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The multifaceted biology of plasmacytoid dendritic cells

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Abstract

Plasmacytoid dendritic cells (pDCs) are a unique dendritic cell subset that specializes in the production of type I interferons (IFNs). pDCs promote antiviral immune responses and have been implicated in the pathogenesis of autoimmune diseases characterized by a type I IFN signature. However, pDCs can also induce tolerogenic immune responses. Here, we review recent progress from the field of pDC biology, focusing on: the molecular mechanisms that regulate pDC development and functions; the pathways involved in their sensing of pathogens and endogenous nucleic acids; the function of pDCs at mucosal sites; and their roles in infections, autoimmunity and cancer.

Keywords

plasmacytoid dendritic cell; interferon; transcription factor; virus; autoimmunity; cancer; mucosal immunity; human

Introduction

Plasmacytoid dendritic cells (pDCs) were originally described in human lymph nodes in the 1950s[1]. These cells were later found to secrete high amounts of type I interferon (IFN; i.e. IFN-α, IFN-β) in response to viruses[2,3], thereby corresponding to the enigmatic natural IFN-producing cells identified in human peripheral blood[4,5]. pDCs specialize in producing type I IFNs following their recognition of viruses or self nucleic acids through Toll-like receptor (TLR) 7 and TLR9[6–8]. However, pDCs can also secrete other pro-inflammatory cytokines and chemokines, including interleukin-6 (IL-6), IL-12, CXC-chemokine ligand 8 (CXCL8), CXCL10, CC-chemokine ligand 3 (CCL3) and CCL4. Moreover, expression of MHC class II and costimulatory molecules enables pDCs to present antigens to $CD4^+$ T cells. Thus, the biology of pDCs is multifaceted (Figure 1).

The identification of pDCs in mice in 2001[9–11] has bolstered research in the pDC field, permitting exploration of the mechanisms involved in their development and their roles in innate and adaptive immunity. In addition to contributing to innate antiviral immunity, pDCs

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can participate in the priming of both immunogenic or tolerogenic adaptive immune responses. Human pDCs also continue to be extensively studied with a view to understanding their potential roles in the pathogenesis of autoimmune diseases, cancer and human immunodeficiency virus (HIV) infection. In this Review, we discuss the recent progress that has been made in these areas of mouse and human pDC biology. We begin by discussing the phenotype of pDCs and the mechanisms that control their development.

Phenotypes of human and mouse pDCs

Human pDCs lack expression of the lineage-associated markers CD3, CD19, CD14, CD16 and CD11c and selectively express the C-type lectin BDCA2 (also known as CLEC4C), and the Ig-superfamily receptor ILT7[12]. Human pDCs also express CD4, CD68, ILT3 and the receptor for IL-3 (also known as CD123). Accordingly, IL-3 mediates human pDC survival *in vitro*[3]. CD2 is a cell adhesion molecule that is expressed by a subset of human pDCs[13]. $CD2^{hi}$ pDCs produce lysozyme, but whether this endows them with the capacity to lyse bacteria is unknown. The markers most commonly used to identify pDCs in mice are CD11c, B220, Ly6C, bone marrow stromal antigen 2 (BST2) and SIGLEC-H. In contrast to human pDCs, which are $CD11c^{-}$, mouse pDCs express intermediate levels of CD11c. BST2 (also known as Tetherin[14]) is fairly specific to pDCs and plasma cells in steady-state conditions but is induced on many cell types following exposure to type I and type II IFNs[15]. In cell suspensions from primary and secondary lymphoid organs, SIGLEC-H expression is mainly confined to pDCs[16,17] and is downregulated upon activation[18]. However, specialized macrophage subsets in the spleen, lymph nodes and brain also express SIGLEC-H[17,19]. In addition, studies in reporter mice have confirmed *Siglech* mRNA transcription in progenitor cells[18,20,21]. Mouse pDCs can also express CD8α and, like human pDCs, CD4. In peripheral tissues, the majority of mouse pDCs express CC chemokine receptor 9 (CCR9), Ly49Q and SCA1; however, in the bone marrow these markers distinguish pDC subsets that differ in developmental stage and/or activation state (Box 1).

Box 1

Heterogeneity of mouse pDCs in the bone marrow

While CCR9, SCA1 and Ly49Q are expressed on the majority of mouse pDCs in the periphery, in the bone marrow these markers have unequal distribution, identifying pDC subsets that differ in their degree of maturation and their capacity to produce IFN-I or pro-inflammatory cytokines. $CCR9^{-}$ cells are pDC-like common DC precursors, whereas $CCR9^{+}$ cells are fully differentiated pDCs. $CCR9^{-}$ pDC-like common DC precursors can respond to TLR stimulation and produce type I IFN and pro-inflammatory cytokines better than mature $CCR9^{+}$ pDCs[29]. While $CCR9^{-}$ pDC-like common DC precursors are $SCA1^{lo}$, $CCR9^{+}$ pDCs in the bone marrow can be further divided into $SCA1^{lo}$ and $SCA1^{hi}$ subsets. $SCA1^{lo}$ pDCs are more efficient at producing IFN-α than $SCA1^{hi}$ pDCs and give rise to $SCA1^{hi}$ pDCs after activation or exposure to type I IFN[217]. $Ly49Q^{-}$ and $Ly49Q^{+}$ pDCs secrete type I IFN in response to the synthetic TLR9 ligand CpG or herpes simplex virus (HSV), a DNA virus, but $Ly49Q^{-}$ pDCs respond poorly to stimulation with influenza virus, a RNA virus. $Ly49Q^{-}$ pDCs also appear to produce lower levels of pro-

inflammatory cytokines after TLR stimulation compared to Ly49Q$^+$ pDCs[218]. Two pDC subsets have been defined by CD9 expression[219]. The CD9$^+$ subset has high type I IFN producing and T cell stimulatory capacities and may partially overlap with the nonplasmacytoid, high type I IFN producing DC subset described in the bone marrow[220] and CCR9$^-$ pDC-like common DC precursors.

In general, studies on bone marrow pDC subsets concur that newly generated pDCs or their close precursors may be more efficient at producing type I IFN than mature pDCs in the bone marrow and in the periphery, at least in response to TLR agonists. However, it has also been reported that pDCs in the periphery and not in the bone marrow are the major source of type I IFN in mice infected with murine cytomegalovirus (MCMV)[221]. Most likely, the relative importance of bone marrow versus peripheral pDCs as sources of type I IFN depends not only on their intrinsic capacity but also on the degree of exposure to viruses or other stimuli that elicit a type I IFN response.

In conclusion, pDC subsets in bone marrow reflect different stages of development and/or activation and differ in their capacity to produce type I IFN versus pro-inflammatory cytokines as well as their impact on T cell activation and T cell effector or regulatory functions. Clonogenic assays and consistency among gating strategies and markers used to define pDCs will be essential to determine which populations contain mature pDCs versus those that are heterogeneous and can give rise to different subsets.

Development of pDCs

Progenitors and cytokines required for pDC development

A common DC progenitor (CDP) that generates both pDCs and classical DC (cDCs) but not other cell lineages has been identified in the bone marrow. The CDP is characterized by lack of lineage markers (LIN) and expression of Fms-like tyrosine kinase 3 (FLT3, also known as CD135), macrophage colony-stimulating factor receptor (M-CSFR, also known as CD115) and the receptor tyrosine kinase KIT (also known as CD117)[22–26]. Recently, a clonogenic progenitor downstream of CDP with prominent pDC potential has been reported[27]. This progenitor is LIN$^-$KIT$^{int/lo}$FLT3$^+$IL-7Rα$^-$ and does not express M-CSFR. It expresses high levels of E2-2 (also known as TCF4), the transcription factor that defines the pDC lineage[28], and can be derived from CDPs under conditions in which is E2-2 is upregulated, i.e. exposure to M-CSF or thrombopoietin. A subsequent step in pDC development is the generation of a CCR9$^-$ pDC-like common DC precursor that expresses some of the phenotypic markers of mature pDCs, such as CD11c, B220 and SiglecH, but has low or negligible levels of MHC class II and CCR9. This CCR9$^-$ pDC-like common DC precursor retains the potential to differentiate into either pDCs or cDCs, depending on the tissue environment[29,30]. Therefore, the conversion of progenitors into pDCs or cDCs may happen not only at the CDP stage of development, but also closer to terminal pDC differentiation.

