

Review

Plasmacytoid Dendritic Cells Act as the Most Competent Cell Type in Linking Antiviral Innate and Adaptive Immune Responses

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Appropriate *in vivo* control of plasmacytoid dendritic cell (pDC) recruitment and activation is a fundamental requirement for defense against viral infection. During this process, a pivotal event that influences the outcome of viral infection is the production of high levels of type I interferon by pDCs. In particular, recent research findings showed that pDCs not only shape the nature of innate resistance, but are also responsible for the successful transition from innate to adaptive immunity for viral resistance. In addition, pDCs can differentiate into antigen presenting cells that may regulate tolerance to a given pathogen. Importantly, in a series of recent clinical studies, pDCs appeared to be defective in number and function in conditions of chronic viral diseases such as infected with HIV-1, HBV or HCV. pDC-associated clinical antiviral therapy is also emerging. This review describes research findings examining the functional and antiviral properties of *in vivo* pDC plasticity. *Cellular & Molecular Immunology*. 2005;2(6):411- 417.

Key Words: virus, plasmacytoid dendritic cell, innate immunity, adaptive immunity

Introduction

Infectious diseases have been a dominant cause of human morbidity and mortality throughout the history of mankind and emerging infectious diseases, led by pathogenic viruses, constitute a growing threat to public health. It is well known that host non-specific innate immunity and specific adaptive immunity play crucial roles in eradicating invading pathogens through the synergistic actions of immune cells and their effector proteins. Increasing evidence has recently shown that plasmacytoid dendritic cells (pDCs) function not only as part of an innate immune response through the release of large amounts of type I interferons (interferon- α , β , ω) after viral infection, but also as participants of an adaptive immune system through differentiation into DCs that can present antigens to T cells. In particular, pDCs have drawn much attention in the study of endogenous systems of defense against virally infectious diseases, as well as in cancer and autoimmune diseases. Through more than two

decades of research, pDCs have finally established their place in the hematopoietic development pathway as the most important cell type in antiviral innate and adaptive immune systems (Figure 1). In this review, we summarize recent progress on the origin, characterization and clinical significance of human pDCs.

Discovery, distribution and origins of pDCs

Since 1958, numerous terms, such as T-associated plasma cells, plasmacytoid T cells, plasmacytoid monocytes, type 2 DC precursors (pDC2) and natural type I interferon-producing cells (IPCs), have been proposed to describe pDCs based on their features of morphology, localization, phenotype and function. Dr. Liu and his team have been leaders in pDC research and have made outstanding contributions to the field (1).

The identification of human pDCs in fetal liver, thymus and bone marrow suggests that pDCs develop from hematopoietic stem cells in these primary lymphoid tissues. During adult life, pDCs are produced constantly in bone marrow and, in steady-state conditions, circulate in the blood and migrate to lymphoid tissues (lymph nodes (LNs), tonsils, spleen, thymus, bone marrow, mucosal-associated lymphoid tissues and Peyer's patches) as well as to certain peripheral tissues (2). Also, pDCs can migrate to and accumulate in inflammatory sites where they may contribute to the ongoing inflammatory response through release of cytokines and chemokines and activation of lymphocytes. Additionally, they may contribute to the induction of tolerogenic responses to tumors. How do pDCs enter LNs and inflammatory sites?

