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REVIEW

Plasmacytoid dendritic cells, a role in neoplastic prevention and progression

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ABSTRACT

Background Plasmacytoid dendritic cells (pDCs) are multifunctional bone-marrow-derived immune cells that are key players in bridging the innate and adaptive immune systems. Activation of pDCs through toll-like receptor agonists has proven to be an effective treatment for some neoplastic disorders.

Materials and methods In this mini-review, we will explore the fascinating contribution of pDCs to neoplastic pathology and discuss their potential utilization in cancer immunotherapy.

Results Current research suggests that pDCs have cytotoxic potential and can effectively induce apoptosis of tumour-derived cells lines. They are also reported to display tolerogenic function with the ability to suppress T-cell proliferation, analogous to regulatory T cells. In this capacity, they are critical in the suppression of autoimmunity but can be exploited by tumour cells to circumvent the expansion of tumour-specific T cells, thereby allowing tumours to persist.

Conclusion Several forms of skin cancer are successfully treated with the topical drug Imiquimod, which activates pDCs through toll-like receptor 7 engagement. Additionally, pDC-based anticancer vaccines have shown encouraging results for the treatment of melanoma in early trials. Future studies regarding the contributions of pDCs to malignancy will likely afford many opportunities for immunotherapy strategies.

Keywords Cancer, immunity, pDC, plasmacytoid dendritic cells, tolerance, tumour.

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Introduction

Plasmacytoid dendritic cells (pDCs) are a unique population of bone-marrow-derived immune cells that bridge the innate and adaptive immune systems. They are remarkable in that they are the only immune cell to serve two professional roles, one as interferon (IFN)-producing cells and the other as antigen-presenting cells (APCs). Although accounting for only 0·3–0·5% of peripheral blood cells, pDCs are responsible for over 95% of type I IFN produced by circulating lymphocytes [1]. Activation of pDCs and the subsequent production of IFN occur as the result of a signalling cascade that initiates through the receptor-ligand interactions of pattern recognition receptors (reviewed by Lombardi and Khaiboullina [2]). pDCs are primarily activated through the engagement of endosomally located toll-like receptors (TLR)-7 and TLR-9, by ssRNA [3,4] or nonmethylated and CpG DNA [5,6], respectively (Fig. 1a), which are common to microbial genomes, such as viruses or their replicative intermediates. pDCs are also known to produce type I IFN in response to double-stranded dsRNA, probably through the engagement of protein kinase R (PRK) [7], although their response to dsRNA is less well characterized. Similar to other TLRs, (with the notable exception of TLR-3), TLR-7 and TLR-9 utilize the universal adapter protein MyD88 (myeloid differentiation primary response 88), which acts via the constitutively expressed transcription factor IRF7 and the inflammatory transcription factor NF-κB, thereby initiating transcription of type I and III IFN, or inflammatory cytokines and chemokines, respectively [8–10]. Upon activation, pDCs also undergo phenotypic changes resulting in the upregulation of costimulatory molecules, including CD40, CD80, CD86. They ultimately develop into more 'conventional' dendritic cells (cDC) with classical DC morphology and the ability to present and cross-present antigens in the context of MHC and costimulatory molecules to naïve and memory T cells [11].

Over the last decade, our understanding of pDCs biology has greatly expanded but this expansion has also resulted in many unanswered questions. Indeed, it is now evident that pDCs play a much larger role in immunology than originally realized. In addition to their ability to produce IFN, pDCs contribute to

