CD40 antibody reverses the outcome from tolerance to prolonged T cell activation and immunity.^{39,42} These experiments elegantly demonstrate that under steady state conditions *in vivo*, DC have the capacity to induce peripheral T cell unresponsiveness, but a robust immune response is induced to the same antigen in the presence of danger signals. Therefore, CD205 provides an efficient receptor-based mechanism for DC to process proteins for MHC class I presentation *in vivo*, leading to tolerance in the steady state and immunity after DC maturation. In addition, *in vivo* targeting of tumour-specific antigen to DC, by exploiting DC specific surface

receptors such as CD205 could potentially replace vaccination with *ex vivo*-generated, antigen loaded DC.

2. Antigen presentation

Major Histocompatibility Complex (MHC) molecules are fundamental in T cell activation, as they bind and present peptide to T cells. MHC class I molecules (HLA-A, -B and -C) are expressed on most nucleated cells but expression varies between cell types. MHC class II molecules (HLA-DP, -DQ, and -DR) are expressed on professional APC, such as DC and B cells. Mature DC express high levels of MHC class I and II molecules that allow efficient antigen presentation.

DC process captured exogenous proteins into peptides, and load them onto MHC class I and II molecules, and these peptide-MHC complexes (pMHC I and II) are transported to the cell surface for recognition by antigen-specific T cells (pMHC I and pMHC II are recognized by CD8⁺ and CD4⁺ T cells, respectively).⁴³ Antigens acquired endogenously (i.e., synthesized within the DC cytosol) are loaded onto MHC I molecules. Processing of endogenous proteins is through a cytosolic pathway that involves ubiquitination, degradation by proteasomes, transport by TAP (transporters for antigen presentation) into the endoplasmic reticulum and loading onto MHC class I molecules. In contrast, exogenously acquired antigens are typically degraded in endosomes/lysosomes, where the peptides are loaded onto MHC II following degradation of the MHC II-associated invariant chain (Ii, which blocks access to the peptide-binding pocket of MHC II). An alternative pathway also exists whereby DC load processed exogenous antigens onto MHC I. This pathway, called “cross-presentation,” permits DC to elicit CD8⁺ T cell responses to exogenous antigens such as apoptotic or necrotic tumour cells, virus-infected cells, and immune complexes.⁴⁴ Cross-presentation is linked to specific DC antigen uptake receptors, which may be targeted in strategies to load exogenous antigens onto both MHC I and II molecules. Cross-presentation provides the immune system with an important mechanism for generating immunity to viruses and tumours.⁴⁵

3. DC maturation

DC maturation is a continuous process initiated in the periphery upon antigen encounter and/or

inflammatory cytokines and completed during the DC-T cell interaction in the lymphoid organs.³ The process of maturation transforms DC from cells specialized for antigen capture into cells specialized for T cell stimulation. Maturation is characterised by reduced antigen uptake, the development of cytoplasmic extensions or “veils” (Fig. 1), migration to lymphoid tissues, and enhanced T cell activation potential. Mature DC develop an enhanced ability to form pMHC II⁴⁶ and pMHC I⁴³ and deliver signals that can also induce cross-presentation.⁴⁵

Mature DC express a number of characteristic antigens, including CD83, a cell surface molecule involved in CD4⁺ T cell development and cell-cell interactions and DC-LAMP, a DC specific lysosomal protein.⁴⁷ Table 2 summarises the important features of immature DC and mature DC.

DC maturation is initiated by antigens (components of pathogens) or by host molecules associated with inflammation or tissue injury. These stimuli are often collectively referred to as “danger signals”.⁴⁸ Maturation stimuli act on DC through receptors, including receptors for host-derived inflammatory molecules such as CD40L, TNF- α , IL-1, and IFN- α and TLR for microbial products.³⁸ Understanding the mechanism of DC activation through TLR triggering may improve strategies for DC-based immunotherapy.