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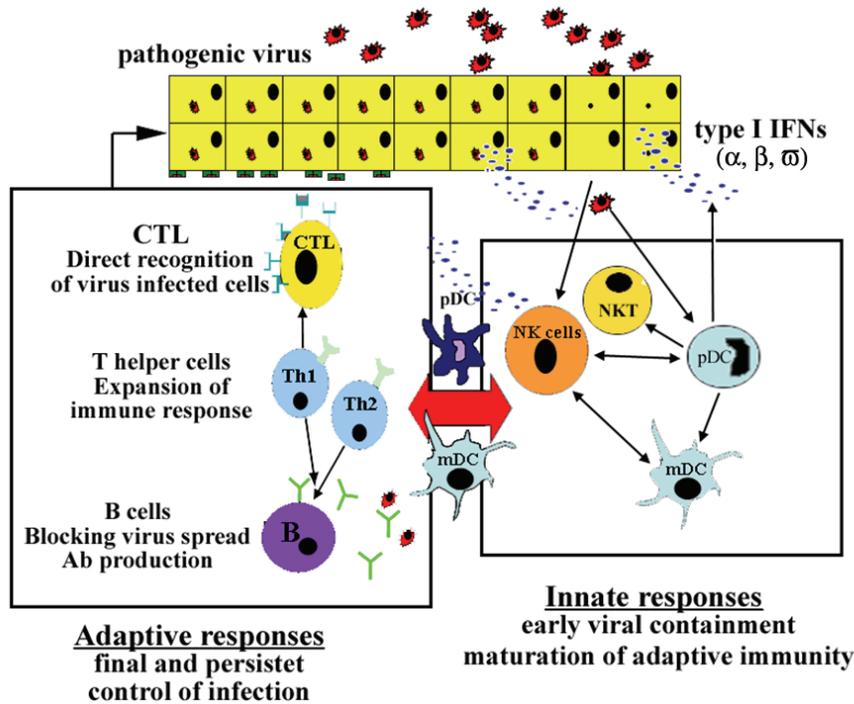


Figure 1. Summary of interaction between innate and adaptive immune responses to a pathogenic virus. The DCs are positioned in the critical intersection between these two systems.

This unique migration property of pDCs appears to be associated with their expression of additional chemokine receptors (e.g., CCR2, CCR5, CXCR3, CXCR4, CD62L and CCR7). Generally, pDCs can migrate efficiently to the CXCR4 ligand SDF-1/CXCL12 expressed on dermal endothelial cells in LN high endothelial venules (HEVs) or in malignant cells. pDCs also migrate to the CXCR3 ligands produced by Th1 cells. The reason pDCs are able to migrate is that mature pDCs can up-regulate the expression of CD62L and CCR7, which interact with L-selectin ligands expressed by HEV and chemokines ELC/CCL19 and SLC/CCL21 expressed by HEV as well as stromal cells within T cell-rich areas (2).

It is unclear whether pDCs are derived from a myeloid or lymphoid progenitor. Although some evidence suggests that pDCs are derived from a lymphoid progenitor that is distinct from that of myeloid DCs (mDCs), this concept is challenged by a recent report (3) that pDCs originated from common myeloid progenitors (CMP), express RAG gene products, and show IgH D-J rearrangement, suggesting that pDCs probably represent a very unique hematopoietic lineage. Currently, the developmental progression and molecular regulation of pDCs are not completely understood. Based on seemingly divergent findings, several different hypotheses have been proposed to depict the possible developmental origins of pDCs, including the existence of a common DC precursor in the blood that can give rise to all DC subsets, pDCs arising as a branch of the committed lymphoid lineage, and pDCs being a population of lymphoid cells undergoing

an *in vivo* cell fate conversion from a lymphoid to a myeloid cell type. Though mDCs are comprised of several subsets, such as Langerhans cells, dermal dendritic cells and interstitial DCs, specific subpopulations of pDCs have not yet been identified.

The phenotypic features of pDCs

pDCs represent 0.2%-0.8% of peripheral blood mononuclear cells and display plasma cell morphology in humans. Recently, a collection of surface molecules were identified on pDCs that express the CD4 molecule and IL-3R α and HLA-DR molecules, but not T cell receptors, CD3 chains, B cell lineages (CD19, CD21) or myeloid markers (CD13, CD14, CD33). Therefore, these phenotypic features, such as the CD4⁺CD45RA⁺CD123⁺ILT3⁺MHCII⁺ILT1⁻CD11c⁻ lineage, suggest that the lineage of pDCs is distinct from that of conventional myeloid CD11c⁺ DCs. In addition, pDCs also express BDCAs and TLRs that associate with pDC function.