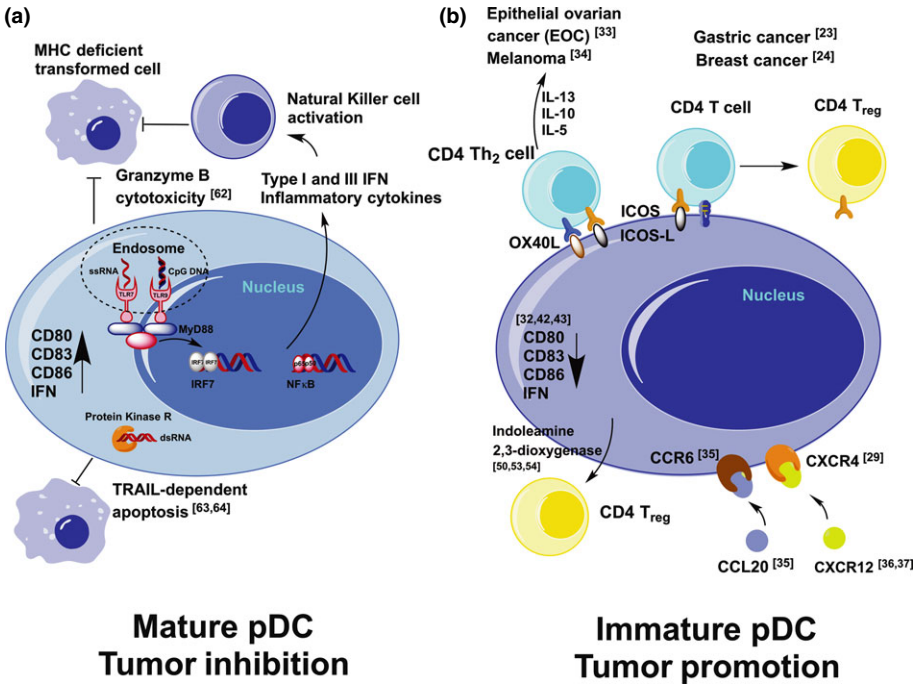

Figure 1 Plasmacytoid dendritic cell (pDC) involvement in tumour inhibition and promotion. (a) Activation of pDCs through the engagement of endosomally located toll-like receptors (TLR) 7 and 9 by ssRNA or nonmethylated CpG DNA, respectively, leads to the MyD88-dependent upregulation of type I interferon (IFN) and inflammatory cytokines as well as expression of costimulatory molecules such as CD80, CD83 and CD86. Type I IFN expression also occurs through the engagement of protein kinase R (PKR) by dsRNA. pDCs possess direct tumoricidal activity in a granzyme B and TRAIL-dependent manner and indirectly through the activation of natural killer (NK) cells by type I IFN. (b) Mechanisms suggested to explain tumour-associated pDC dysfunction include the recruitment of immature pDCs as characterized by the lack of expression of costimulatory molecules and tumour secretion of immunosuppressive factors. In addition to being immature, these pDCs are shown to promote tolerance by activating Tregs, express anti-inflammatory cytokines such as IL-13 and are refractory in IFN production.

tolerance but when dysregulated can also contribute to autoimmunity. Current research suggests that pDCs have the capacity to induce apoptosis of neoplastic cells and, therefore, may also contribute to cancer surveillance. Conversely, it has also been shown that the tolerogenic functions of pDCs may be utilized by tumours to their advantage, allowing them a way to evade the immune system. For these reasons, an understanding of pDC function in the context of neoplastic pathology and the tumour microenvironment will likely provide a greater understanding of malignancy in general and suggest potential treatment strategies.

Neoplastic progression and the tumour microenvironment

The historical paradigm of cancer development and propagation is based upon the presence of mutations that lead to cell cycle dysregulation. According to this model, a single mutation in a cell cycle gene allows the cell to grow uncontrolled whereby it rapidly expands to form a tumour. However, this model is an oversimplification in that as the tumour expands, it forms its own microenvironment that differs from that of healthy non-malignant tissue [12]. Indeed, cross-talk between stromal and epithelial cells is essential for maintaining homoeostasis of malignant as well as non-malignant tissue [13,14]. In the last three decades, our knowledge regarding the role of immune effector cells in maintaining a protumorigenic microenvironment has increased substantially. For instance, tumour-derived colony-stimulating factor-1 (CSF-1), VEGF and endothelial monocyte activating polypeptide II (EMARII) have been shown to facilitate the infiltration of tumour tissue by monocytes [15–17]. Within the tumour, monocyte-derived macrophages polarize into the M2 stage, which is strongly associated with proangiogenic and protumorigenic properties [18,19]. Furthermore, tumour-associated macrophages contribute to an immunosuppressive environment by releasing interleukin (IL)-10 and TGF-β [20] and additionally, promote the infiltration of T regulatory cells (Tregs) by releasing the

chemoattractant CCL22 [21,22]. A positive correlation has been observed between inducible costimulator (ICOS)-expressing Tregs and pDCs in the peripheral blood and peritumour tissue of subjects with gastric cancer [23] (Fig. 1b). Additionally, ICOS-driven interaction between CD4+ T cells and pDCs has been reported to lead to the upregulation of Tregs and IL-10 secretion in breast tumours [24]. These observations underscore the contributions of immune effector cells to an immunosuppressive tumour microenvironment, thus supporting the maintenance and propagation of malignancy.