Although many studies have focused on pDC differentiation within the myeloid lineage, there is also evidence that pDCs can originate from the lymphoid lineage. The lymphoid-primed multipotent progenitor, delineated as LIN$^-$KIT$^+$SCA1$^+$CD34$^+$FLT3$^+$, can generate the M-CSFR$^-$ progenitor with prominent pDC potential described above[27], which

subsequently can differentiate into both Rag1-positive and Rag1-negative mature pDCs. pDCs of lymphoid origin may be distinct from pDCs of myeloid origin, but it has been reported that both myeloid- and lymphoid-derived pDCs can activate lymphoid-specific genetic programs[31] such as nonproductive rearrangements of immunoglogulin or TCR genes and expression of pre-T cell receptor alpha[32–34].

FLT3 and its ligand (FLT3L) are crucial for pDC development[35]. In the absence of FLT3 signaling, pDCs are reduced in number in the lymphoid organs and bone marrow of mice[36,37]. FLT3L promotes pDC development through activation of signal transducer and activator of transcription 3 (STAT3)- and phosphoinositide 3-kinase (PI3K)-dependent activation of mammalian target of rapamycin (mTOR)[38,39]. LAMTOR2 is a member of the Ragulator complex known to inhibit mTOR signaling. Conditional deletion of LAMTOR2 in $CD11c^+$ cells (which include cDCs and pDCs) causes accumulation of FLT3 on the cell surface and massive expansion of pDC and cDC populations in older mice[40]. Recently, it was shown that FLT3L and type I IFN act synergistically to promote pDC development from common lymphoid progenitors by inducing upregulation of FLT3[41]. In addition to FLT3 signaling, M-CSF (also known as CSF1) may support the generation of pDCs[42]. Indeed, pDCs can be derived from $M\text{-}CSFR^+$ CDP[23,27] and *Csf1*$^{-/-}$ mice have reduced numbers of both pDCs and cDCs[43]. By contrast, the growth factor GM-CSF inhibits FLT3L-driven pDC development[44]. GM-CSF-induced STAT5 signaling abolishes pDC-related gene expression in $FLT3^+$ progenitors and inhibits the transcription factor interferon regulatory factor 8 (IRF8), which is crucial for pDC development, as discussed below[45].

E2-2 expression guides pDC differentiation

E2-2 is the transcription factor essential for committing CDPs to the pDC lineage in both mice and humans[28,46] (Figure 2). E2-2, along with the transcription factors E12, E47 and HEB (also known as TCF12), make up the E family of class I basic helix-loop-helix transcription factors[47]. E proteins form homodimers or heterodimers with each other and bind to conserved E box sequences, a process that can be perturbed by inhibitor of differentiation (ID) proteins. E2-2 binds to a large fraction of pDC-enriched genes and continuous expression of E2-2 in pDCs is required to maintain cell fate[28,48]. Deletion of E2-2 in mature pDCs causes the loss of pDC-associated markers, spontaneous differentiation into cDC-like cells, upregulation of MHC class II molecules and, ultimately, an increase in the ability to prime T cells[48]. These cDC-like cells are similar to the steady-state noncanonical $CX3CR1^+CD8\alpha^+$ DC population that is related to pDCs[49]. $CX3CR1^+CD8\alpha^+$ DCs require E2-2 for development, harbor Ig heavy-chain (IgH) D-J rearrangements and are enriched for pDC-specific genes; however, they do not produce type I IFN in response to influenza virus and lack surface expression of classical pDC markers like B220 and BST2. Thus, noncanonical $CX3CR1^+CD8\alpha^+$ DCs may represent an alternative fate of pDC-committed cells that are unable to reach or maintain high enough levels of E2-2.

ID2, a repressor of E2-2, is essentially absent in pDCs, but is prominently expressed in cDCs and supports $CD8\alpha^+$ DC development from CDPs[28,50]. pDC numbers are increased in *Id2*$^{-/-}$ mice[50] whereas overexpression of ID2 or ID3 inhibits pDC development *in vitro*[51].

STAT3 and STAT5 help to regulate the balance between E and ID proteins during DC development by controlling the expression of E2-2 and ID2, respectively[52]. STAT3 stimulates FLT3L-dependent expression of E2-2, whereas STAT5 induces GM-CSF-dependent expression of ID2 (Figure 2). A recent study also identified a transcriptional cofactor of the ETO family, MTG16, as a key factor that represses ID2, thereby driving pDC differentiation and restricting cDC development (Figure 2)[53].

Transcription factors targeted by E2-2

Transcriptional targets of E2-2 in pDCs encode proteins involved in the development, homeostasis and function of pDCs, including the transcription factors SPIB, BCL11A, IRF8, RUNX2 and CIITA; the cell surface markers BDCA2, ILT7, SIGLEC-H; and the nucleic acid sensors TLR7, TLR9 and PACSIN1[28,48,54] (Figure 2).

SPIB is a transcription factor critical for the differentiation of hematopoietic progenitors into pDCs and controls the survival of pDCs and their progenitors through induction of the anti-apoptotic gene *BCL2-A1*[55–58]. pDC numbers are reduced in the bone marrow of *Spib*$^{-/-}$ mice but are increased in the periphery, suggesting impaired retention in the bone marrow[59]. Furthermore, *Spib*$^{-/-}$ pDCs exhibit attenuated expression of pDC-specific genes and display defects in type I IFN production following TLR7 or TLR9 stimulation. SPIB was also recently shown to be a novel immunohistochemical marker for the identification of human blastic pDC neoplasms[56].

BCL11A is necessary for the expression of IL-7R and FLT3 in early hematopoietic progenitor cells and wildtype mice reconstituted with *Bcl11a*$^{-/-}$ fetal liver have severely reduced numbers of pDCs[60]. Moreover, genome-wide analyses of DNA binding has revealed that BCL11A regulates transcription of *E2-2*, *Id2* and *Mtg16*[61], thereby inducing a positive feedback loop for pDC development. IRF8 is essential for pDC and CD8α^{+} DC development: IRF8 deficiency results in the absence of pDCs and a reduction in the numbers of CD8α^{+} DCs[62], as well as a decrease in all splenic DC subsets after competitive reconstitution of irradiated mice with wildtype and *Irf8*$^{-/-}$ bone marrow[63]. Loss of pDCs, cDCs and monocytes in an individual with an *IRF8* mutation that impairs DNA binding and transactivation has also been reported[64].

RUNX2 is a Runt family transcription factor that is required for expression of several pDC-enriched genes including the chemokine receptors CCR2 and CCR5[65]. Mature pDCs in *Runx2*$^{-/-}$ mice accumulate in the bone marrow and fail to populate peripheral compartments due to reduced CCR5 expression. Additional transcription factors enriched in pDCs have been identified[54,66], but their roles in pDC development, maintenance and activation have yet to be characterized. Other regulators of pDC development including microRNAs are discussed in Box 2.

Box 2

Additional regulators of pDC development

Other transcriptional regulators are involved in pDC development, including IKAROS, PU.1, X box-binding protein 1 (XBP1), hypoxia-inducible factor 1α (HIF1α) growth

factor independent 1 (GFI1) and nuclear polyadenylated RNA-binding protein 2 (NAB2). Mice that express low levels of IKAROS lack peripheral pDCs, but not other DC subsets, and are impaired in their ability to produce type I IFNs in response to TLR7 or TLR9 ligands[222]. PU.1 directly regulates FLT3 expression in a concentration-dependent manner and is necessary for both pDC and cDC development[223]. XBP1 is a target of the unfolded protein response sensor IRE-1α that maintains endoplasmic reticulum (ER) homeostasis and prevents activation of cell death pathways[224]. XBP1 deficiency results in impaired survival and reduced numbers of pDCs and cDCs[225]. Treatment of pDCs with the proteasome inhibitor Bortezomib reduces the active form of XBP1 and suppresses pDC survival and immunostimulatory functions by targeting ER homeostasis and the intracellular trafficking of TLRs[226]. Hypoxia-inducible factors influence hematopoiesis and maintain HSC function. Recently, it was shown that low oxygen content upregulates ID2 and suppresses FLT3L-induced pDC development, in a manner dependent on HIF1α[227]. GFI1 controls the development and functions of pDCs and cDCs in a STAT3-dependent manner[228] and represses *Rag* transcription in pDCs[229]. NAB2 is a transcriptional corepressor that induces TRAIL expression in activated pDCs[230]. TRAIL-expressing pDCs are tumoricidal[231] and induce apoptosis of $CD4^+$ T cells in HIV viremic patients or after exposure to HTLV1[232–234].