4. DC activation through ligation of TLR

There are 9 known TLR so far, each recognizing different pathogen-associated molecular patterns (PAMP). TLR expression varies amongst different human DC subsets, reflecting functional specialisation between DC subsets. In humans, PB-MDC express TLR 1,2,3,4,5,7 and 8, while PB-PDC express TLR 1, 7, and 9.^{50,51} Some TLR are expressed on the cell surface, whereas others such as TLR 3, 7, 8, and 9 are found within endosomes. After ligation of TLR by PAMP, a signal-transduction cascade is induced, and nuclear factor- κ B (NF- κ B) and MAP kinase are activated resulting in costimulatory and adhesion molecules upregulation and cytokine production. The distinct signalling pathways triggered can induce different patterns of DC activation and consequently different T cell responses.⁵² TLR agonists, therefore, can be used to target DC activation to induce the desired T cell responses. Recent data show that targeting specific DC subsets using TLR ligands can enhance their ability to stimulate antigen-specific CD4⁺ and

TABLE 2: Essential differences between immature and mature DC.

Features	Immature DC	Mature DC
HLA-class I, class II molecules	+ (surface expression), +++ (intracellular expression)	+++ (surface expression)
<i>Costimulatory/activation molecules</i>		
CD86 (B7.2),CD40	+ (low)	+++ (high)
CD80(B7.1)	-	++
CD83	-	++
CMRF-44	-	+
CMRF-56	-	+
<i>Chemokine receptors</i>		
CCR1, CCR5, CCR6	+++	+
CCR7	+	+++
<i>Adhesion molecules</i>		
CD54, CD58	+	+++
<i>Motility</i>	+	++
<i>Endocytosis</i>	++	+/-
<i>T cell stimulation</i>	+	+++
<i>Cytokines</i>	Maturation inhibited by IL-10	Abundant IL-12 production, resistance to IL-10

*Adapted with modification from.*⁴⁹

CD8⁺ T cell responses,⁵³ providing rationales for the use of TLR ligands as adjuvants for immune modulating therapy. Commonly used TLR ligands to stimulate human DC include poly[I:C] (a TLR3 ligand)⁵⁴ and CpG-rich DNA motif (a TLR9 ligand).⁵⁵ Poly[I:C] triggers the maturation of Mo-DC into a phenotype that strongly supports the development of T_h1 cells.⁵⁶ Importantly, in contrast to other maturation stimuli (*e.g.* cytokines), treatment of Mo-DC with poly[I:C] results in mature Mo-DC with stable phenotype.⁵⁷ Poly[I:C] has been used in various clinical trials with little or no toxicity.^{58,59}

5. DC migration

Unlike T cells, most types of DC, with the possible exception of PDC, do not seem to enter LN directly from the blood.⁶⁰ Instead, they gain access from the afferent lymphatic vessels that drain peripheral tissues, rendering this migratory pathway a key event in the induction of immune responses. The capacity of DC to migrate to the sites of inflammation and

subsequently migrate to the local LN is regulated by the expression of different chemokines and chemokine receptors.

MIP-3 α (CCL19) appears to be the most potent chemokine regulating DC precursor migration to tissues.⁶¹ Upon initial stimulation in tissues, immature DC produce inflammatory chemokines (MIP-1 α , MIP-1 β , MCP-2, MCP-4, RANTES, TECK, and stromal cell-derived factor-1 (SDF-1) and express receptors for these chemokines), presumably to enhance neutrophil and monocyte recruitment to sites of infection. As they mature, DC lose the capacity to produce these inflammatory cytokines and upregulate the chemokine receptor CCR7, and accordingly acquire responsiveness to its ligand, MIP-3 β and SLC (CCL21) that regulate the trafficking of DC to the lymphoid vessels and secondary lymphoid organs.⁶² Mature DC entering the draining LN traffic to the paracortical T cell areas in response to the production of CCL19 and CCL21 by cells in the T cell zone.⁶³ The newly arriving DC produce chemokines (CCL18

and CCL22) which chemoattract naïve and memory T cells, respectively. Upon contact with T cells, DC receive additional signals provided by CD40/40L, RANK/TRANCE, or 4-IBB/4-IBBL interactions, which help DC terminal maturation. After interaction with T cells and terminal maturation, DC are believed to die by apoptosis.