Plasmacytoid dendritic cells, immunity and cancer

All DCs are professional APCs with the capacity to prime and activate naïve T lymphocytes [25]. By controlling the outcome of antigen presentation to T cells, DCs also play a central role in the maintenance of peripheral tolerance. Through the activation of pattern recognition receptors, such as TLRs, they also produce cytokines such as interferons and interleukins, thus modulating the balance between humoral immunity, cell-mediated immunity and tolerance [26]. For these reasons, it is not surprising that DCs may play a pivotal role in antittumour immunity. Involvement of pDCs in neoplastic disorders became evident upon the observation that several tumours including ovarian, head and neck, and breast tumours and primary melanoma are infiltrated with pDCs [27–32]. In some instances, the presence of infiltrating pDCs is associated with a poor prognosis; for example, while investigating epithelial ovarian cancer (EOC), Conrad and co-workers observed that a significant number of Foxp3+ Tregs present in the tumour microenvironment expressed ICOS [33]. They further observed that the ability of these cells to suppress T-cell proliferation was strictly dependent on ICOS-L costimulation provided by infiltrating pDCs and therefore suggested that pDCs and ICOS+ Foxp3+ Tregs were strong predictors of EOC progression. As a further example, Aspord *et al.* reported that Th2-promoting pDCs were associated with the progression of melanoma and that the frequency of IL-5, 10 and 13-producing T cells in melanoma cases was correlated with a high proportions of OX40L- and ICOSL-expressing pDCs [34].

Dendritic cells play a central role in orchestrating immune responses, and numerous studies have reported that tumour tissue is often infiltrated with various populations of DCs including pDCs. For example, as previously stated, pDCs have been reported to be among the cellular infiltrate of several tumours [27–32]. It is believed that the recruitment of pDCs into tumour tissue is governed by chemokines secreted by neoplastic cells. Zou *et al.*, as well as others, have reported that tumours infiltrated with pDCs express high levels of chemokines such as CXCL12 (stromal cell-derived factor 1) and CCL20 (macrophage inflammatory protein-3) [29,32,35]. Zou and co-workers additionally reported that tumour-derived pDCs express high levels of CXCR4 [29], the specific receptor for CXCL12 [36,37]. Charles *et al.* reported that tumour-associated pDCs express high levels of the chemokine receptor CCR6, the receptor for CCL20 [35], a requirement for the rapid recruitment of dendritic cells into tissue [38]. Indeed, the multiple receptor-ligand interactions that occur between tumour cells and immune effector cells contribute to the complex microenvironment that allows tumours to maintain their own persistence.

pDCs are essential for recognition of altered self-antigens and for triggering immune responses directed towards transformed cell. Therefore, it would be expected that the increased presence of pDCs in tumour tissue should promote immune recognition of tumour antigens and, in turn, lead to tumour rejection. Contrary to this supposition and unexpectedly, increased pDC tumour infiltration is often associated with tumour progression and persistence [24,39]. Furthermore, it has been shown that increased pDC infiltration is associated with poor prognosis in some cancer cases [40,41]. Therefore, it has been suggested that tumour-associated pDCs are often incompetent with respect to tumour-specific immune surveillance.

Several mechanisms have been suggested to explain tumour-associated pDC dysfunction including the recruitment of immature pDCs, promotion of pDC tolerance and tumour secretion of immunosuppressive factors. Numerous studies have shown that tumour-associated pDCs are immature as characterized by the lack of expression of costimulatory molecules such as CD80, CD83 and CD86 [32,42,43]. In addition to being immature, these pDCs are shown to be defective in IFNα production [32,44] and it has been suggested that defective IFNα production is the result of a downregulation of Flt3, TLR9 or IRF7 [44–47]. Tsukamoto *et al.* [48] proposed that tumour-associated immunoglobulin-like transcript 7 ligands (ILT7L) can downregulate IFNα production in pDCs via interaction with the ILT7 receptor. IFNα is a pleotropic cytokine with strong tumour inhibitory activity [49]. Therefore, by producing less IFNα, pDCs may significantly impair local immune surveillance allowing tumours to escape IFNα-associated immune responses. Several studies have suggested that tumour-associated pDCs are indeed tolerogenic [28,50]. For example, animal tumour models have shown that tumour-infiltrating pDCs can activate mature Tregs [51,52]. Additionally, it is well documented that malignant cells and tumour-associated pDCs release indoleamine 2,3-dioxygenase (IDO) which is a powerful promoter of Treg activation, and can lead to anergy, thus allowing tumour cell to escape immune surveillance [50,53,54].