MicroRNAs represent another class of regulatory molecules that control different aspects of pDC development and function. miR-22 is induced in progenitor cultures by GM-CSF and it targets *Irf8* mRNA for post-transcriptional repression[235]. Overexpression of miR-22 during DC development promotes the expansion of $CD11b^+$ cDC populations at the expense of pDCs. Epigenetic or chromatin modifiers may also have a prominent role in DC lineage commitment and development. In support of this, histone deacetylation is required for PU.1 recruitment to target genes (including *Flt3*, *Irf8* and *PU.1* itself) and for pDC differentiation from progenitors[236].

In conclusion, while E2-2 is the master transcription factor for pDC development, it is becoming clear that E2-2 is a component of a multi-protein complex that includes both positive and negative regulators. More studies are required to define the various components of this complex as well as the genomic regions that interact with this complex and drive the expression of molecules that define pDC phenotype and function.

Trafficking of pDCs

Initial studies showed that pDC migration is quite different from that of cDCs. Following their development in the bone marrow, pDCs circulate in the blood and reach T cell areas of lymph nodes mainly through high endothelial venules (HEVs) and not through afferent lymphatics[67,68]. In addition to secondary lymphoid organs, pDCs also migrate from blood into peripheral tissues. pDC migration involves CD62L, PSGL1, β1 and β2 integrins and multiple chemokine receptors, such as CXCR4, CCR7, CXCR3, CCR5, CCR2, CCR6, CCR10 and CCR9 (Figure 3)[68,69].

CXCR4 is required for the retention of pDCs in the bone marrow stromal niche and their development[70]. CXCR4 and CCR7 mediate the migration of pDCs into the splenic white

pulp[71]. CXCR4 also promotes pDC recruitment to tumors that produce CXCL12[72]. During inflammation, CXCR3 and CCR5 drive pDC migration into inflamed tissues[73,74]. CCR2, which is expressed with a bimodal distribution on pDCs[65], drives recruitment of pDCs to the skin following inflammation induced by topical application of imiquimod[75]. CCR6 and CCR10 are expressed by a subset of pDCs in human tonsil and facilitate migration to inflamed epithelia in response to CCL20 and CCL27[76]. pDC recruitment to the thymus and small intestine requires CCR9[77,78], whereas pDC migration into the colon is CCR9-independent[79]. MAdCAM-1 and β7 integrin were recently shown to promote pDC trafficking into the intestinal intraepithelial compartment[80]. pDCs can also migrate in response to engagement of receptors for chemerin (ChemR23), adenosine, as well as C3a and C5a, which are released at sites of tissue damage[68]. CX3CR1 is expressed by pDCs; however, its role in pDC migration and homeostasis is not well characterized.

Several studies have recently identified intracellular proteins that selectively impact pDC trafficking in the steady-state and in disease. Human and mouse pDCs express CD2-associated protein (CD2AP)[81], an intracellular protein that regulates actin dynamics and promotes pDC migration to lymph nodes during inflammation[82]. DOCK2, a hematopoietic cell-specific CDM (Caenorhabditis elegans Ced-5, mammalian DOCK180 and *Drosophila melanogaster* myoblast city) protein, is necessary for migration of pDCs to lymph nodes and the periarteriolar lymphoid sheaths of the spleen[83]. Chemokine-induced Rac activation is severely impaired in *Dock2*$^{-/-}$ pDCs, resulting in the reduction of motility and the loss of polarity during chemotaxis[83].

Innate sensing by pDCs

Sensing of pathogens and self nucleic acids by TLR7 and TLR9

The recognition of viruses or self nucleic acids by pDCs is mainly mediated by TLR7 and TLR9, which are located in endosomal compartments. Activation of these receptors in pDCs results in secretion of type I IFNs via the MyD88-IRF7 pathway as well as production of pro-inflammatory cytokines and chemokines via the MyD88-NF-κB pathway (reviewed in[6–8]). TLR7 senses RNA viruses, endogenous RNA and synthetic oligoribonucleotides, whereas TLR9 detects DNA viruses containing unmethylated CpG-rich DNA sequences, endogenous DNA and synthetic CpG oligodeoxyribonucleotides. Since pDCs constitutively express high levels of IRF7 and produce type I IFNs[84], they are largely resistant to viral infection unless type I IFN signaling is compromised[85]. Therefore, uptake and recognition of viral nucleic acids by pDCs likely occurs via mechanisms that are independent of viral infection. Such mechanisms are not yet completely understood and are the subject of intensive investigation. In the case of hepatitis A virus, virions coated in host membrane enter pDCs through the phosphatidylserine receptor TIM1[86], while pDCs sense hepatitis C virus (HCV) via exosomes containing HCV RNA that are released from infected cells[87]. Direct contact of pDCs with vesicular stomatitis virus (VSV)-infected cells triggers type I IFN production in a manner that is TLR-dependent and more robust than free virus alone[88]. Viruses and endogenous nucleic acids can enter pDCs through Fc receptors when bound by antibodies during an immune response. Recently, it was documented that the inhibitory Fc receptor FcγRIIB promotes the uptake of Sendai virus immune complexes during a memory

response and prevents type I IFN production by pDCs[89]. By contrast, endogenous nucleic acids are internalized as a complex with antinuclear antibodies via the activating Fc receptor FcγRIIA[90–92].

Whether engagement of TLR7 or TLR9 results in the production of type I IFN or pro-inflammatory cytokines depends on the type of compartment in which these TLRs encounter their ligands (Figure 4)[93,94]. Multimeric CpG-A oligonucleotides aggregate in early endosomes where they activate the MyD88-IRF7 pathway that induces type I IFNs. In contrast, monomeric CpG-B is transferred to an endolysosomal compartment, where it activates the MyD88-NF-κB pathway that triggers expression of costimulatory molecules and pro-inflammatory cytokine secretion. IkappaB kinase-alpha (IKKα)[95], osteopontin[96] and mTOR[97] are key components of the MyD88-IRF7 signaling pathway, while IRF5 is essential for MyD88-NF-κB signaling[98,99]. Trafficking of TLR9 to the subcellular compartment for type I IFN production is dependent on adapter protein 3 (AP3), as virus- or CpG-induced type I IFN expression is abolished in the absence of AP3[100,101]. Forward genetic approaches have further identified *Slc15a4*, which encodes the peptide/histidine transporter 1 (PHT1), and BLOC1 and BLOC2 Hermansky-Pudlak syndrome proteins as key components required for TLR9 signaling in pDCs but not in other cell types[101]. However, TLR9-mediated recognition of large DNA-containing immune complexes is independent of AP3[102] and requires the convergence of phagocytic and autophagic pathways. This process is called microtubule-associated protein 1A/1B-light chain 3 (LC3)-associated phagocytosis (LAP) and involves autophagy-related proteins but not the conventional autophagic pre-initiation complex. Recognition of live, single stranded RNA (ssRNA) viruses by TLR7 in pDCs also occurs in a distinct subcellular compartment that requires transport of cytosolic viral replication intermediates into the lysosome by autophagy[103].

Several additional molecules involved in TLR7/9 signaling have been identified in pDCs. The scavenger receptor SCARB2/LIMP-2 is highly expressed in pDCs and regulates TLR9-dependent type I IFN production by mediating TLR9 endosomal translocation and IRF7 nuclear translocation[104]. Phospholipid scramblase 1 (PLSCR1) is a TLR9-interacting protein that regulates TLR9 trafficking to endosomal compartments and type I IFN production in pDCs[105]. PACSIN1 is an endocytic adapter specifically expressed in mouse and human pDCs that is essential for TLR9-induced type I IFN secretion but not pro-inflammatory cytokine production[54,106]. VIPERIN is an IFN-inducible antiviral protein that promotes TLR7 and TLR9 signaling in pDCs by recruiting IL-1 receptor-associated kinase 1 (IRAK1) and TNF receptor-associated factor 6 (TRAF6) to lipid bodies and inducing nuclear translocation of IRF7[107]. In addition to having a role in pDC migration, DOCK2 is essential for TLR7- and TLR9-mediated type I IFN production in pDCs, most likely because of its influence on actin cytoskeleton and vesicular trafficking[108]. In the absence of DOCK2, phosphorylation of IKKα and nuclear translocation of IRF7 are impaired in pDCs, but the capacity to produce pro-inflammatory cytokines is maintained. TLR9, but not TLR7, signaling is also dependent on Bruton's tyrosine kinase (BTK)[109]. Blocking BTK inhibits all TLR9-induced responses in pDCs, including cytokine production and expression of costimulatory molecules. Additional studies are required to provide an integrated view of

how all these molecules coordinate the trafficking and signaling properties of TLR7/9-containing vesicles in pDCs.