MDC and PDC isolated from human PB differ in their capacity to migrate to chemotactic stimuli. These different migration programmes and chemokine production profiles of MDC and PDC point to their divergent roles in the induction and regulation of immune responses. A detailed understanding of the role of chemokines in DC function should allow us to effectively employ the potential of DC as therapeutic agents. For example, *ex vivo* conditioning of DC with gene encoding chemokines could provide an alternative approach to improve the migratory capacity of DC *in vivo*.

DC-T CELL INTERACTION

Naïve T cells located in paracortical regions of lymph node (LN) and spleen (SP), migrate directly from the vasculature. Because naïve T cells do not (for the most part) enter peripheral tissues, they interact with DC migrating into secondary lymphoid organs such as LN, SP, or mucosal lymphoid tissues.⁶⁴ There, DC attract T and B cells by releasing chemokines, maintain the viability of recirculating T cells and complete their maturation.

There are two main outcomes of DC-T cell interaction: (i) the induction of immunity after T cell priming results in elimination of antigen and (ii) the induction of peripheral tolerance. The fate of naïve T_h cells is determined by three signals that are provided by pathogen-primed mature DC (Fig. 6). Signal 1 is the antigen-specific signal that is mediated through TCR; signal 2 is the costimulatory signal, mainly mediated by triggering of CD28 by CD80 and CD86, and Signal 3 is the polarising signal that is mediated by various soluble or membrane bound factors, such as IL-12 (Fig. 6).

The strength of the T cell response is dependent on many factors, including (i) the concentration of antigen presented by DC; (ii) the affinity of the TCR for the corresponding pMHC; (iii) the state of DC maturation, and (iv) the type of maturation stimulus.⁶⁵ For example, T cell stimulation by immature DC leads to initial T cell proliferation but only short-term survival

(“abortive proliferation”), whereas stimulation by mature DC results in long-term T cell survival and differentiation into memory and effector T cells.⁶⁵ Enhanced survival following priming, referred to as T cell “fitness,” is characterised by resistance to cell death in the absence of cytokines and by responsiveness to the “homeostatic” cytokines IL-7 and IL-15, which promote T cell survival in the absence of antigen.^{65,66}

DC POLARISATION OF T CELL RESPONSES

The decision of naïve $CD4^+$ T cells to become T_h1 or T_h2 has important consequences in the development of an immune response and the progression of disease. A T_h1 response *via* generation of T cells secreting IFN- γ is needed to rid the body of viruses, intracellular bacteria and tumour cells, and a T_h2 response, *via* generation of T cells secreting IL-4, IL-5, and IL-13 is needed to clear parasitic worms or dampen down excessive T_h1 reactions that might cause unnecessary damage or lead to autoimmunity.⁶⁷ The type of T_h polarisation is mainly determined by the secreted cytokine profile of the mature DC, which in turn depends on the DC subtype, the anatomic location of the DC and the type of maturation stimulus.^{46,52} These factors control other characteristics of the T cell response as well, such as tolerance induction or T cell homing.⁶⁸ T_h1 polarising stimuli such as LPS induce production of IL-12p70 by mature DC, which together with IFN- γ induce $CD4^+$ T cells to differentiate into IFN- γ -secreting T_h1 effector cells.⁶⁹ T_h1 polarisation is mediated largely by activation of transcription factor Stat4.⁷⁰ T_h2 polarising stimuli such as schistosome eggs cease production of IL-12p70 by DC and in the presence of IL-4, induce naïve T_h cells to differentiate into IL-4, IL-5, and IL-13-secreting T_h2 effector cells.⁶⁹ It is not clear whether T_h2 polarisation is induced by specific DC cytokines or if it is a default programme carried out in the absence of a T_h1 polarisation signal from the DC. The T_h2 programme in $CD4^+$ T cells is dependent on transcription factors GATA-3 and c-Maf.⁷⁰ A better understanding of the role of DC in T_h cell polarisation is of pivotal importance for the design of more powerful vaccines to combat infectious disease and cancer.

