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REVIEW ARTICLE

Dendritic Cells-Nature and Classification

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ABSTRACT
Dendritic cells (DCs) are antigen (Ag)-presenting cells (APCs) characterized by a unique capacity to stimulate naïve T cells and initiate primary immune responses. Recent studies suggest that DCs also play critical roles in the induction of central and peripheral immunological tolerance, regulate the types of T cell immune responses, and function as sentinels in innate immunity against microbes. The diverse functions of DCs in immune regulation depend on the heterogeneity of DC subsets and their functional plasticity. Here we review recent progress in our understanding of the nature and classification of DCs.

KEY WORDS
Dendritic cells, Development, Functional plasticity, Immune response, Lineage, Subset heterogeneity

INTRODUCTION

The discovery of skin dendritic cells (DC) by Langerhans in 1868 was followed by much speculation as to their function. Steinman and Cohn identified spleen cells having distinct morphological features with dendrites prepared from mouse peripheral lymphoid organs in 1973, and termed these potent stimulators of primary immune responses DCs.[1] The observation that similar leukocytes were present in the nonlymphoid tissues of both rodents and humans,[2] combined with early evidence suggesting that they played an important role in heart and kidney transplant rejection as well as immune responses,[2] generated further interest in DCs. However, a low frequency in the body (1–2% of the total leukocytes)[1] and lack of markers for DCs caused problems with the purification of DCs as well as slow progress in their further characterization. For the past decade, advances in the *in vitro* culture of DCs using a cocktail of cytokines and methods to purify DCs with the availability of monoclonal antibodies (mAbs) have provided a reliable source of these cells for functional and development studies. It has become clear that DCs follow various hematopoietic pathways of differentiation and maturation, and the multiple and heterogeneous subsets of DCs vary in cell surface marker expression.[3-6] The extensive studies to date have revealed that DCs are critical APCs for not only the induction of primary immune responses, but also for the regulation of the type of T cell-mediated immune response, including T type 1 helper (Th1) cell- and Th2 cell-responses.[3,4,7] In addition to their role in adaptive responses, DCs serve as sentinels, recognizing the presence of invading pathogens through various pattern-recognition receptors (PRRs), and become activated by microbial products to secrete proinflammatory cytokines involved in host defense, thereby linking innate and adaptive immunity.[8,9] Conversely, DCs could be also important for the induction of immunological tolerance, and the mechanisms involved include clonal deletion of self-reactive T cells in thymus (central tolerance) and clonal deletion and anergy as well as active suppression by the production of T regulatory (T_R) cells in the periphery, a function of likely importance in autoimmunity and transplant rejection as well as self-tolerance (peripheral tolerance).[10-13] The diverse functions of DCs in immune regulation reflect the heterogeneous subsets with different lineages and maturity, and functional plasticity. In this review, we attempt to highlight the nature and classification of DC subsets in humans and mice.

NATURE OF DCS

Hematopoietic progenitors in the bone marrow (BM) give rise to circulating DC precursors that home to