TLR2- and TLR12-mediated sensing in pDCs

Although TLR7 and TLR9 are important for pDC detection of viruses and self nucleic acids, recent evidence indicates that mouse pDCs also express TLR12 and TLR2, which permit detection of *Toxoplasma gondii* profilin[110] and bacterial polysaccharide A (PSA)[111], respectively. Although TLR11 and TLR12 can both recognize profilin, TLR12 is sufficient for profilin detection in pDCs and triggers the production of IL-12 and type I IFNs, which activate NK cells[110]. Furthermore, in contrast to *Tlr11*$^{-/-}$ mice, *Tlr12*$^{-/-}$ mice are highly susceptible to *T. gondii* infection, which suggests a critical role for pDCs in the induction of the innate immune response and host resistance. PSA is an immunomodulatory molecule expressed by the ubiquitous gut microorganism *Bacteriodes fragilis*[112]. pDCs exposed to PSA via TLR2 express MHC class II, inducible costimulatory ligand (ICOSL) as well as CD86, and stimulate IL-10 production by CD4^{+} T cells, all of which are required for protection against colitis[111].

Other cytosolic sensors expressed by pDCs

Although TLRs have been established as the main innate receptors involved in pDC activation, the impact of cytosolic nucleic acid sensors on type I IFN or pro-inflammatory cytokine production by pDCs has just begun to be explored. CpG-A oligonucleotides selectively bind to the DExD/H-box helicase 36 (DHX36) and this is associated with the nuclear translocation of IRF7 and type I IFN production in pDCs[113]. By contrast, CpG-B oligonucleotides are bound by DHX9, and this results in NF-κB activation and the secretion of pro-inflammatory cytokines in pDCs. Additionally, in the absence of type I IFN signaling, viruses can replicate in pDCs, producing viral nucleic acid that can be detected in a RIG-I-like helicase-dependent manner[85]. Major cytosolic sensors of nucleic acids, such as cGAS and STING[114], are likely to be investigated in pDCs in the near future as well.

Regulation of TLR7/TLR9 responses in pDCs

Regulatory receptors expressed by pDCs

Human and mouse pDCs express cell surface receptors that control the amplitude of type I IFN production and pDC activation state in response to TLR7 or TLR9 ligands[115]. Many of these receptors either contain intracellular tyrosine-based inhibitory motifs (ITIM) or they deliver intracellular signals through an associated adaptor, usually DNAX activation protein 12 (DAP12) or the γ chain of Fc receptors (FcRγ), which have an intracellular tyrosine-based activation motif (ITAM). Regulatory receptors associated with human pDCs include BDCA2[116], ILT7[117], NKp44[118], CD300A/C[119], DCIR (also known as CLEC4A)[120], CD32[90–92], BST2[121] and leukocyte-associated immunoglobulin-like receptor 1 (LAIR1)[122]. Recently, it was shown that CD2-associated protein (CD2AP) positively regulates BDCA2 and/or FcRγ signaling by forming a complex with SHIP1 to inhibit the E3 ubiquitin ligase CBL[123]. Receptors that have been shown to regulate TLR7- and TLR9-driven responses in mouse pDCs include SIGLEC-H[16], BST2[15], PDC-TREM[124], Ly49Q[125], PIR-B[126] and EBI2[127].

Regulation by microRNAs and hormones

MicroRNAs and hormones can also impact TLR7 and TLR9 signaling and pDC functions. Expression of miR-146a is induced upon engagement of TLR7 and TLR9, and this suppresses NF-κB activation and TLR-mediated signaling in pDCs[128]. miR-155 and its star-form partner miR-155* are expressed in pDCs after TLR7 signaling and have opposing effects on type I IFN production[129]. Shortly after TLR7 stimulation, miR-155* is induced and augments type I IFN expression by inhibiting IRAKM, whereas miR-155 induction occurs later and shuts down type I IFN production by targeting TAB2. miR-126 has a prominent role in promoting the survival and function of pDCs[130]. miR-126 targets the mTOR pathway and regulates the expression of genes including *Tlr7*, *Tlr9*, *Nfkb1* and *Kdr*, which encodes the growth factor receptor VEGFR2. pDC homeostasis and the capacity to respond to TLR ligands are impaired in both *miR-126*$^{-/-}$ and *Kdr*$^{-/-}$ mice, indicating a role for VEGFR2 in supporting type I IFN production[130].

Recently, it was shown that TLR7-mediated signaling in pDCs is enhanced by estrogen[131]. Notably, this would provide a mechanistic basis for the observation that pDCs from women produce more type I IFN in response to HIV than pDCs from men[132].

pDCs as antigen-presenting cells

Because pDCs express MHC class II molecules as well as the costimulatory molecules CD40, CD80 and CD86, they can present antigens to CD4$^+$ T cells, albeit not as efficiently as cDCs (reviewed in[133,134]). Antigen presentation by pDCs can lead to CD4$^+$ T cell activation or tolerance induction, depending on the context (Figure 1). When pDCs receive activation signals through TLRs or other pattern recognition receptors, they act as immunogenic cells. When pDCs are either unstimulated or alternatively activated such that they express indoleamine 2,3-dioxygenase (IDO)[135–138], ICOSL[139], OX40L[140], programmed death-ligand 1 (PD-L1)[141] and/or granzyme B[142], they promote tolerance to tumor cells, alloantigens and harmless antigens. Recent studies have proposed that pDCs that capture antigens in peripheral tissues utilize CCR9 to migrate to the thymus, where they induce deletion of antigen-specific thymocytes, thus contributing to immune tolerance[77]. It has also been reported that CCR9$^+$ pDCs are potent inducers of regulatory T (T_{Reg}) cell function and can inhibit acute graft-versus-host disease induced by allogeneic CD4$^+$ donor T cells in irradiated recipients[143]. This study defined pDCs as being either B220$^+$CD11c$^+$CCR9$^+$ or B220$^+$CD11c$^+$CCR9$^-$, the former being the tolerogenic subset; however, a later study argued that B220$^+$CD11c$^+$CCR9$^-$ cells are not a subset of pDCs, but rather precursors of lymphoid tissue-resident cDCs[144]. In a mouse model of lung inflammation, adoptive transfer of CD8α$^+$ pDCs suppressed the development of airway hyper-reactivity by inducing T_{Reg} cells[145]; however, it has been reported that CD8 subunit expression by mouse pDCs is variable and does not define stable subsets[146]. Corroborating this, earlier studies found that CD8α is expressed by a fraction of pDCs in the steady-state but is induced on the majority of pDCs after activation[9,147]. Thus, the role of pDC subsets in tolerance remains to be further explored.

To selectively examine pDC antigen presentation capacity, antigens have been specifically targeted to pDCs by conjugating them to antibodies specific for pDC cell surface molecules.

A recent study found that targeting antigens to BDCA2 promotes immunological tolerance by suppressing antigen-specific $CD4^+$ T cell and antibody responses upon secondary exposure to antigen in the presence of adjuvant, a process that involves both preservation of TReg cells and a decrease in effector $CD4^+$ T cells[148]. Antigens targeted to pDCs through DCIR are also presented to T cells[120]. In mice, it was shown that targeting a T cell epitope derived from myelin oligodendrocyte glycoprotein (MOG) to pDCs via SIGLEC-H delays onset and decreases disease severity in a model of MOG-induced experimental autoimmune encephalomyelitis (EAE) by reducing the expansion and polarization of Th1 and Th17 cell populations[149]. In contrast, antigen delivery to pDCs via BST2 in combination with TLR agonists generates robust cellular and humoral immunity and protects mice against subsequent viral infection or tumor growth[150]. Thus, targeting antigen to pDCs can either promote or inhibit antigen-specific immune responses, depending on the antigen formulation and mode of stimulation or delivery.