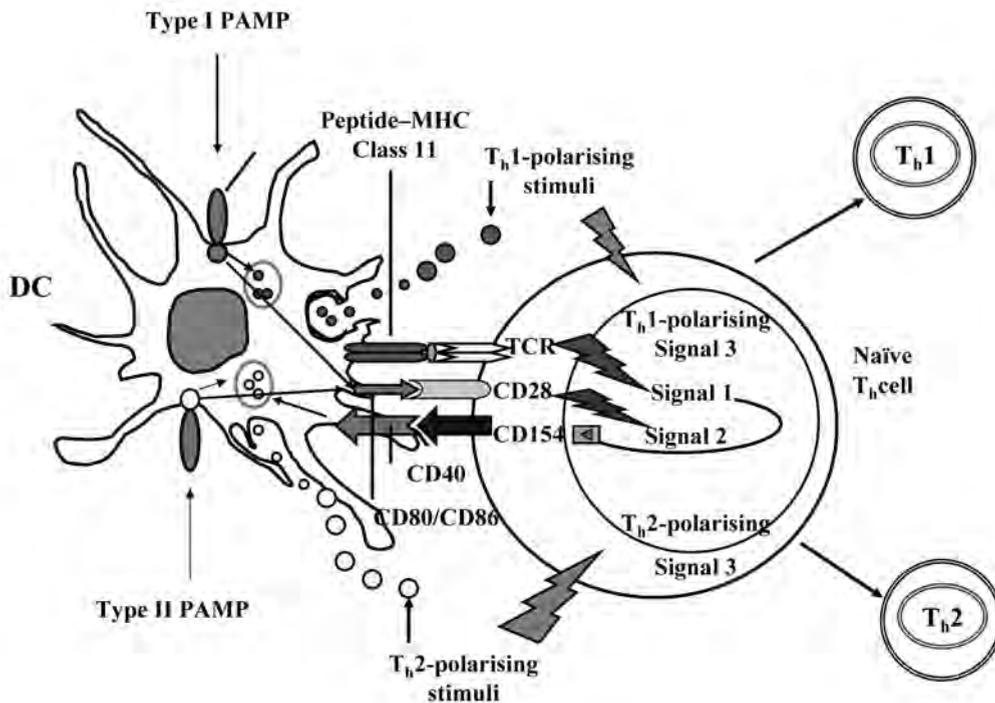


FIG. 6: T cell stimulation and T_h cell polarisation requires three DC-derived signals.

Signal 1 is the antigen-specific signal that is mediated by triggering of TCR by MHC class II-associated peptides. Signal 2 is the costimulatory signal mediated by triggering of CD28 by CD80 and CD86 that are expressed by DC after ligation of TLR by pathogen associated molecular pattern (PAMP). Signal 3 is the polarising signal that is mediated by various soluble or membrane bound factors, such as IL-12 and CCL2, which promote the development of T_h1 or T_h2 cells, respectively. The nature of signal 3 depends on PAMP. Type I and type II PAMP can be defined as those that selectively prime DC for the production of T_h1 cell-polarising factors or T_h2 cell-polarising factors, respectively. Whereas, the profile of T cell-polarising factors is determined by PAMP, optimal expression of this profile often requires feedback stimulation by CD40L expressed by T cells after activation by signals 1 and 2.^{65,66}

CYTOKINES PRODUCED BY DC

1. IL-12

DC and phagocytes, in response to microbial stimulation, are the main producers of IL-12.⁷¹ The physiologically most important target cells of IL-12 are i) NK cells, NKT cells and T cells, for which IL-12 induces proliferation, enhancement of cytotoxicity, expression of cytotoxic mediators, and the production of cytokines, particularly IFN- γ ; ii) B cells, for which IL-12, directly or through the effects of type-1 cytokines such as IFN- γ , enhances the activation and production of T_h1-associated classes of Ig.⁷² IL-12 has been shown to have an important role in the T_h1 response that sustains organ-specific autoimmunity in several mouse experimental models, and to be instrumental in the resistance to many infections, particularly

with bacteria and intracellular parasites.⁷³ The ability of IL-12 to facilitate T_h1 responses has led to its use as an adjuvant in vaccination to induce a T_h1-biased memory.⁷⁴