BDCAs

pDCs express BDCA-2 (CD303) and BDCA-4 (CD304), dectin-1, and possibly DEC-205, but lack DC-SIGN and langerin. These surface receptors belong to the C-type lectin (CLRs) family of transmembrane glycoproteins on DC subsets, but their physiological functions have not yet been resolved. Anti-BDCA-2 antibodies are rapidly internalized and efficiently presented to T cells, suggesting a role in

antigen capture and presentation (4). The monoclonal antibody against BDCA-2 is highly specific for human pDCs. However, its use for pDC enrichment is limited, as BDCA-2 engagement results in inhibition of type I IFN production. Another pDC marker, the BDCA-4/neuropilin-1 receptor, is a member of the semaphorin family and functions as a co-receptor for vascular endothelial growth factor (VEGF). Antibodies to BDCA-4 are now commonly used for magnetic bead enrichment of human pDCs from blood.

Toll like receptors (TLRs)

TLRs serve as primary sensors for recognizing conserved structures of bacteria, fungi and viruses through pathogen associated molecular patterns (PAMPs) to activate innate immune cells in the host. Human pDCs express only TLR7, 8 and 9, and do not express TLR2, TLR3, TLR4 and TLR5. Therefore, human pDCs respond only to some DNA and RNA viruses.

TLR7 mediates pDC responses through binding to guanosine analogs as well as imidazoquinolines, which are used as antiviral compounds in the treatment of human papilloma virus infections. Guanosine- and uridine-rich ssRNA, including RNA derived from the HIV-1 U5 region, are also recognized by human TLR8 (5). In response to binding of small ssRNA segments of 20 nucleotides, human pDCs can secrete IFN- α , while mDCs produce IL-12p40, IL-6, and TNF- α (5).

TLR9 is engaged by unmethylated CpG-rich DNA that is common in the genomes of DNA viruses and bacteria. Interestingly, the response of human pDCs is dependent upon the class of CpG ODNs to which they are exposed. For example, stimulation of CpG A and CpG B can induce two distinct pathways of IFN- α/β production (6). Stimulation with CpG A (D)/2216 ODN induces pDCs to produce large amounts of type I IFN but minimal up-regulation of cell surface maturation markers such as CD80, CD86, and HLA-DR (MHC-II). On the other hand, stimulation with CpG B (K)/2006 results in increased expression of surface co-stimulatory and antigen presenting molecules and heightened IL-8 and TNF- α secretion but low levels of IFN- α production by pDCs. pDCs constitutively express IRF-7 and synthesize high levels of IFN- α in response to CpG A, which also triggers an autocrine feedback loop involving the IFN receptor-dependent pathway. In contrast, IFN- α/β induction by CpG B is independent of the IFN- α/β receptor loop. A recently developed class of CpG ODNs, CpG C, in which structural elements of CpG A and CpG B have been combined and emerged, activates B cells and also induces IFN- α production by pDCs (7).

Functional plasticity of pDCs in linking innate immunity and adaptive immunity

In comparison with mDCs that capture antigens in peripheral tissues and then initiate immune responses in secondary lymphoid organs, pDCs may specialize in modulating the

strength, duration and quality of natural killer (NK), NKT, T and B cell response by presenting antigens as well as releasing appropriate cytokines and chemokines. At the early stage of viral infection, pDCs act as effector cells and promptly secrete massive amounts of type I interferon, activating NK cells, NKT cells, B cells, T cells, and myeloid DCs. pDCs then differentiate into unique, mature dendritic cells that regulate the function of T cells through directing T cell polarization and up-regulating expression of MHC class II and other T cell-stimulating molecules.

The functional interaction between pDCs and other immunocytes may physiologically be critical in the regulation of both innate resistance and adaptive immunity to infections. pDCs could amplify innate immunity through IFN- α/β -mediated activation of additional immune cells including NK and NKT cells. Recent studies suggest that human pDCs, following engagement of TLR9 or inactivated influenza virus, activate and recruit autologous NK cells and enhance their cytotoxicity. Similar findings showed that pDCs amplified the NKT cell mediated-immune response and directed the development of NKT cells (9). Moreover, pDCs have been shown to amplify adaptive immunity of other antigen-presenting cells including B cells, mDCs and monocytes, through IFN- α/β -mediated activation. Results of one study showed that IFN- α and IL-6 secreted by influenza virus-activated pDCs acted sequentially to drive virus-specific B cells to differentiate into plasma blasts and then mature plasma cells (10). pDCs also control TLR7 sensitivity of naïve B cells *via* type I IFN (11). In addition, human pDCs can induce a bystander maturation of mDCs in response to HIV infection *in vitro* by producing type I IFN and TNF- α (12). pDCs also amplify antimicrobial responses through IFN- α -mediated effects on monocytes. When monocytes are cultured with GM-CSF and IFN- α in place of IL-4, they differentiate into DCs with the capacity to drive a Th1 response that is independent on IL-12 and partially dependent on IFN- α .