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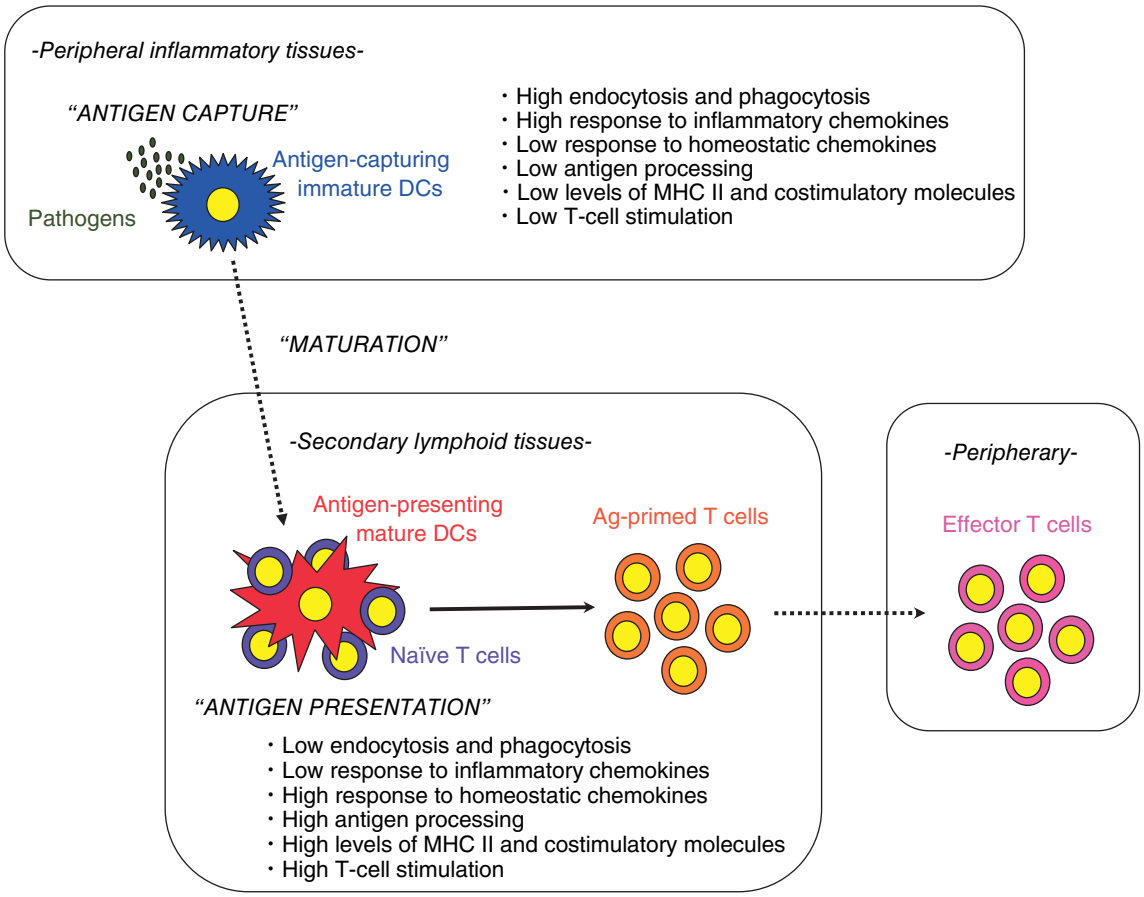

Fig. 1 DCs act as natural adjuvants to generate Ag-specific T cells from naïve T cells. Immature DCs sense the presence of invading pathogens via various PRRs and process the pathogens intracellularly in inflammatory tissues, and they develop into mature DCs with change of the various functions. Subsequently, mature DCs home into secondary lymphoid tissues where they present the processed Ags to naïve T cells to generate effector T cells.

blood as well as non-lymphoid and lymphoid tissues, where they reside as immature cells. Immature DCs possess high endocytic and phagocytic capacity permitting Ag capture, but express low levels of MHC class II (MHC II) molecules and costimulatory molecules on their surface (Fig. 1). Following a microbial infection and tissue damage, immature DCs migrate to the inflammatory regions in response to the production of a large spectrum of inflammatory chemokines upon local inflammation through specific receptors (Fig. 1). Immature DCs recognize pathogen-associated molecular patterns (PAMPs) of microbial products via PRRs, including the families of Toll-like receptor (TLR) and mannose-like receptors, and inflammatory compounds released by damaged tissues (Fig. 1). Upon recognizing a pathogen, they release large amounts of proinflammatory or antiviral cytokines resulting in the activation of innate immune cells, thereby limiting the spread of infection (Fig. 1). Simultaneously, DCs acquire a "mature" phenotype, and the maturation process is associated with several coordinated events such as (*a*) loss of endocytic and phagocytic receptors, (*b*) high-level expression of MHC II at the cell surface and increased production of costimulatory molecules, including CD40, CD80, and CD86, (*c*) changes in morphology, and (*d*) activation of the Ag-processing machinery, including a shift in lysosomal compartments and increase in DC-lysosome-associated membrane protein (DC-LAMP) (Fig. 1). In addition, mature DCs reprogram chemokine receptor expression and responsiveness, including the loss of the responsiveness to inflammatory chemokines (e.g., CCL3, CCL5, and CCL20) through either receptor downregulation or receptor desensitization (e.g., CCR1, CCR5, and CCR6) and acquisition of the responsiveness to homeostatic chemokines, including CCL19 and CCL21 via upregulation of CCR7 (Fig. 1). Consequently, mature DCs move via the afferent lymphatics into the T-cell area of local draining lymph nodes (LNs), where they select rare Ag-specific T cells and induce their activation and differentiation into effector cells, thereby in-