Although pDC infiltration of tumours is often associated with disease progression, their activation with TLR-agonists is proving to be an effective treatment for some forms of neoplasm. For instance, the topical treatment of basal cell carcinoma, superficial squamous cell carcinoma and some superficial malignant melanomas, with the synthetic TLR-7 agonist Imiquimod, has been shown to lead to the increased infiltration of activated pDCs and a significant reduction in neoplastic cells and in some cases, a complete regression [31,55–58].

Antineoplastic functions of pDCs

DCs have the potential to invoke antitumour immunity in multiple ways. Similar to cytotoxic CD8 T lymphocytes (CTLs), natural killer (NK) cells and gamma/delta T cells; DCs have the capacity for direct cytotoxic killing of susceptible target cells such as virus-infected cells and transformed cells. The focus of our discussion is the contribution of pDCs to anticancer immunity; notwithstanding, significant body of research also addresses the cytotoxic capacity of cDCs. These topics are excellently reviewed by Tel *et al.*, and Larmonier *et al.*, with respect to humans and animal models [59,60] and thus we will only discuss their tumoricidal activity in conjunction with, or in comparison to pDCs.

Classic cytotoxic cells, such as NK cells and CTLs, express perforin (PRF1) and the proapototic enzyme granzyme B (GZMB). Although initially believed that PRF1 was required for entry of GZMB into target cells, current research suggests that both proteins may enter cells through an alternative mechanism. For instance, Veugelers and colleagues proposed a mannose 6-phosphate receptor as a potential entry mechanism for PRF1 and GRZB [61]. The definitive role for pDC-GRZB is currently the subject of ongoing investigations. However, as pDCs do not express PRF1, an alternative PRF1-independent entry method would support the possibility of pDC cytotoxicity in a GZMB-dependent but PRF1-independent manner. Indeed, using a human asthma model of segmental allergen challenge, Bratke and co-workers reported that pDCs upregulate GRZB in response to IL-3 and additionally showed that IL-3 activated pDCs killed MHC deficient K562 cells [62]. Furthermore, they reported that the observed killing was abrogated in the presence of GRZB and caspase inhibitors. Interestingly, they also observed that engagement of the TLR-7 or -9 receptor suppressed GRZB expression, suggesting that the classical IFN-induced pathway of pDCs is not involved in GRZB-associated cytotoxicity. Tel *et al.* reported that human pDCs activated with the preventative vaccine to tick-borne encephalitis virus FSME upregulated the neural cell adhesion marker CD56, a classic NK marker, and were empowered with the tumoricidal ability to lyse K562 and Daudi cells in a contact-dependent manner [63]. They additionally reported that the expression of CD56 on the surface of pDCs coincided with elevated expression of programmed death-ligand 1 (PD-L1), GRZB and TNF-related apoptosis-inducing ligand (TRAIL).

TRAIL-dependent apoptosis has been implicated in the tumoricidal capacity of pDCs by other researchers as well. For instance, Stary and co-workers reported that, upon treatment of basal cell carcinoma with Imiquimod, a cellular infiltrate of GRZB and PRF1 positive cDCs and TRAIL positive pDCs was observed [64]. However, in contrast to the observations of Bratke *et al.*, the contribution of pDC killing was strictly TRAIL dependent, as TRAIL neutralizing antibody abrogated the killing of TRAIL-sensitive Jurket cells. Consistent with the observations of Stary *et al.*, Kalb and co-workers reported that pDCs stimulated with agonists for TLR-7 and 9, but not other TLRs, upregulated the surface expression of TRAIL in a type I IFN-dependent manner [65]. They additionally reported that pDCs treated with TLR7/9 agonists as well as pDCs treated with type I IFN efficiently lysed Jurkat cells, as well as the melanoma cell lines SKMel2 and WM793, in a TRAIL and contact-dependent manner. Using a mouse model of melanoma, Drobits and co-workers showed that topical Imiquimod treatment resulted in tumour clearance in a TLR7/MyD88-dependent and IFN-α/β receptor 1-dependent manner, with a concomitant upregulation of the chemokine CCL2 in mast cells [66]. They additionally observed that Imiquimod treatment promoted the secretion of both TRAIL and GRZB and that blocking these molecules led to impaired pDC-mediated tumour killing. These data strongly implicate both TRAIL and GRZB in pDC-mediated tumour killing and further suggest that the tumoricidal ability of pDCs is independent of adaptive immunity.