While in mouse models, pDCs have been mostly studied for their impact on antigen presentation to $CD4^+$ T cells, human studies have focused on pDC antigen presentation to $CD8^+$ T cells[151,152]. Human pDCs have recycling endosomes in which peptides can be continuously loaded on to MHC class I molecules, which enables efficient presentation of viral antigens to $CD8^+$ T cells[153]. Through this mechanism, pDCs may contribute to adaptive responses during viral infections.

pDCs as a source of type I IFNs in viral infections

Acute viral infections

Type I IFN production by pDCs in response to acute viral infections is usually limited in time and amplitude (reviewed in[154]). pDC-secreted type I IFN is mostly evident at early timepoints in systemic infections such as MCMV, VSV, lymphocytic choriomeningitis virus (LCMV) and HSV-1, and mediates immediate containment of viral replication but becomes less important later on in the infection as other host cells become more dominant producers of type I IFNs. The impact of pDCs also depends on the route of infection. pDCs provide an important source of type I IFN in systemic infections, when the virus reaches the circulation. In contrast, the requirement for pDCs in type I IFN-mediated antiviral defense during local viral infections appears to be necessary only if other lines of defense are broken. For example, in pulmonary infection with Newcastle disease virus (NDV), alveolar macrophages are the primary source of type I IFN; if they are eliminated then pDC-derived type I IFN becomes important[155]. Only in the case of mouse hepatitis virus (MHV) and HSV-2 infections has pDC-derived type I IFN been shown to be critical for viral control in mice, reducing both morbidity and mortality[156–158]. A recent study found that individuals carrying inactivating mutations in the *IRF7* gene have a severe defect in pDC function and are selectively susceptible to influenza virus infections[159]. It remains to be determined whether the pDC defect is sufficient to cause susceptibility to influenza or if IRF7 has a more general impact on the capacity of all cells to respond to the virus. Paradoxically, pDC responses to acute viral infections may not always be beneficial. Recent evidence indicates that excessive production of type I IFNs, probably by pDCs, during influenza virus infection in 129 mice can result in uncontrolled inflammation and TNF-related apoptosis-inducing

ligand (TRAIL)-mediated apoptosis of bronchial epithelium[160]. Thus, the impact of pDCs on acute viral infections may vary considerably depending on the virus, route of infection and genetic background.

Chronic viral infections

The role of pDCs in chronic viral infections, such as HIV-1, is even more complex, involving pDC activation, trafficking, type I IFN production and interaction with $CD4^+$ T cells (reviewed in[161]). HIV induces pDC activation through TLR7 but can also directly infect pDCs as they express CD4, CXCR4 and CCR5, which are co-receptors for viral entry. HIV promotes pDC trafficking to peripheral lymph nodes, and this is reflected by the reduced numbers of pDCs found in the blood of patients with HIV[161]. Moreover, new evidence indicates that pDCs upregulate the gut-homing markers α4β7 and CD103 and accumulate in the gut mucosa during SIV and HIV infections[162,163]. Interestingly, pDCs from elite controllers also maintain a gut-homing phenotype, suggesting that gut trafficking of pDCs is independent of viral load[164].

pDCs may contribute to the chronicity of HIV infection through dysregulated activation and type I IFN production (reviewed in[165]). HIV-stimulated pDCs express low levels of maturation molecules, induce weak T cell responses and persistently secrete type I IFNs, due to HIV trafficking to early endosomes[166]. In addition, HIV-stimulated pDCs produce chemokines and express TRAIL, thereby promoting recruitment and apoptosis of T cells. HIV can also activate the noncanonical NF-κB pathway in pDCs, resulting in the expression of IDO and induction of TReg cells[167]. Noncanonical NF-κB signaling and induction of a tolerogenic pathway in pDCs stimulated with high doses of CpG has also been reported[168]. Studies in humanized mice have shown that depletion of pDCs prior to or during chronic HIV-1 infection severely reduces type I IFN production and increases viral replication[169]. However, HIV-1-induced $CD4^+$ T cell death is curbed despite elevated viral loads, suggesting that pDCs suppress HIV-1 replication but also contribute to HIV-1 immunopathogenesis. Conversely, studies in simian immunodeficiency virus (SIV) infection have provided a different view. Blockade of TLR7- and TLR9-mediated IFN-α production by pDCs did not diminish immune activation during SIV infection[170], suggesting that other sources of type I IFN may contribute to immunopathology.

The beneficial or detrimental effects of pDCs and type I IFNs in chronic viral infections may also depend on the timing of their action. During SIV infection, the timing of type I IFN production has a marked effect on the disease course[171]. Exogenous administration of IFN-α2a early on augments expression of antiviral genes and prevents systemic infection; however, sustained IFN-α2a treatment induces type I IFN desensitization, decreases antiviral gene expression, increases viral load and accelerates $CD4^+$ T cell loss. In mouse, constitutive absence of pDCs or loss of their ability to signal through TLR7 and TLR9 in chronic LCMV infection appears to negatively impact T cell priming and viral clearance[157,172]. Moreover, early administration of exogenous type I IFN prevents chronic LCMV infection. However, late type I IFN administration has no beneficial effects[173]. In fact, blockade of type I IFN signaling during chronic LCMV infection improves T cell function and diminishes viral persistence[174,175]. Thus, there is a window of opportunity - i.e.

early during infection - when type I IFNs and pDCs can be both beneficial and necessary to prevent or control chronic viral infection and preserve T cell numbers and functions.

Pathogenic functions of pDCs in autoimmunity

Role of pDCs in systemic lupus erythematosus

pDCs have been implicated in the pathogenesis of autoimmune diseases that are characterized by a type I IFN signature, such as systemic lupus erythematosus (SLE) (reviewed in[176]). In SLE, antinuclear antibodies form immune complexes with endogenous nucleic acids, which are delivered to pDC endosomal compartments via the Fc receptor CD32, where they activate TLR7 and TLR9[90–92]. This process is enhanced by high mobility group box 1 (HMGB1)[177]. Neutrophils have been proposed to be a prominent source of endogenous nucleic acids for activation of pDCs in SLE. After exposure to anti-ribonucleoprotein antibodies, neutrophils release extracellular traps (NETs) containing chromatin DNA, the antimicrobial peptide LL-37 and HMGB1, which trigger the TLR9 pathway in pDCs, resulting in type I IFN secretion[178,179]. Counter to this hypothesis, lupus-prone MRL.*Fas*^{lpr} mice have markedly exacerbated lupus when crossed with mice lacking NADPH oxidase (NOX2), such that neutrophils are incapable of forming NETs[180]. Thus, endogenous nucleic acids that stimulate pDCs in SLE may be derived from other modes of cell death, such as necroptosis or pyroptosis, as well as from NADPH-independent release of mitochondrial DNA[181].

Sustained activation of pDCs is also detrimental in SLE because it results in the resistance of pDCs to therapeutic glucocorticoids (GC), which are often administered to patients with SLE to control inflammation[182, 183]. Bioinformatics and functional analyses have suggested that TLR activation prevents GC-induced pDC apoptosis by inhibiting miR-29b and miR-29c, which promote pDC apoptosis by targeting MCL1 and BCL2[184]. Endogenous GC also have an impact on pDC homeostasis and survival[185]. Regulation of endogenous GC concentrations involves conversion of inactive substrates to active 11-hydroxyglucocorticoids by an enzyme complex containing 11β-hydroxysteroid dehydrogenase type 1 (11βHSD1) and hexose-6-phosphate dehydrogenase (H6PDH). Under physiologic conditions, 11βHSD1-H6PDH increases the sensitivity of pDCs to GC-induced apoptosis; however, following activation with TLR ligands, the effects of enzyme activity are overridden.

Studies in various rodent models of SLE have confirmed that type I IFNs and pDCs contribute to disease pathogenesis. Male mice of the BXSB lupus-prone strain have a duplication of TLR7 and develop spontaneous autoimmunity. Blockade of type I IFN signaling early on in these mice reduces disease manifestations and extends survival[186]. Disruption of type I IFN signaling in MRL.*Fas*^{lpr} mice also provides some therapeutic benefit, however, it does not impact mortality. Additionally, autoimmune skin inflammation induced by tape stripping in lupus-prone (NZBxNZW)F(1) mice is ameliorated by antibody-mediated pDC depletion or by blocking TLR7 and TLR9 signaling with a bifunctional inhibitor[187].

Genetic models that specifically address the contribution of pDCs in autoimmunity have been lacking until recently. Deletion of *Irf8* in NZB mice and a mutation in *Slc15a4* in C57BL/6.*Fas*^{lpr} mice abolish production of autoantibodies and disease manifestations[188]. While this study provides compelling evidence that pDCs and their TLR7/TLR9-mediated responses are damaging in SLE, IRF8 and SLC15A4 are not exclusively expressed by pDCs. In fact, B cell expression of SLC15A4 appears to be necessary for TLR7-induced expression of type I IFN and pathogenic antibody production in a mouse model of lupus[189]. Analysis of MRL.*Fas*^{lpr} mice lacking MyD88 showed that MyD88-dependent production of IFN-α by pDCs contributes to SLE pathogenesis, particularly B lymphopenia, but not to nephritis[190]. Two recent studies using lupus-prone mice that specifically lack pDCs have confirmed that pDCs are pathogenic in SLE. Global or CD11c-specific *Tcf4* haplodeficiency in lupus-prone mice nearly abolishes autoantibody production and glomerulonephritis[191], whereas transient depletion of pDCs prior to disease initiation in BXSB mice impairs expansion and activation of B and T cells and reduces antinuclear antibodies and expression of type I IFN-induced genes in tissues[192]. Taken together, these studies validate pDCs as a therapeutic target in SLE.

pDCs in other autoimmune diseases

Chronic activation of pDCs and dysregulated type I IFN production also appear to be contributing factors in initiating and/or promoting psoriasis and type I diabetes (T1D). In psoriasis, self nucleic acids form complexes with antimicrobial peptides which activate pDCs through TLR7 and TLR9[193–195]. Interestingly, the Vitamin D analogue calcipotriol, which is used to treat psoriatic skin lesions, triggers tolerogenic responses in cDCs and was recently shown to impair the capacity of both human and mouse pDCs to induce T cell proliferation and effector T cell differentiation[196]. In the nonobese diabetic (NOD) mouse model of T1D, activated B-1a cells produce DNA-reactive antibodies that stimulate release of cathelin-related antimicrobial peptide (CRAMP) from neutrophils; CRAMP subsequently binds to self DNA and activates pDCs through TLR9[197].