Positive regulation of IL-12 production

Because excessive T_h1 cell responses may result in severe inflammation, the control of IL-12 production is important in immune system regulation. Products from micro-organisms including bacteria, intracellular parasites and fungi, dsRNA, bacterial DNA, and CpG ODN are strong inducers of IL-12 production by DC.⁷⁵ The relative efficiency of the various inducers depends on the differential expression by DC subsets of the TLR that these products engage. IL-12 in cooperation with IFN- γ , induces the T cell clones that are expanding in response to specific antigens to differentiate into T_h1. Apart

from IFN- γ , IL-4 and IL-13 are also potent enhancers of IL-12 production.⁷⁶ The effect of these two cytokines is particularly noteworthy, because IL-4 and IL-13 are often used for the generation of DC *in vitro* from monocytes or hematopoietic precursors, thereby explaining the ability of DC obtained in these culture systems to produce more IL-12 than *ex vivo*-purified DC.⁷⁷

Negative regulation of IL-12 production

Several reports have shown that the ability of MDC to produce IL-12 can be stably suppressed by inflammatory mediators such as IL-10⁷⁸ and PGE₂,⁷⁹ resulting in DC populations with T_h2-promoting capacity.

IL-10 is a crucial factor for the maintenance of the fine balance between effective resistance against pathogens and detrimental systemic inflammation. It is a potent inhibitor of IL-12 production. The essential regulatory role of IL-10 is shown by the uncontrolled, lethal, systemic inflammatory response to various pathogens in IL-10-deficient mice.⁸⁰ TGF- β is also an inhibitor of IL-12 production that, unlike IL-10, also reduces the stability of IL-12p40 mRNA.⁸¹ Although IFN- α and IFN- β have some functions that overlap with those of IL-12 in terms of the induction of IFN- γ production and T_h1 responses, they suppress IL-12 production.⁷³

2. IL-6

In contrast to IL-10, most DC populations produce IL-6. Murine LC, DC isolated from LN, as well as *in vitro*-generated BM DC are good producers of this cytokine.⁸² IL-6 gene expression is also detected in freshly isolated human BDC, Mo-DC, and CD34⁺-derived DC.⁸³

IL-6 is a pleiotropic cytokine, the functions of which include induction of acute phase proteins, co-stimulation of proliferation and antibody production by B cells, participation in the development of CTL responses and enhancement of the survival of naïve T cells.⁸⁴ IL-6 derived from APC is able to polarise naïve CD4⁺ T cells into T_h2 cells by inducing the initial production of IL-4 in CD4⁺ T cells.⁸⁵

3. Type I IFN

Type I IFN (IFN- α , IFN- β , and IFN- ω) are promptly produced upon invasion of pathogens, and activate a broad range of effector cells in the innate and adaptive immune system. PDC

produce enormous amounts of type I IFN in response to viruses (herpes simplex virus [HSV], influenza virus).⁸⁶ In addition to viral stimulation, various bacterial stimulations, such as *Staphylococcus aureus*, *Escherichia coli*, *Mycobacterium tuberculosis*,⁸⁷ and CpG DNA also induce production of type I IFN by PDC.⁸⁶ Thus, PDC play a significant role in anti-viral as well as anti-bacterial immune responses by virtue of their enormous potential to produce type I IFN.

