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Review Article

# Plasmacytoid dendritic cell in immunity and cancer

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ABSTRACT

Plasmacytoid dendritic cells (pDCs) comprise a subset of dendritic cells characterized by their ability to produce large amount of type I interferon (IFN-I/α). Originally recognized for their role in modulating immune responses to viral stimulation, growing interest has been directed toward their contribution to tumorigenesis. Under normal conditions, Toll-like receptor (TLR)-activated pDCs exhibit robust IFN-α production and promote both innate and adaptive immune responses. In cancer, however, pDCs demonstrate an impaired response to TLR7/9 activation, decreased or absent IFN-α production and contribute to the establishment of an immunosuppressive tumor microenvironment. In addition to IFN-α production, pDCs can also act as antigen presenting cells (APCs) and regulate immune responses to various antigens. The significant role played by pDCs in regulating both the innate and adaptive components of the immune system makes them a critical player in cancer immunology. In this review, we discuss the development and function of pDCs as well as their role in innate and adaptive immunity. Finally, we summarize pDC contribution to cancer pathogenesis, with a special focus on primary malignant brain tumor, their significance in the era of immunotherapy and suggest potential strategies for pDC-targeted therapy.

## 1. Introduction

The role of the immune system in reacting to tumor tissue has been described as early as the eighteenth century (Parish, 2003), however cancer immunotherapy as a potentially viable field of its own, did not come into existence until the 1960s. With a deeper understanding of T cell and antigen presenting cell (APC) biology, as well as the discovery of tumor associated antigens, over the past several years cancer immunotherapy has emerged as one of the most promising avenues in the treatment of cancer, including primary malignant brain tumors (malignant gliomas). Malignant gliomas (MG) are highly aggressive, incurable tumors of glial origin and carry dismal prognosis for patients suffering from this disease (Tivnan et al., 2017). The goal of cancer immunotherapy is to overcome tumor-induced immunosuppression and augment an individual's own anti-tumor immune response using various strategies such as adoptive T cell transfer, vaccination using tumor specific peptides or tumor pulsed dendritic cells (DC), oncolytic virotherapy and immune checkpoint inhibitors (Tivnan et al., 2017).

DCs are professional antigen presenting cells (APCs) and play a critical, decisive role in determining the final outcome of the immune response to antigens. Broadly, DCs can be classified into two subsets: myeloid DCs (mDCs) or classical DCs (cDCs) and plasmacytoid DCs (pDCs). This, however, is an oversimplification, as cDCs and pDCs can further be divided into subpopulations based on surface antigens, function and location within tissues (Collin et al., 2013; O'Keeffe et al., 2015). For the purpose of this review we will only discuss recent studies of pDC sub-classification. A thorough review of DC subsets can be found in Collin et al., 2013 and O'Keeffe et al., 2015.

Recently, several studies have demonstrated that pDCs can further be divided into subsets. Alculumbre et al., demonstrated that activated pDCs could be separated into three subpopulations based on CD80 and PD-L1 expression following stimulation by a single stimulus; P1-pDCs (PD-L1$^+$, CD80$^-$), P2-pDCs (PD-L1$^+$, CD80$^+$), and P3-pDCs (PD-L1$^-$, CD80$^+$) (Alculumbre et al., 2018). High levels of PD-L1 expression by pDCs (P1-pDC) were found to be a marker for interferon production, which suggests an immunogenic, not tolerogenic, function for the P1-pDC subset (Alculumbre et al., 2018). Villani et al., also isolated a unique subset of DCs, AS DCs, which are able to stimulate T cell proliferation and are morphologically similar to cDCs, but express pDC markers, CD123 and CD303 (Villani et al., 2017). Further supporting this finding, See et al., recently distinguished pre-DCs from pDCs and demonstrated that these pre-DCs, which express pDC markers (CD123, CD303, CD304), were able to induce proliferation and polarization of naïve CD4 T cells, whereas “pure” pDCs could not (See et al., 2017).

pDCs were initially recognized as important regulators of immune responses to viral infections due to their ability to produce large amounts of IFN-α in response to viral pathogens (Megjugorac et al., 2004). Upon activation of Toll-like receptors 7 or 9 (TLR7/9) by viral

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DNA or RNA, pDCs promote both innate and adaptive immune responses through induction of natural killer (NK) cell migration, macrophage and dendritic cell maturation, T cell response, antigen presentation and differentiation of antibody-producing plasma cells (Jego et al., 2003, Megjugorac et al., 2004, Tough et al., 1996).