Several studies have shown that activated pDCs are capable of priming CD8⁺ T cell responses to endogenous antigens or peptides (but not responses to exogenous antigens). They also induce expansion of virus-specific memory CD8⁺ T cell populations and CD4⁺ T cell populations for specific endogenous antigens and an influenza virus (13). Importantly, both virus- or IL-3- and CD40L-activated pDCs have been shown to elicit a potent Th1 polarization by producing large amounts of type I interferon, or to elicit Th2 polarization by an OX40L-dependent mechanism (14). Thus, pDCs can display functional plasticity in terms of priming different effector T cell responses, depending upon the maturation stage, nature, concentration of the antigen and the circumstances.

Recently, much attention has been paid to the tolerogenic characteristics of pDCs. pDCs stimulated with the inhibitory ligands CTLA4-Ig or OX2(CD200)-Ig can induce the production of indoleamine 2,3-dioxygenase, which profoundly inhibits T cell proliferation, potentially contributing to the tolerogenic effects of pDCs (15). Human pDCs can induce

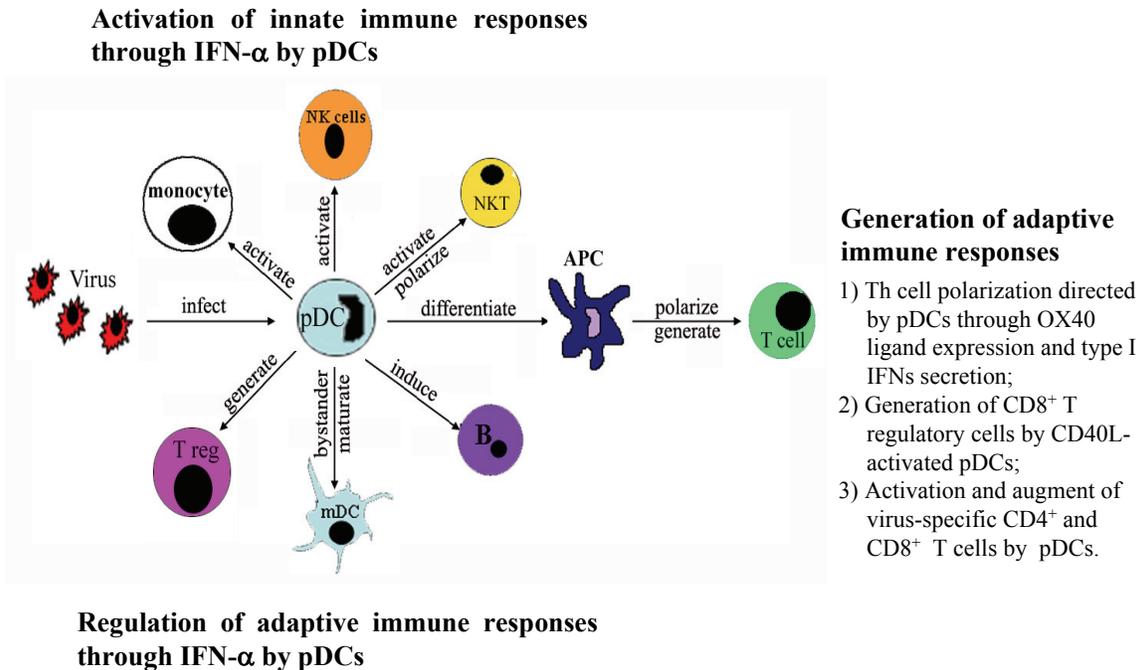


Figure 2. Central role of pDCs: activation of the innate and adaptive immune systems. Upon being encountered with virus, pDCs release an amount of type I IFNs immediately, which activates innate immune responses and regulates adaptive immune responses. Sequentially, pDCs differentiate into a unique type of mature dendritic cells that directs the polarization of T cells as antigen-presenting cells.