Type I IFN has been shown to exert immunomodulatory effects *in vitro*. These include upregulation of MHC class I,⁸⁸ promotion of T_h1 responses⁸⁹ and enhancement of the maturation/activation state of DC generated from human PB or mouse BM precursors.⁹⁰ In addition, IFN- α , was shown to act as a survival factor for PB-PDC, although it did not induce their maturation.⁸⁶

TOLERISATION OF T CELLS BY DC

In addition to their role in the induction of effector T cell responses, DC have an important role in peripheral tolerance. The induction of antigen-specific tolerance in the periphery is critical for the prevention of autoimmunity and maintenance of immune homeostasis. The role of DC in peripheral tolerance includes induction of T cell apoptosis, T cell anergy and induction of T_{reg} cells. The induction of T cell anergy by DC might be due to incomplete maturation, blockade of B7 co-stimulatory molecules and the influence of non-inflammatory cytokines such as IL-10 and TGF- β . The anergic T cells induced by IL-10-modulated DC are characterised by inhibited antigen-specific proliferation, a reduced production of IL-2 and IFN- γ and downregulated expression of CD25.⁹¹ Finally, DC may control peripheral tolerance by inducing differentiation of T_{reg} cells. Once activated, CD4⁺CD25⁺ T_{reg} cells are able to suppress responses of both CD4⁺ and CD8⁺ T cells. T_{reg} cells have been shown to inhibit autoimmune diabetes in mice,⁹² induce tolerance to alloantigens⁹³ and inhibit the development of colitis in severe combined immunodeficiency (SCID) mice.⁹⁴ Studies in animal models provide strong evidence for a role of IL-10 and TGF- β for the suppressive effects of T_{reg} cells.⁹⁴ The capacity of DC to induce tolerance offers new therapeutic perspectives for its clinical application in the treatment of allergy, autoimmune and chronic inflammatory diseases and allotransplant rejection.

DC EFFECT ON DIFFERENT CLASSES OF LYMPHOCYTES

DC can contribute to the expansion and differentiation of most classes of lymphocytes; not just T cells, but also B cells,⁹⁵ innate NK cells⁹⁶ and NKT cells.⁹⁷ Activated MDC can directly induce B-cell proliferation, Ig isotype switching, and plasma cell differentiation through the production of the B-cell activation and survival molecules BAFF (B-cell-activating factor belonging to the TNF family) and APRIL (a proliferation-inducing ligand).⁹⁸ Activated PDC can induce the differentiation of CD40-activated B cells into plasma cells through the secretion of IFN α/β and IL-6.⁹⁹ Conversely, B cells have a profound regulatory effect on the antigen-presenting function of DC *in vivo*.¹⁰⁰ Indeed, DC from B-cell depleted animals have an impaired capacity to induce antigen-specific differentiation of IL-4-secreting T cells.¹⁰⁰ Therefore, B cells (by controlling IL-12 production by DC) may favour the development of T_H2 cells that provide helper activity for antibody synthesis, thereby promoting their own effector function.

DC can also activate and induce the expansion of resting NK cells.¹⁰¹ These activated NK cells can kill immature DC, but not mature DC and can stimulate DC to induce protective CD8⁺ T cell responses.¹⁰² Finally, DC presenting the synthetic glycolipid α -galactosylceramide (α -GalCer) on CD1d can activate NKT cells to produce IFN- γ and promote resistance to tumours.¹⁰³ Activated NKT cells can in turn induce full maturation of DC and can directly interact with DC to enhance both CD4⁺ and CD8⁺ T cell responses.¹⁰³ These observations show that a bi-directional interaction occurs between DC-B and DC-NK cells. This interaction could be exploited in many ways to generate immunotherapies that are more effective. For example, vaccination with melanoma cells in adjuvant can activate CD1d-restricted NKT cells that recognize tumour-associated gangliosides¹⁰⁴ and intravenous delivery of a soluble antigen together with the synthetic CD1d-binding glycolipid α -GalCer can lead to *in vivo* activation of NKT cells and induction of anti-tumour T cell immunity. Trials to test the activating potential of α -GalCer-pulsed DC are under way in cancer patients.¹⁰⁵

CONCLUSION

DC are the most potent antigen presenting cells. Depending on their type, state of

differentiation and maturation, DC can induce immunostimulatory as well as tolerogenic responses. This dual function has made them potential targets in vaccine development in cancer and infection as well as in the prevention of allograft rejection and autoimmune diseases. A better understanding of DC immunobiology using *in vitro* and *in vivo* models is required for the development of new therapeutic approaches to induce immunity or tolerance.

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