The deleterious role(s) of pDCs in psoriasis and T1D have not been confirmed yet in genetic models, but blockade of type I IFN or antibody-mediated depletion of pDCs appears to confer protection in mouse models of these diseases[197–199]. Future studies will be necessary to address whether either blockade of type I IFN or depletion of pDCs are potential therapeutic avenues for intervention in psoriasis and T1D in human.

Gut-associated pDCs

As mucosal immunity and microbiome research is rapidly progressing, pDCs that reside within gut and gut-associated lymphoid tissues are also becoming the focus of increasing interest. In the TNBS-induced colitis model, PSA-induced protection is highly dependent on the ability of pDCs to induce the generation of IL-10-producing T_{Reg} cells[111]. pDCs from the liver and mesenteric lymph nodes are also important for suppression of T cell responses and induction of oral tolerance[200]. In contrast to splenic pDCs, pDCs from Peyer's patches are unable to produce type I IFN after TLR7 or TLR9 stimulation[201]. pDCs that have been developed in the presence of type I IFN resemble Peyer's patch pDCs, produce inflammatory cytokines, stimulate Th17 cell generation and fail to secrete IFNα after TLR

engagement[202]. Therefore, factors expressed at mucosal sites inhibit type I IFN secretion by pDCs, but do not block the ability of pDCs to prime naïve T cells and trigger T_{Reg} and Th17 differentiation[203,204].

Gut-associated pDCs are also efficient at generating mucosal B cell responses under normal conditions and during infection with a gut-tropic virus. In the steady-state, pDCs from Peyer's patches and mesenteric lymph nodes induce T cell-independent IgA production[205]. Specifically, the microbiota activates stromal cells to produce type I IFN, which drives pDC expression of BAFF and APRIL that facilitate IgA responses in the gut. Viral clearance and protection against reinfection with rotavirus, an intestinal pathogen, is highly dependent on B cells[206]. Type I IFN production by human and mouse pDCs exposed to rotavirus appears to be necessary for B cell activation, virus-specific antibody secretion and viral clearance during infection[206]. How the microbiota influences pDC functions is largely unknown, but an earlier study found that colonization of the intestine with a restricted microflora resulted in lysis of pDCs by activated cytotoxic T lymphocytes[207]. Therefore, specific microbial communities are likely to have a profound impact on pDC homeostasis and function through both direct and indirect mechanisms.

Concluding remarks

Although many immune functions of pDCs have now been described, many questions still remain concerning their complex biology. Tuberculosis in humans is associated with a type I IFN signature[208] and pDC accumulation in lymph nodes[209]. So what is the role of pDCs in tuberculosis and in bacterial infections in general? Can bacterial products activate pDCs and what are the sensors for these products? Although pDCs accumulate in the microenvironment of some tumors, they are reduced in other cancers such as chronic lymphocytic leukemia (CLL)[210]. What is the impact of pDCs in anti-tumor immune responses and can we exploit them to generate effective T cell responses (Box 3)?

Box 3

pDCs and cancer

The recruitment of pDCs to tumors is often associated with poor prognosis as tumor-infiltrating pDCs tend to be tolerogenic rather than immunogenic. Unstimulated or alternatively activated pDCs can induce T_{Reg} cells through expression of IDO[135–138] or ICOSL[139]. The accumulation of IDO-expressing cells in tumor draining lymph nodes has been associated with worse clinical outcomes in patients with malignant tumors including breast carcinoma[137]. The infiltration of pDCs in primary tumors of patients with invasive nonmetastatic breast cancer correlates with shorter survival times, suggesting that pDCs contribute to the progression of breast cancer[237]. Breast tumor-associated pDCs are poor producers of type I IFN and favor the expansion of T_{Reg} cells[238]. The mechanisms suppressing type I IFN production and immunogenic capacity of breast tumor infiltrating pDCs include tumor cell-derived TGF-β and TNF-α[239], both TGF-β and TNF-α inhibit type I IFN secretion by pDCs[201,240]. ICOSL expression on pDCs also appears to correlate with breast cancer progression by supporting amplification of IL-10 producing T_{Reg} cells[241].

Recruitment of pDCs to ovarian tumors is driven by CXCL12 and is associated with impaired T cell responses and poor prognosis[72,242]. Compared to pDCs in blood or ascites, ovarian tumor-associated pDCs produce less type I IFN and pro-inflammatory cytokines/chemokines in response to TLR stimulation and induce IL-10 production from allogeneic naive $CD4^+$ T cells, suggesting that they play a critical role in the induction of immune tolerance and progression of ovarian cancer[243].

Human pDCs may also contribute to cancer progression via the production and release of the pro-apoptotic molecule granzyme B[12], which suppresses T cell proliferation. Activation of pDCs through TLR agonists and CD40 ligand negatively regulates granzyme B expression and the ability of pDCs to suppress T cells[142]. In contrast to NK cells, pDCs do not release the pore-forming protein perforin, and therefore are unable to kill tumor target cells by release of lytic granules.

On the other hand, pDCs can promote immunogenic anti-tumor responses if appropriately stimulated. Injection of activated pDCs loaded with tumor-associated peptides into metastatic melanoma patients leads to favorable CD4 and CD8 T cell responses, indicating that vaccination strategies employing activated pDCs might be an attractive therapeutic strategy to overcome immune tolerance in certain types of cancer[244]. In an orthotopic murine mammary tumor model, intratumoral administration of a TLR7 ligand results in tumor-associated pDC activation and has a potent anti-tumor effect[245]. In the B16 melanoma mouse model, TLR-stimulated pDCs mediate tumor killing through expression of TRAIL and granzyme B[75] as well as through the activation of NK cells[246]. The ability of pDCs to produce cytolytic mediators and the functional relevance of this capacity in the elimination of target cells and immune regulation has been extensively debated; however, it has been shown that the FSME-IMMUN vaccine against tick-borne encephalitis stimulates pDCs to upregulate neural cell adhesion molecule 1 (NCAM1, also known as CD56) and TRAIL expression, thereby generating a subset of pDCs that combines the abilities of interferon production and antigen presentation with that of lysing target cells[247]. CD56 expression is also typical of several neoplastic human pDC tumors[248].

Although SLE is generally associated with a type I IFN signature, it has been recently shown that pDC production of type I IFN is impaired in patients carrying a variant of the *PTPN22* gene predisposing to SLE[211]. This observation raises the question of whether pDC and type I IFN depletion is a valid strategy for all SLE patients or should be adopted after stratification of the patients for pDC activity. Recent studies suggest that pDC dysfunction in autoimmune and inflammatory diseases may extend to scleroderma[212], Sjögren syndrome[213], Wiskott-Aldrich syndrome[214] and atherosclerosis[215]. Is there a role for pDCs in other inflammatory diseases such as IBD? Is manipulation or elimination of pDCs a feasible therapeutic strategy for some of these diseases? Whether pDCs are valid targets during viral infections remains a matter of debate. While pDC activation may be beneficial at early stages of infection, depletion of pDCs may be considered during chronic viral infections, such as HIV, when type I IFN may exert negative effects.

Addressing these and other questions in mouse models will require advanced tools to deplete pDCs *in vivo*. The development of CLEC4C-DTR transgenic mice has provided a method for specific but transient pDC depletion[216]. Conditional targeting of E2-2 in $CD11c^+$ cells has enabled constitutive depletion of pDCs[157], although $CX3CR1^+CD8\alpha^+$ DCs[49], which are closely related to pDCs, and possibly B cell subsets expressing CD11c may also be affected. It will be important to continue to identify pDC-specific genes that can be used for development of pDC lineage-specific deficient mice and pDC-specific Cre deleters. With the recognition of pDC involvement in human pathologies, it is predicted that these cells will be more extensively evaluated for diagnostic and prognostic purposes. Indeed, it will be interesting to explore the therapeutic potential of monoclonal antibodies that selectively target pDCs in many of the disease scenarios discussed above.