itiating primary immune responses (Fig. 1).

CLASSIFICATION OF DCS BY ANATOMICAL LOCALIZATION

The level of heterogeneity is reflected by anatomical localization. DCs and DC precursor cells circulate in low numbers (approximately 1% of peripheral blood mononuclear cells [PBMCs]). In non-lymphoid tissues, skin epidermal Langerhans cells (LCs) and dermal DCs exist in the skin, and interstitial DCs (intDCs) are found in most organs, including liver, kidney, heart, and other connective tissues and tend to be associated with vascular structures. Mucosal surface-associated DCs are found in the mucosa of the oral cavity, intestinal tract, and respiratory tract. These cells develop from precursors in the blood and provide a sentinel system in the peripheral tissues. In lymphoid tissues, the germinal center (GC), which is the microenvironment that allows the generation of B cell memory, also contains follicular DCs (FDCs) and GC DCs (GCDCs). FDCs have the unique capacity to trap Ag in the form of immune complexes for long periods of time and promote the activation and selection of GC B cells. In contrast, GCDCs are strong APCs for T cells. DCs which have captured Ag, and migrated from the skin, other nonlymphoid interstitial sites, and the mucosal surfaces into afferent lymph are recognized as afferent lymphatic DCs (veiled cells). These cells migrate through the afferant lymphatics and become interdigitating DCs (IDCs) in T-cell areas in the paracortex, which can initiate immune responses by activating naïve T cells, and probably B cells. In the thymus, thymic DCs are suggested to be involved in the negative selection of T cells. Although certain phenotypic differences have been observed among these different DC subsets, the cell's origins, maturation stages, and functional differences have not been clearly established. A DC lineage-specific marker has not yet been identified, and the subsets of DCs in humans and mice are therefore currently defined by linage$^-$MHC II$^+$cells in combination with various cell surface markers.