CD4⁺CD25⁺ T regulatory cells (16). CD40L-activated pDCs can also induce naïve CD8⁺ T cells to differentiate into IL-10-producing CD8⁺ T suppressor cells (17). Such findings suggest that pDC-derived DCs have an intrinsic ability to induce naïve T cells to become tolerogenic, although the exact mechanisms of these functions remain unknown.

In summary, many studies indicate that pDCs represent a unique cell lineage with flexible functions in the immune system. They first rapidly secrete massive amounts of type I IFNs in response to viral infection and promote the function of natural killer cells, NKT cells, B cells, monocytes and myeloid DCs and they subsequently differentiate into professional antigen-presenting cells, which regulate the function of T cells by directing polarization, thus linking innate and adaptive immune responses (Figure 2).

Activation and antiviral activity of pDCs mediated by invading viruses

To activate pDCs, viruses do not need to be replication competent, but the envelope structure must remain intact. Extensive investigations have shown that IFN- α/β are produced by pDCs in response to a wide range of enveloped viruses, including herpes simplex virus (HSV), Sendai virus, human immunodeficiency virus type 1 (HIV-1), influenza virus, Newcastle disease virus, and vesicular stomatitis virus, as well as parasites (*Plasmodium falciparum*), bacteria (e.g., SAC), and DNA containing unmethylated CpG sequences,

typical of microbial DNA. In fact, all types of enveloped viruses affect secretion of IFN- α/β . The amount, kinetics and type depend to a large extent on the cell type, with pDCs being the unique “professional IFN- α/β -producing cells” (1).

HIV-1 infection and AIDS

It has been demonstrated that human pDCs express CD4, CXCR4 and CCR5 that are targeted as receptors and co-receptors for entrance of HIV-1 into the cells. Thus pDCs are highly susceptible to HIV-1 (18) and can transfer HIV-1 preferentially to antigen-specific CD4⁺ T cells (19).

Several studies have demonstrated that both acute and chronic HIV-1 infections resulted in the alteration of phenotypes, functional impairment of IFN- α -release and T-cell activity, and reduced number of pDCs in both adult and pediatric individuals (20-23). The decline of circulating pDCs is inversely correlated with viral load and associated with a fall in CD4⁺ T-cell counts (20). In addition, the loss of pDCs correlates with the occurrence of opportunistic infections and Kaposi sarcoma (22) and may predict progression of the disease (24). Antiretroviral treatment can partially recover pDC number and function in HIV-1-infected adults and children (20, 23). Taken together, these data suggest that pDCs actively participate in the pathogenesis of HIV-1 infection.

Viral hepatitis induced by HCV/HBV

pDCs are also involved in immune responses against HBV/HCV infection. In acute HCV infection, circulating pDC

number and IFN- α -producing capacity are dramatically reduced and are inversely correlated with alanine aminotransferase levels and with the degree of liver inflammation (25). In chronic HCV infection, some studies have shown that low IFN- α -producing capacity and impaired T helper 1 polarization ability of pDCs might be responsible for viral persistence, the typical low anti-HCV immune responses and chronic liver disease (25-27). The loss of circulating pDC number and function was considered to be associated with antiviral therapy (27) and enrichment for pDCs within the intrahepatic compartment (26). Similar observations have been made for patients with HCV-HIV-1 co-infection (28). However, a recent study showed that pDCs are not impaired (29) and lower numbers of pDCs results in decreased IFN- α production in HCV-infected patients (30).

Similar findings indicating a numerical decrease and functional impairment of pDCs were observed in chronic HBV-infected humans. Therefore, impaired DC subsets probably due to chronic HBV infection favor viral persistence and disease chronicity (31, 32). We have observed, in our laboratory, that lamivudine antiviral therapy restored the number of circulating pDCs and there was a reversal of pDC frequency with the control of HBV replication in chronic HBV patients, indicating these subjects are unlikely to be totally immunocompromised. The decrease of pDC frequency was found to be related to nosocomial infections in patients with liver cirrhosis. Our results suggest that chronic HBV-infected patients probably have a quantitative and qualitative impairment of circulating pDCs or NK cells, which may be associated with HBV persistent infection as well as the nosocomial infections that arise in LC patients (31).