pDCs as potential targets in cancer immunotherapy

CTLs are considered to be the most critical mediators of anticancer immune responses, and CTL infiltration of tumours is typically associated with a positive diagnostic outcome [67,68]. The use of immunomodulating drugs to increase CTL responses has been shown to be an effective strategy for improving the induction of long-term memory CTLs. For instance, one strategy targets the blockade of the inhibitory receptors such as the cytotoxic T lymphocyte-associated antigen 4, the programmed death-1 receptor (PD-1) or its ligand, PD-L1. This approach is often referred to as 'immune-check-point blockade'. The use of anti-PD1 antibodies in combination with the anti-B cell drug rituximab has led to encouraging results both in preclinical models and in clinical applications [69,70]. However, the nonspecific nature of these 'check point inhibiting' drugs and the broad mechanism by which they exert

their actions can lead to activation of autoreactive T cells and, in turn, lead to potentially severe side effects [71,72]. DC therapies are an attractive alternative to check point-inhibiting drugs in that they have few side effects, and natural DC therapy is generally less costly.

Ex vivo DCs are capable of inducing CTL responses against tumours when loaded with tumour-associated antigens and given as a vaccine. Therefore, the primary goal of cancer vaccine immunotherapy is the induction of long-term memory CTLs that are capable of facilitating immune surveillance and promoting tumour rejection. Although the use of cancer vaccines to generate antitumour immune responses is theoretically promising and appears fairly straightforward, the clinical success of such vaccines has been less than encouraging [73]. Although previous studies have largely employed monocyte-derived DCs (moDCs) for this purpose, a pioneering study conducted in the laboratory of Dr. Jolanda De Vries utilized activated pDCs preloaded with tumour-associated antigens to vaccinate subjects with melanoma [74]. Although the overall magnitude of antimelanoma immune responses was comparable to that of previous moDC trials, a number of encouraging observations were made as a result of this study. The pDC vaccine produced a systemic type I IFN response, which is critical to NK activation and subsequent inhibition of tumour metastasis [75]. Additionally, pDCs were observed to migrate efficiently to the lymph nodes and, subsequently, T-cell clones with high avidity could be identified after vaccination, indicative of a strong functional response. Lastly and most importantly, the overall survival of subjects treated with the vaccine greatly increased when compared to matched controls that only received a standard chemotherapy treatment. With regard to the mechanism of the observed efficacy, one could speculate that the improved treatment outcome may have been the result of pDC-mediated activation of innate immune cells such as NK cells, or perhaps T cells induced by pDCs may be more potent immune effectors. Nevertheless, these observations clearly suggest that pDC-based anticancer vaccines will likely provide advantages over moDC vaccinations or may even supplement moDC vaccinations when used in combination therapy.

Concluding remarks

The current model of tumour neogenesis holds that the tumour microenvironment provides favourable conditions that support malignant cell growth and propagation, while at the same time, allows them to evade the immune system. pDCs that infiltrate tumours are often dysfunctional and, accordingly, do not produce IFNα. Furthermore, they often display an immature or naïve phenotype and promote a tolerogenic microenvironment through the activation of Tregs. In this context, pDCs likely contribute to neoplastic homoeostasis and, accordingly, represent a very attractive target in cancer immunotherapy. Indeed, activating pDCs with the TLR-7 agonist, Imiquimod is highly effective in treating some forms of skin cancer and exemplifies the potential impact of pDC immunity in neoplastic disease. Additionally, pDCs have been shown to have tumoricidal properties in culture; therefore, potentiating this ability in vivo may prove to be an effective treatment strategy. Future studies regarding the contributions of pDCs to malignancy will likely afford many opportunities for immunotherapy strategies.

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Conflict of interest

The authors declare no conflict of interests.

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