HETEROGENEITY OF MOUSE DC SUBSETS

SUBSETS OF CONVENTIONAL MOUSE DCS

Multiple DC subsets have now been defined in mouse lymphoid organs on the basis of cell surface marker expression. Mouse DCs are basically defined by their expression of CD11c and MHC II in combination with CD4, CD8α, CD11b, and CD205. The T-cell markers CD4 and CD8 are expressed on mouse DCs and are useful for segregating subsets. CD8 on DCs is in the form of an αα-homodimer rather than the αβ-heterodimer that is typical of T cells. In the past, CD8α$^+$DCs have been referred to as "lymphoid DCs" and CD8α$^-$DCs as "myeloid DCs", although this concept is now thought to be inappropriate.[14] Other markers that are useful for segregating mouse DC subsets include the myeloid cell marker CD11b and the interdigitating DC marker CD205. Using these cell surface markers, five conventional subsets of CD11c$^+$MHC II$^+$DCs can be found in lymphoid tissue under steady-state conditions. The spleen contains three subsets, including CD4$^-$CD8α^{high}CD205$^+$CD11b$^-$lymphoid DCs (20% of spleen DCs), CD4$^+$CD8α$^-$CD205$^-$CD11b$^+$myeloid DCs (40% of spleen DCs), and CD4$^-$CD8α$^-$CD205$^-$CD11b$^+$myeloid DCs (15% of spleen DCs)[15] (Table 1). Both CD4$^+$CD8α$^-$CD205$^-$CD11b$^+$myeloid DCs and CD4$^-$CD8α$^-$CD205$^-$CD11b$^+$ myeloid DCs of spleen and LNs that also express 33D1 and F4/80, are located in the marginal zone between white and red pulp,[15] and migrate to the T-cell area upon stimulation.[16] Both CD8α$^-$subsets are efficient stimulators of CD4$^+$ and CD8$^+$T cells *in vitro*, and can direct a Th2-type immune response *in vivo* (Fig. 2).[17] CD4$^-$CD8α^{high}CD205$^+$CD11b$^-$ lymphoid DCs occupy the T-cell area of spleen, and are also found at moderate levels in LNs, but are the dominant subset among thymic DCs.[15,18,19] Freshly isolated CD4$^-$CD8α^{high}CD205$^+$CD11b$^-$lymphoid DCs have a regulatory effect on T cells in which they activate both CD4$^+$ and CD8$^+$T cells, but can induce apoptosis in CD4$^+$T cells and a limited CD8$^+$T cell response.[20] Moreover, they are responsible for maintaining peripheral tolerance under steady-state conditions through the induction of cross-tolerance.[21,22] In this role, they may function to maintain T-cell tolerance in lymphoid organs in the absence of infection. In contrast, under conditions consistent with activation, CD4$^-$CD8α^{high}CD205$^+$CD11b$^-$lymphoid DCs can not only activate CD8$^+$T cells but also cross-present for the stimulation of cytotoxic T cells (Fig. 2).[23,24] In addition, they can trigger the development of Th1 cells *in vivo* (Fig. 2), consistent with activated CD4$^-$CD8α^{high}CD205$^+$CD11b$^-$lymphoid DCs being the major producers of interleukin (IL)-12.[17,25-27] In addition to the three subsets described in spleen, LNs contain two extra DC subsets that are not normally found in spleen, which apparently arrived via the lymphatic system.[18,28,29] They represent the mature form of interstitial tissue DCs which are CD4$^-$CD8α$^-$CD205$^+$CD11b$^+$myeloid subsets, and LCs which are CD4$^-$CD8α^{low}CD205^{high}CD11b$^+$myeloid subsets (Table 1). Mature LCs are restricted mainly to the skin-draining LNs, while mature interstitial tissue DCs are common to all LNs. LCs in LNs are distinguished by their expression of langerin and very high levels of MHC II and costimulatory molecules. They appear to be fully activated DCs and efficient stimulators of naïve CD4$^+$T cells to possibly generate Th1 cells (Fig. 2).[29] However, the migration of DC subsets from peripheral tissue into LNs occurred in the absence of infection,[30] suggesting that the steady-state migration of semi-mature DCs from tissue to LNs occurs naturally during the maintenance of peripheral tolerance.[31]

Table 1 Heterogeneity of murine DCs

DC Subsets	Conventional					* Plasmacytoid
	Spleen and Lymph nodes			Lymph nodes		IPCs
	CD4 $^-$ CD8α^{high}	CD4 $^-$ CD8α^-	CD4 $^+$ CD8α^-	CD4 $^-$ CD8α^{low}	CD4 $^-$ CD8α^-	
Lineage	Lymphoid	Myeloid	Myeloid	Myeloid	Myeloid	Lymphoid/Myeoild
Phenotype						
CD8α	+++	−	−	var.	−	var.
CD4	−	−	++	−	−	var.
CD11b	−	var.	++	++	++	−
CD11c	+++	var.	+++	+++	+++	++
CD205	+++	−	−	+++	+	+
33D1	−	−	++	−	−	−
B220	−	−	−	−	−	++
Gr-1	−	−	−	−	−	++
128G	−	−	−	−	−	+++
440c	−	−	−	−	−	+++
mPDCA-1	−	−	−	−	−	+++
CD40	+	+, −	+	+++	++	−
CD80	+	+, −	+	+++	++	−
CD86	+	+, −	+	+++	++	−
MHCII	+++	+++	+++	+++	+++	++
Langerin	−	−	−	+++	−	−
Anatomical site	T-cell area	Marginal zone	Marginal zone	Skin-draining lymph nodes	All lymph nodes	All area

−, negative; +, low; ++, intermediate; +++, high; var., heterogenous.
* Plasmacytoid DCs can be CD4 $^-$ CD8α^+, CD4 $^+$ CD8α^-, CD4 $^-$ CD8α^-, CD4 $^+$ CD8α^+ subsets, and they are found mostly in the periarteriolar lymphoid sheaths, but scattered plasmacytoid DCs are present in the marginal zone and red pulp.