SARS

SARS-CoV can infect both immature and mature human monocyte-derived DCs. SARS-CoV infected DCs showed low expression of antiviral cytokines, moderate upregulation of proinflammatory cytokines and significant upregulation of inflammatory chemokines. The lack of an antiviral cytokine response against a background of intense chemokine up-regulation could represent a mechanism of immune evasion by SARS-CoV (33). Another study showed that mDCs could not be infected by SARS-CoV, but these cells were able to transfer the virus to susceptible target cells through a synapse-like form of transmission (34). We found that SARS patients exhibited a rapid, dramatic decrease in the number of circulating pDCs during the first 2 weeks of illness, followed by a slow return to normal cell numbers during convalescence. These findings provide the framework for further studies of the immunopathogenesis of SARS (35).

Dengue virus infection

A recent study showed that an increase of circulating pDC frequency soon after dengue virus infection was associated with mild or asymptomatic disease in nonhuman primates and mild symptomatic acute infection in children. An early decrease of circulating pDC frequency predicts the development of dengue hemorrhagic fever in children.

Therefore, the blunted circulating pDC responses to dengue virus infection may lead to severe disease (36).

Antiviral immunotherapy associated with pDCs

CpG ODNs

It is known that CpG ODN A and B bind to TLR9 presenting in pDCs, and induce the release of large amounts of IFN- α / β and pDC maturation. Recent findings indicated that CpG ODNs or other TLR ligands inhibited HBV replication within 24 h, in an α / β interferon-dependent manner, without cytopathic effects in mice that received a single intravenous injection of various TLR ligands. This finding suggests that TLR signaling activation could represent a powerful and novel therapeutic strategy for treatment of chronic HBV/HCV infection (37). A recently developed hypothesis proposed that TLR9⁺ pDCs were able to release large amounts of type I IFNs in HIV-1 infected and AIDS patients treated with CpG ODN-A and B or CpG ODN-C (38). The clinical safety of CpG ODN has been confirmed in phase I of clinical studies as it appears to be well-tolerated in patients at various doses (39).

FLT3

FLT-3 ligand (FLT-3L) is a key differentiation factor for pDCs derived from hematopoietic progenitor cells (HPCs). *In vivo* injection dramatically increases the numbers of both mDCs and pDCs in human blood samples. FLT-3L and thrombopoietin synergistically induce the generation of large numbers of pDCs, in addition to CD11c⁺ immature DCs and CD14⁺ monocytes from human fetal liver- or blood-derived HPCs (40). These data suggest that FLT-3L treatment may improve antiviral immune responses by increasing the number of pDCs.

Adoptive immunotherapy of pDCs

Since pDCs may serve as targets for therapeutic intervention in viral infectious diseases, an expansion of functional pDCs *in vitro* has occurred, making adoptive pDC immunotherapy to treat virus-infected diseases possible. A recent study showed that thrombopoietin (TPO) cooperates with FLT3-L, inducing CD34⁺ HPCs to undergo a 400-fold expansion in cell numbers of pDCs within 30 days in culture. This new finding will allow *in vitro* generation of large numbers of human pDCs to facilitate future studies of the immune function of pDCs and for examination of their clinical applications in immune-based therapies (41).

Future directions

The number of investigations focusing on the antiviral role of pDCs in immune system defense against invading pathogens is increasing. Gaining a better understanding of pDC biology in particular how the pDC population links innate and adaptive immune defense responses may enable cures for chronic viral infectious diseases induced by such viruses as HIV-1, HBV and HCV to be developed. However, despite

some important findings in the field, there are still mysteries regarding the origins of pDCs and the mechanisms through which they contribute to the control of acute or chronic viral infections. In the pathogenesis of chronic viral infections, the signaling pathway, molecular regulation and influence on tolerance development by pDCs in innate and adaptive immune responses against invading viruses are not fully understood. These and other questions are likely to keep pDCs in the limelight for future investigations of virally infectious diseases.

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