Depending on the environment and the type of stimulation, pDCs are capable of engaging either immunogenic or tolerogenic functions (Kerkmann et al., 2003; Villadangos and Young, 2008). This functional variability has posed an interesting challenge and it has been shown that cancer cells capitalize on the tolerogenic capacity of pDCs to establish an immunosuppressive tumor microenvironment (TME) and promote tumorigenesis (Aspord et al., 2013). pDC dysfunction is demonstrated in cancer by impaired IFN-α secretion and upregulation of immune checkpoint mediators (Aspord et al., 2013). Additionally, in several types of cancers, an increase in tumor-associated pDCs (TApDCs) is associated with an increase in regulatory T cells (Tregs) and decreased overall survival (Gousias et al., 2013; Labidi-Galy et al., 2012; Sisirak et al., 2013b).

These findings have sparked interest in investigating pDCs as potential targets in cancer immunotherapy, either through induction of IFN-α production or ablation of their immunosuppressive mechanisms. In this review, we provide a comprehensive overview of pDCs and their immunogenic role, followed by a discussion of their contribution to cancer pathogenesis and potential therapeutic interventions for targeting their dysfunction.

## 2. pDC origin, development and regulation

### 2.1. Origin of pDC

pDCs arise from hematopoietic stem cells in the bone marrow, are morphologically round, with a well-developed rough endoplasmic reticulum (RER) and Golgi apparatus (Ghosh et al., 2010). Upon in vitro stimulation with IL-3, pDCs are shown to assume a cDC-like morphology, mature into antigen presenting cells and acquire the ability to stimulate $T_H2$ responses (Ghosh et al., 2010, Grouard et al., 1997). Human pDCs are identified phenotypically by the absence of CD11c, ILT-1, and leukocyte lineage markers (e.g. CD3, CD14, CD19, CD56), as well as by the presence of CD4, CD123, HLA-DR, CD68 and ILT-3 (Dzionek et al., 2001). Additionally, human pDCs are known to express ILT-7, a cell surface receptor involved in the negative modulation of IFN-α production, and BDCA-2, a C-type lectin involved in ligand internalization and inhibition of IFN-α/β synthesis (Cao et al., 2006, Dzionek et al., 2001). The expression of CD2, a surface adhesion molecule, further distinguishes two subsets of pDCs (Matsui et al., 2009). CD2hi pDCs have been shown to secrete higher levels of IL-12, express higher levels of CD80 and possess a greater capacity to initiate T cell-mediated immune responses (Matsui et al., 2009). Further, Zhang et al. recently demonstrated subsets of pDCs within the CD2hi population that differ in their morphology, function and expression of CD5 and CD81. CD5 + CD81+ pDCs were shown to express more IRF-5, less IRF-7 and produce less type I interferon than CD5-CD81- pDCs (Zhang et al., 2017). These CD5 + CD81+ pDCs were also suggested to be superior in triggering T cell proliferation as well as Treg and plasma cell differentiation (Zhang et al., 2017). As discussed previously, Villani et al., recently classified these CD2 + CD5+ cells, previously thought to be pDCs, as AS DCs, which are functionally distinct from pDCs, but maintain expression of pDC markers, CD123 and CD303 (Villani et al., 2017). Additionally, Alculumbre et al., demonstrated that activated pDCs, which were subsequently classified into three subsets, arise from CD2-CD5- pDCs (Alculumbre, Saint-Andre, 2018). These findings contrast those of Zhang et al., and suggest that these CD2 + CD5+ cells may be distinct from pDCs (Alculumbre et al., 2018, Villani et al., 2017, Zhang et al., 2017). Murine pDCs, in contrast, are CD11c+, and do not express the Flt3 receptor unless treated with Flt3L. They also express cell surface antigens B220, Ly6C, BST2, mPDCA-1 and SIGLEC-H (Asselin-Paturel et al., 2001; Blasius and Beutler, 2010; Blasius et al., 2006a; Blasius et al., 2006b).