MOUSE PLASMACYTOID DCS

Plasmacytoid DCs, which were originally identified in humans, represent a distinct class of DCs newly identified in spleen (25% of spleen DCs) as well as BM, thymus and lymph nodes in mice.[32-34] Plasmacytoid DCs display characteristics different from other "conventional" DC subsets, but share most of the morphological and functional characteristics of their human counterparts. While conventional DC subsets enter LNs from peripheral tissues, plasmacytoid DCs enter directly from blood by crossing the high endothelial venule (HEV) though CD62L.[32] Freshly isolated plasmacytoid DCs have a morphology typical of that of large, round cells with a diffuse nucleus and few dendrites, and express B220 and Gr-1 together with CD8 α, CD11c, CD205 and MHC II and lack costimulatory molecules (Table 1). Recently, mouse plasmacytoid DC-restricted surface markers have been identified with three additional Abs, 120G8 Ab, 440c Ab, and mPDCA-1 Ab (Table 1). They exhibit poor capacity to stimulate T cells, and may play a role in the maintenance of peripheral tolerance under steady-state conditions.[35] Activation of plasmacytoid DCs improves their viability in culture and upregulates their expression of CD8α, MHC class II, and costimulatory molecules, to a lesser extent than conventional DCs. In addition, like their human counterpart, mouse plasmacytoid DCs produce a large amount of type I interferon (IFN) following a viral infection and so may play an important role in antiviral responses rather than the APC function of T-cell immune response once activated (Fig. 2).

It has been suggested that mouse plasmacytoid DCs as well as thymic CD8α^+DCs arise as a lymphoid lineage.[36] However, recent studies have proposed several different hypotheses regarding the complexity of the developmental origin of mouse plasmacytoid DCs,[36] and the cells may represent a unique hematopoietic lineage, whose development may be much more flexible than that of conventional lymphoid (B, T and NK) and myeloid (monocyte and granulocyte) cells.

The mobilization of mice with FLT3-ligand (Flt3L) resulted in strikingly increased numbers of plasmacytoid DCs in BM and spleen, whereas Flt3L-deficient mice have fewer plasmacytoid DCs.[37-39] Therefore, Flt3L is the main cytokine for the development of plasmacytoid DCs from hematopoietic stem cells in mice. In steady-state conditions, plasmacytoid DCs in mice have an average turnover of about 2 weeks[40] whereas conventional DCs have a rapid turnover, within 3 to 5 days.[41]