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Glossary terms

star-form partner	The less abundant of the two strands of microRNA (miRNA) that are generated during miRNA biogenesis. Star-form miRNAs may have important functions in miRNA regulatory networks
noncanonical NF-κB pathway	Nuclear factor-κB (NF-κB) signaling that is induced by certain members of the tumour necrosis factor receptor superfamily and predominantly activates p52–RELB NF-κB complexes. By contrast, canonical (or classical) NF-κB signaling typically involves the activation of p50–RELA dimers and is induced by a wider range of pro-inflammatory stimuli
B-1a cells	A subset of innate-like B cells

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Biographies

Marco Colonna obtained his MD degree from Parma University, Italy, and completed post-doctoral training at Roswell Park Memorial Institute and Harvard University. He established an independent laboratory at the Basel Institute for Immunology in 1994 and became Professor of Pathology and Immunology at the Washington University in St. Louis in 2001. He focuses on innate immunoreceptors, a field in which his accomplishments encompass identification of the KIR receptors and HLA-C polymorphisms as their inhibitory ligands, as well as the LILR and TREM receptors. Through analysis of the cellular distribution of these

receptors, he identified pDCs and Innate Lymphoid Cells, which are a major focus of his laboratory.

Melissa Swiecki received her PhD in Microbiology from the University of Alabama at Birmingham in 2007. She joined the Colonna laboratory in 2007 as a postdoctoral trainee and then as Research Instructor. She studied the impact of pDCs on antiviral responses as well as surface receptors that influence pDC homeostasis and function. She is currently a Scientist in Discovery Immunology at Janssen Research & Development, LLC.

Key Points

- This Review provides an introduction to human and mouse plasmacytoid dendritic cells (pDCs) and their importance in the immune system.
- We discuss the phenotypes of human and mouse pDCs and the mechanisms that control their development.
- We explain how pDCs sense nucleic acids and microbial products as well as their potential role in viral infections and diseases characterized by a type I interferon (IFN) signature such as SLE, psoriasis and type I diabetes.
- We discuss the emerging field of microbiome research and the impact of pDCs on mucosal immune responses.
- We describe how pDCs can be tolerogenic or immunogenic in the context of cancer.
- Finally, we end by raising several questions regarding the validity of targeting pDCs or their type I IFN production as a therapeutic strategy.

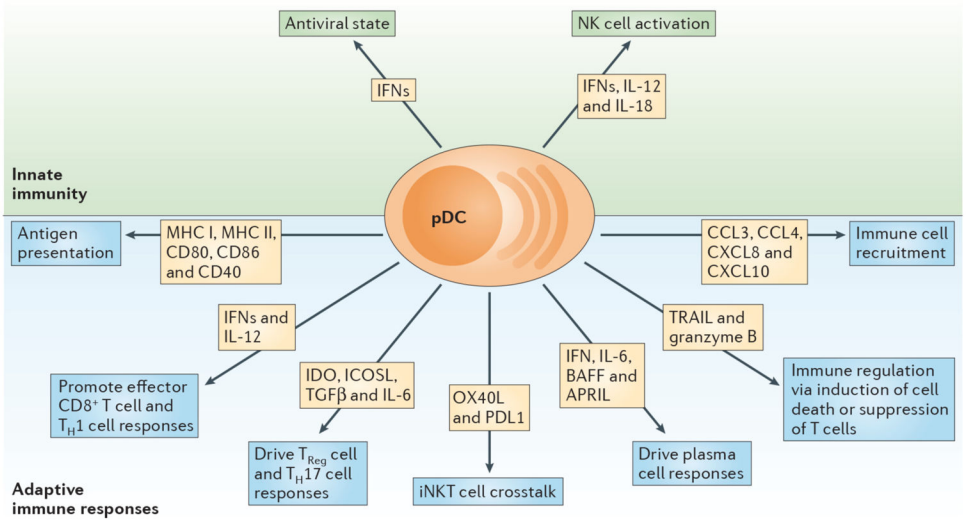

Figure 1. Diverse functions of pDCs

pDCs are a prominent link between innate and adaptive immune responses. Their ability to rapidly produce type I IFNs (IFN-Is) during viral infections promotes an antiviral state by inducing expression of interferon stimulated genes and apoptosis of infected cells. Moreover, IFN-I, IL-12 and IL-18 enhance NK cell activation and effector functions such as IFN-γ secretion and lysis of target cells. Expression of MHC class I (MHCI) and MHC class II (MHCII) along with costimulatory markers including CD80, CD86 and CD40 enable pDCs to cross prime $CD8^+$ T cells and present Ag to $CD4^+$ T cells. Production of IFN-I and IL-12 by pDCs supports accumulation and effector functions of $CD8^+$ T cells as well as the polarization of $CD4^+$ T cells into T helper 1 cells (Th1). pDC expression of IDO and ICOSL or production of TGF-β and IL-6 promotes T_{Reg} or Th17 commitment, respectively. Crosstalk between pDCs and invariant NKT (iNKT) cells occurs via OX40/OX40L and PD-1/PD-L1 interactions and dampens antiviral adaptive immune responses. pDCs impact B cell activation, plasma cell generation and antibody secretion through production of IFN-I, IL-6, BAFF and APRIL. TRAIL and Granzyme B (GrB) serve as immunoregulatory factors that endow pDCs with the capacity to kill tumor cells, induce apoptosis of infected $CD4^+$ T cells and suppress T cell proliferation. Finally, pDCs secrete chemokines such CXCL8, CXCL10, CCL3 and CCL4, which attract immune cells to sites of infection or inflammation.

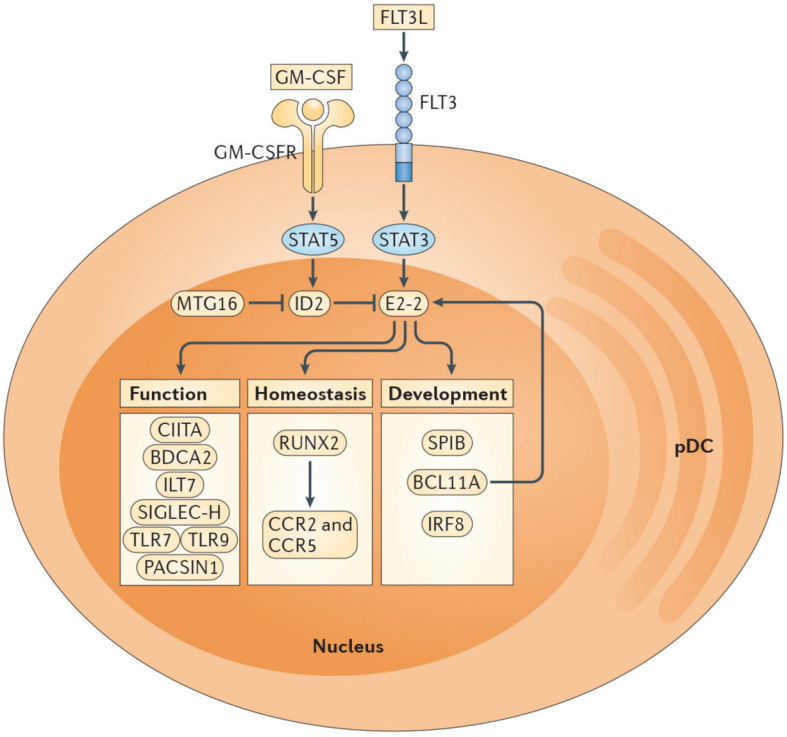

Figure 2. Regulation of pDC development and function

The development of pDCs from CDPs is regulated by specific cytokines and transcription factors. FLT3L-STAT3 signaling promotes expression of E2-2, which is the master transcription factor required for pDC development. The absence of E2-2 or its deletion in mature pDCs results in the complete loss of pDCs or the differentiation of pDC-committed cells into cDC-like cells, respectively. In contrast, GM-CSF-STAT5 signaling blocks pDC differentiation during DC development by inducing expression of ID2, which is an antagonist of E2-2. The transcriptional cofactor MTG16 promotes pDC differentiation and restricts cDC development in part by repressing ID2. Transcriptional targets of E2-2 encode proteins associated with pDC development, homeostasis and function. SPIB, BCL11A and IRF8 are necessary for pDC differentiation and/or survival while RUNX2 controls pDC homeostasis through expression of the chemokine receptors CCR2 and CCR5, which permit egress from the bone marrow. CIITA promotes MHC class II expression. BDCA2, ILT7 and SIGLEC-H are markers that are selectively expressed by human or mouse pDCs and are involved in regulation of type I IFN production. TLR7, TLR9 and PACSIN1 enable pDC recognition of nucleic acids and pathogens (i.e. RNA and DNA viruses), resulting in type I IFN secretion and/or pro-inflammatory cytokine production.