Both DC subsets arise from the same progenitor hematopoietic stem cell. It has been suggested that both common myeloid (CMP) and common lymphoid progenitors (CLP) can give rise to pDCs via an intermediate common DC progenitor (CDP), characterized by the surface phenotype Lin- CD115 + CD117+ CD135+ (Chicha et al., 2004; Naik et al., 2007). Schlitzer et al. demonstrated the existence of an intermediate CCR9-MHCIIlow precursor that is capable of differentiation into both pDCs and mDCs (Schlitzer et al., 2011). These CCR9-MHCIIlow precursors differ from the pro- and pre- DCs described by Naik et al., in that they express lineage markers B220, CD11c, CD4, CD8α and CD86 (Schlitzer et al., 2011). Additionally, Ishikawa et al. have proposed the possibility of a common DC development program that is independent of the conventional myeloid and lymphoid pathways (Ishikawa et al., 2007).

### 2.2. Regulation of pDC development

Regardless of origin, only precursors expressing CD135 (Flt3 receptor) are thought to be capable of producing pDCs (D'Amico and Wu, 2003; Karsunky et al., 2005). FLT3 and its ligand, FLT3L, act via the activation of transcription factor E2–2 in a STAT3-dependent mechanism to control the expression levels of transcription factors necessary for pDC development and function (Fig. 1) (Cisse et al., 2008; Laouar et al., 2003; Li et al., 2012). E2–2 directly binds to promoter regions of the genes responsible for encoding BDCA-2, TLR-9 and ILT-7 (CD127), and to the 5′ regions of IRF-8 and IRF-7 (Cisse et al., 2008, Li et al., 2012). The functions of TLR-9, ILT-7 and IRF-7 is discussed later in the paper, however, there is conflicting information in the literature regarding the role of IRF-8. Previously, IRF-8 was suggested to be critical for pDC development. However, Sichien et al. has recently suggested that IRF-8 plays a role in regulating pDC function, but is not required for development (Schiavoni et al., 2002; Sichien et al., 2016). Their group showed that deletion of IRF-8 resulted in pDCs with increased T cell stimulatory function and decreased IFN-I production, but it did not influence pDC development or survival (Sichien, Scott, 2016). SpiB and BCL11A, are also direct targets of E2–2. SpiB plays an important role in both pDC differentiation and survival, whereas activation of BCL11A is shown to direct CDP commitment to pDC lineage and regulate transcription of E2–2, Id2, Id3 and Mtg16 via a positive feedback loop (Ippolito et al., 2014; Karrich et al., 2012; Schotte et al., 2004).

pDC development is inhibited by GM-CSF through STAT5-mediated inhibition of *Irf8* and upregulation of *Id2* (Esashi and Liu, 2008; Esashi et al., 2008). *Id2* subsequently binds to and prevents E2–2 association with target DNA sequences. It has been suggested that a balance of GM-CSF-STAT5 and Flt3L-STAT3 activation drives differentiation toward one of the two DC subsets (Li, Yang, 2012). Zeb2, a zinc finger transcription factor that interacts with *Smad* proteins, regulates this balance and controls the commitment to pDC or cDC lineage through *Id2* expression regulation (Scott et al., 2016; Wu et al., 2016). Zeb2 also promotes the expression of M-CSFR, which may drive pDC development in a Flt3-L independent manner (Fancke et al., 2008).

In addition to its role in pDC development, E2–2 may be critical in the maintenance of pDC identity. Deletion of E2–2 in pDCs is shown to result in a loss of pDC-associated cell markers and spontaneous cDC-like differentiation. While there is evidence that E2–2 directly binds to and controls the gene expression program of pDCs, it has also been suggested that E2–2 may inhibit commitment to the cDC cell fate by direct repression of cDC-associated genes (Ghosh et al., 2010).

### 2.3. pDC migration

Following development in the bone marrow, pDCs migrate to secondary lymphoid tissues via high endothelial venules (HEV) (Cella