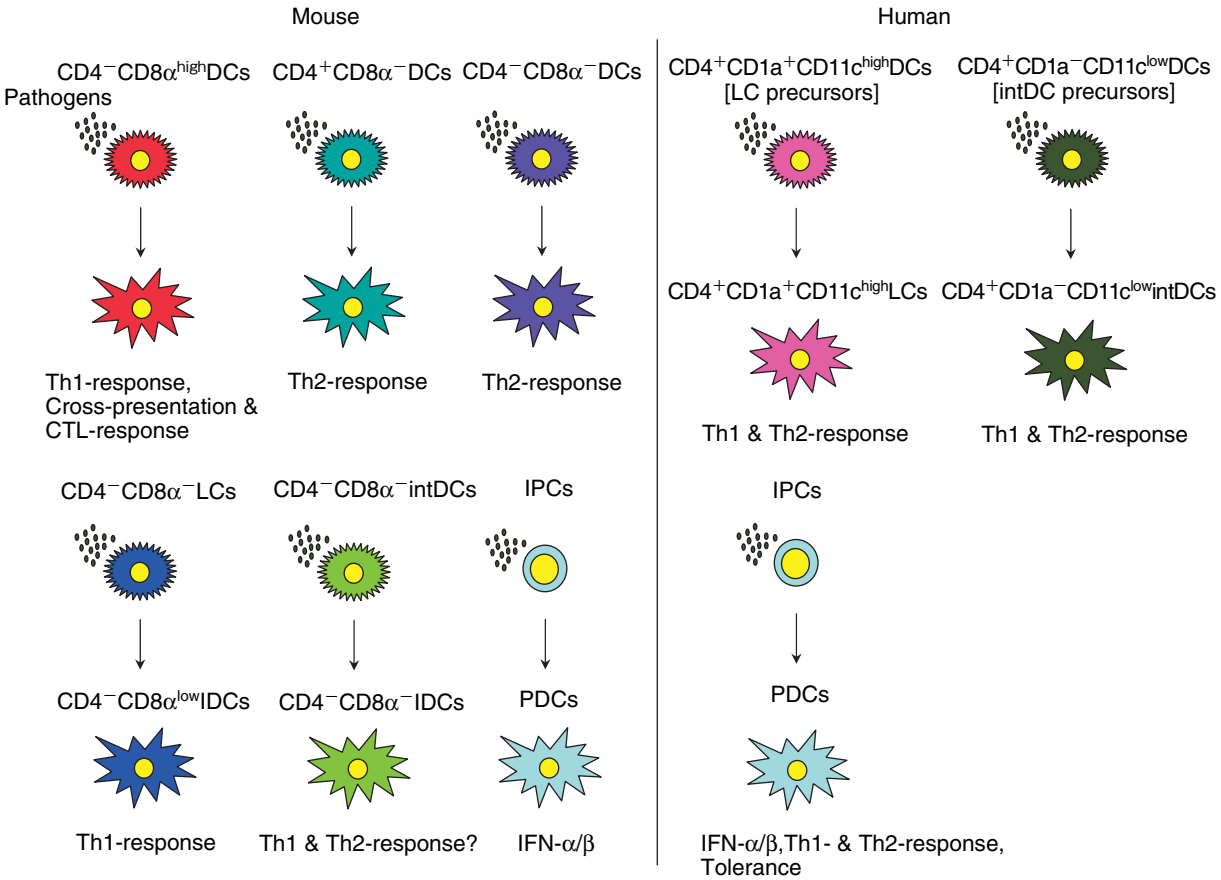

Fig. 2 DC subsets and *in vivo* major function. Immature DC subsets are involved in the induction of peripheral tolerance under steady-state conditions possibly mediated through the induction of clonal T-cell deletion as well as anergic T cells and T_R cells. Following bacterial and viral infections, each immature DC subsets recognizing the pathogens are changed into mature DCs, and they elicit various immune responses, depending on their lineage and activation signals.

HETEROGENEITY OF HUMAN DC SUBSETS

SUBSETS OF CONVENTIONAL HUMAN DCS

In contrast to the extensive research on mouse DCs, there have been relatively few studies on human DCs freshly isolated from tissues. Blood samples are the only readily available source, but human DCs are also isolated from lymphoid tissues derived from tonsil, thymus, and spleen in rare cases. Similar to mouse DCs, human DCs also comprise multiple subsets in terms of the expression of a range of cell surface markers, but these might reflect differences in the maturation status rather than separate sublineages. Human DCs are also defined by lineage$^-$MHC II$^+$ cells, and all of them express CD4 but lack the expression of CD8. In addition, human DCs have two CD11c$^+$ myeloid lineage subsets of conventional DCs and CD11c$^-$ lymphoid lineage subsets of plasmacytoid DCs, whereas all subsets of mouse DCs express CD11c regardless of their developmental lineages.