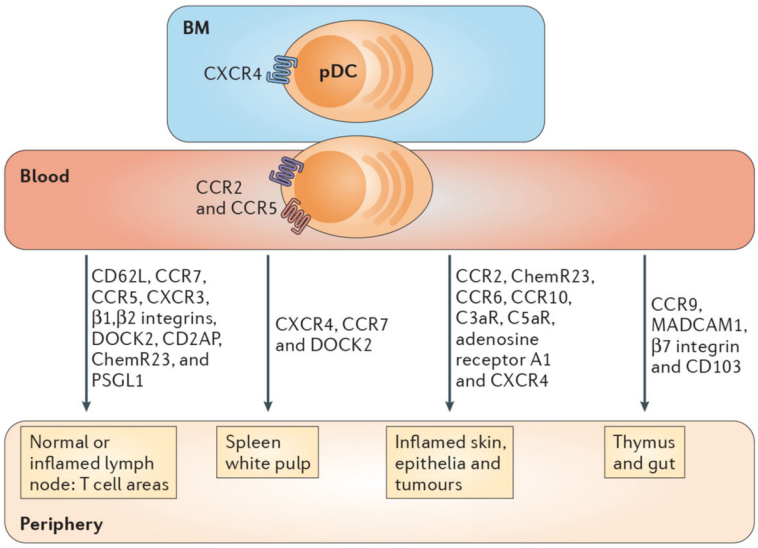

Figure 3. Factors influencing pDC migration

pDCs express chemokine receptors and homing molecules that promote recruitment in the steady-state and during inflammation. Development of pDCs in bone marrow (BM) stromal cell niche requires CXCR4 expression, while pDC egress from the BM into the blood is dependent on CCR5 and CCR2. pDCs are attracted to tumors that produce CXCL12 (not shown) and the splenic white pulp via CXCR4. DOCK2, a hematopoietic cell-specific CDM family protein involved in CXCR4 signaling, is also necessary for pDC migration to spleen and lymph nodes (LNs). pDCs express CD62L, PSGL-1, β1/β2 integrins and the chemokine receptors CCR5, CXCR3 and CCR7 which mediate adhesion and chemotaxis to peripheral LNs and the splenic white pulp under normal and/or inflammatory conditions. Blood pDCs express the chemerin receptor, ChemR23, as well as A1-R, C3aR and C5aR, which may guide them to peripheral LNs and damaged tissues. CD2AP is an intracellular protein that regulates actin dynamics and promotes pDC migration to LNs under inflammatory settings. CCR2 drives the recruitment of pDCs to the skin following inflammation induced by the TLR7 agonist, Imiquimod. CCR6 and CCR10 are expressed by a subset of human tonsil pDCs and enable migration to inflamed epithelia producing CCL20 and CCL27 (not shown). CCR9 and its ligand CCL25 (not shown) promote trafficking of peripheral pDCs to the thymus and are required for pDC recruitment to the small intestine under both normal and inflammatory conditions. MAdCAM-1, β7 integrin and CD103 also influence pDC trafficking to the gut. Finally, pDCs express CX3CR1 (not shown), which may impact their homeostasis.

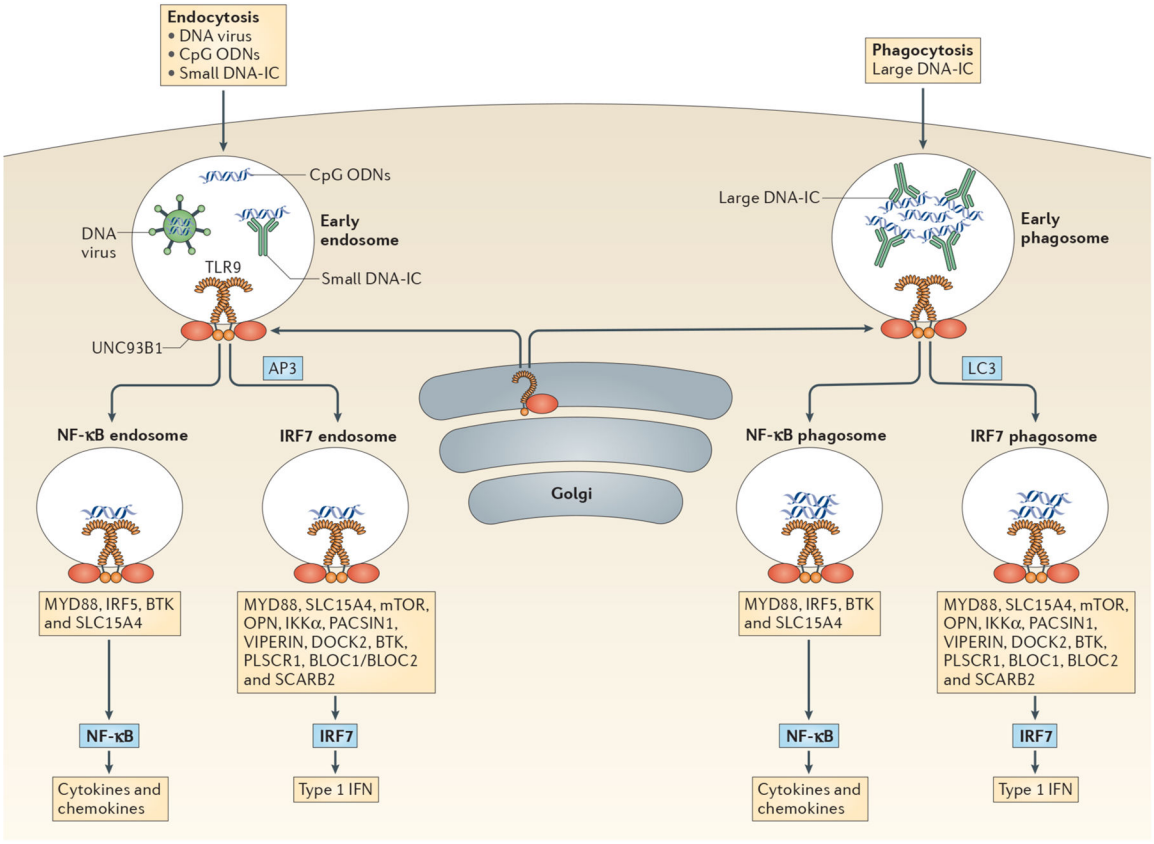

Figure 4. TLR9 signaling in pDCs

pDCs sense DNA viruses, synthetic CpG oligodeoxyribonucleotides (ODN) and endogenous DNA through TLR9. All TLR9 signaling requires MyD88 but additional factors determine whether TLR9 engagement will result in type I IFN (IFN-I) or pro-inflammatory cytokine production. These factors include mode of ligand entry, and the intracellular compartment where TLR9 encounters its ligand. TLR9 is transported to appropriate intracellular compartments by UNC93B[249,250] and requires cleavage in order to recruit MyD88[251,252]. DNA viruses, CpG ODN and small DNA immunecomplexes (DNA-ICs) enter pDCs through endocytosis and meet TLR9 in the early endosome. In contrast, large DNA-ICs are internalized by phagocytosis and encounter TLR9 and UNC93B in the early phagosome. If AP3 or LC3 are recruited then the IRF7 endosome or IRF7 phagosome is formed which leads to IFN-I production. Several molecules/pathways are involved in this process including IKKα, osteopontin (OPN), SLC15A4, BTK, BLOC1, BLOC2, DOCK2, PACSIN1, PLSCR1, VIPERIN, SCARB2 and the mTOR pathway. Alternatively, TLR9-containing compartments can form NF-κB endosomes or NF-κB phagosomes resulting in the production of pro-inflammatory cytokines and chemokines (i.e. IL-6, IL-12, TNF-α, etc). This process requires IRF5, BTK and SLC15A4. CpG ODN have different structures which results in trafficking to different compartments: CpG-A is transported to the IRF7 endosome and is a strong inducer of IFN-I, while CpG-B aggregates in the NF-κB endosome and is a potent stimulator of maturation and cytokine/chemokine production. CpG-C exhibits properties of both CpG-A and CpG-B in that it can induce both IFN-I and

pro-inflammatory cytokines. TLR7 stimulation by viral and endogenous RNA may follow similar pathways.