Human peripheral blood contains two conventional myeloid DCs. CD4$^+$CD1a$^+$CD11c^{high}BDCA-1/CD1c$^+$ subsets (0.6% of PBMCs) and CD4$^+$CD1a$^-$CD11c^{low}BDCA-3/CD141$^+$ subsets (<0.05% of PBMCs)[42-44] (Table 2). CD4$^+$CD1a$^+$CD11c^{high}BDCA-1$^+$ subsets express CD2, CD11b, CD13, CD32, CD33, CD64, CD45RO and CD116 whereas CD4$^+$CD1a$^-$CD11c^{low}BDCA-3$^+$ subsets express CD13, CD33, CD45 RO and CD116, but lack the expression of CD2, CD 11b, CD32 and CD64. These conventional myeloid DC subsets have the capacity to stimulate T cells, and stimulation with granulocyte-macrophage colony-stimulating factor (GM-CSF) causes further enhancement.[44] CD4$^+$CD1a$^+$CD11c^{high}BDCA-1$^+$ subsets, but not CD4$^+$CD1a$^-$CD11c^{low}BDCA-3$^+$ subsets, give rise to cells with characteristics with expression of E-cadherin, Langerin, Lag Ag, and typical Birbeck granules under the appropriate culture conditions, and so CD4$^+$CD1a$^+$CD11c^{high}BDCA-1$^+$ subsets and CD4$^+$CD1a$^-$ CD11c^{low}BDCA-3$^+$ subsets are possibly the direct precursors of LCs and intDCs in peripheral blood, respectively.[42] These conventional DC subsets can elicit Th1- or Th2-response, depending on the inflammatory environments (Fig. 2).[45-47]

Table 2 Heterogeneity of human peripheral blood DCs

DC Subsets	Conventional		* Plasmacytoid
	$CD4^+CD1a^+CD11c^{high}$	$CD4^+CD1a^-CD11c^{low}$	IPCs
Lineage	Myeloid	Myeloid	Lymphoid
Phenotype			
CD1a	++	−	−
CD2	++	−	−
CD4	++	++	++
CD8	−	−	−
CD11b	+	−	−
CD11c	+++	++	−
CD13	++	++	−
CD32	++	−	−
CD33	++	+	−
CD45RA	−	−	++
CD45RO	+	+	−
CD64	+	−	−
CD116	++	++	−
CD123	++	var.	+++
BDCA-1	++	−	−
BDCA-2	−	−	++
BDCA-3	−	++	−
BDCA-4	−	−	++
CD40	+	+	−
CD80	+	+	+
CD86	+	+	−
MHCII	+++	+++	++

− , negative; + , low; ++ , intermediate; +++ , high; var., heterogenous.

HUMAN PLASMACYTOID DCS

Plasmacytoid DCs are recognized as unique DC subsets of lymphoid origin in humans.[4] More than 40 years ago, a previously unrecognized rare cell type was identified in the paracortical areas of reactive lymph nodes with a morphology similar to that of plasma cells and with T-cell and monocyte markers, and therefore designated plasmacytoid T cells or plasmacytoid monocytes. Although these leukocytes are poor stimulators of T cells, they developed into cells with a mature IDC morphology and acquired the ability to activate naïve T cells when cultured with IL-3 and CD40L, and were identical to immature DCs. Moreover, such circulating immature DCs were found to represent a small leukocyte population that produced large amounts of type I IFN in response to certain viruses.[3,48,49] Finally, plasmacytoid monocytes, natural IFN-producing cells (IPCs), and immature DCs in peripheral blood and tonsils were confirmed to be the same cell type termed plasmacytoid DCs (Fig. 2). Human plasmacytoid DCs were originally identified by $lineage^-MHC\ II^+CD11c^-CD4^+CD45RA^+CD123^+ILT3^+ILT1^-$ subsets (0.4% of PBMCs) with a lack of other myeloid markers observed in conventional DC subsets, and two additional markers, BDCA-2/CD303 and BDCA-4/CD304, are restricted to human plasmacytoid DCs in peripheral blood and BM[4,97] (Table 2). Analysis of the ability of human plasmacytoid DCs to prime naïve $CD4^+$T cells suggest that they induce not only Th1- and Th2-responses but also tolerance *in vivo* (Fig. 2).[46,47]

In contrast to the survival effect of GM-CSF on two (conventional human DC) subsets through the CD 116/GM-CSF receptor α-chain (GM-CSFRα), the survival effect of IL-3 on human plasmacytoid DCs depended on the expression of the CD123/IL-3 receptor α-chain (IL-3Rα).[50] The administration of Flt3L to healthy human volunteers dramatically increases both myeloid and plasmacytoid DCs, whereas granulocyte colony-stimulating factor (G-CSF) only increases plasmacytoid DCs.[51]

IN VITRO GENERATION OF DCS FROM HUMAN AND MOUSE PRECURSOR CELLS

Because DCs are rare in the body and therefore the isolation procedures are time consuming and cell yields are low. The methodology for generating a large numbers of DCs from precursor cells and their analysis has proven crucial for a better understanding of the biology of natural occurring DCs.

IN VITRO GENERATION OF MURINE CONVENTIONAL AND PLASMACYTOID DCS

Mouse conventional myeloid DCs are generated from mouse BM or from peripheral blood monocytes with GM-CSF, or GM-CSF plus IL-4.[38,52] On the other hand, the development of murine plasmacytoid DCs as well as myeloid DCs is promoted by Flt3L in total BM cultures, while GM-CSF or tumor necrosis factor (TNF)-α inhibits the development of murine plasmacytoid DCs, but promots the development of myeloid DCs *in vitro*.[37] Although naturally occurring mouse DCs consist of $CD4^+$subsets, $CD8\alpha^{high}$subsets and $CD4^-CD8\alpha^-$ subsets, cultures only generate $CD4^-CD8\alpha^-$ subsets regardless of lineage and the developmental pathway.

IN VITRO GENERATION OF HUMAN CONVENTIONAL AND PLASMACYTOID DCS

Two different precursors have been used to generate human DCs in culture. The earliest precursors used are the $CD34^+$ fraction isolated from BM or umbilical-cord blood. Liquid culture with GM-CSF and TNF-α leads to intDC-like subsets and LC-like subsets.[4,53] In addition, c-kit ligand increases the total number of cultured myeloid DCs in synergistic combination with GM-CSF and TNF-α,[54] and transforming growth factor (TGF)-β1 promotes *in vitro* development of LC-like subsets from $CD34^+$ hemopoietic progenitors.[55] On the other hand, Flt3L, but not G-CSF, has been shown to induce a proportion of human $CD34^+CD45RA^-$ early hematopoietic stem cells to differentiate into human plasmacytoid DCs in culture.[56]

Blood monocytes are the most commonly used precursor cells for generating human DCs in culture. Blood monocytes cultured with GM-CSF and IL-4 differentiate into immature myeloid DCs[57] whereas they develop into LC-like subsets in the presence of GM-CSF, IL-4 and TGF-β1,[58] and little or no proliferative expansion is involved. The final maturation of these DC subsets, which is associated with the expression of CD83 as well as upregulation of costimulatory molecules and MHC II, is achieved by stimulating the cells with bacterial products such as lipopolysacccharide (LPS) and proinflammatory cytokines such as TNF-α for experimental procedures and cytokine cocktails containing TNF-α, IL-1β, IL-6 and prostaglandin E2 (PGE2) for clinical use.[59]

CONCLUDING REMARKS

Many important aspects of the biology of DCs have been revealed through the extensive recent study of the characteristics and role of these cells in acquired and innate immune responses. The remarkable functional diversity of DC subsets endows the immune system with the flexibility to mount appropriate immune responses, and the dynamics of such a flexible and responsive DC system might be dictated by subset heterogeneity dependent on maturity and lineage origin, and functional plasticity. However, there are still unsolved mysteries regarding origin, function, and the molecular events governing their characteristic features. Answering these questions will provide us with a better understanding of not only the relationship between subset heterogeneity and functional plasticity but also the precise role of DCs in immunity and tolerance. In addition, they may also point to novel targets for the improvement of DC-based vaccines in order to enhance insufficient immune responses in patients with infectious diseases and cancer, and to attenuate excessive undesired immune responses in graft-versus-host diseases (GVHD), graft rejection, allergy and autoimmunity.

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