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Review

Tumor microenvironment converts plasmacytoid dendritic cells into immunosuppressive/tolerogenic cells: insight into the molecular mechanisms

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ABSTRACT

Human pDCs represent a rare population of circulating cells characterized by a rapid and massive TLR-dependent secretion of type I IFN in response to pathogenic agents or danger signals. Through their capacity to bring together innate and adaptive immunity and to secrete soluble factors controlling cancer development, these cells could represent important actors in antitumor immunity. However, accumulating evidence suggests that pDCs recruited to the tumor microenvironment often display a nonactivated state and are associated with the development and maintenance of immunosuppression. Here, we present an overview of neoplastic lesions associated with an infiltration of immunosuppressive/tolerogenic pDC. Moreover, as the proper response of pDC against cancer depends on a critical balance between immune-activating and immune-suppressing mechanisms, we summarize current knowledge about the molecular pathways developed by tumors to prevent antitumoral pDC immune responses. A better understanding of the mechanisms regulating pDC function in tumors could aid in the development of new therapies. Indeed, effective cancer vaccines or therapies could combine immunoactivating strategies (i.e., TLR agonists) with elimination of immune-suppressing mechanisms, leading to pDC reprogramming and thus, allowing tumor rejection in a clinical setting.
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Introduction

pDCs represent a subset of DCs specialized in the secretion of large amounts of type I IFN in response to pathogens or motifs, such as synthetic CpG ODNs. Phenotypic characterization revealed that human pDCs are Lin^- $CD4^+$ $CD45RA^+$ $CD123^+$ $BDCA2^+$ and $BDCA4^+$ cells. Following TLR7 and -9 activation by their cognate agonists, the key adaptor molecule MyD88 is recruited and binds to the TLRs. MyD88 then activates multiple signaling pathways in pDCs, such as those involving IRFs, NF-κB, and MAPKs (for review, see ref. [1]). This results in the transcription of genes encoding type I IFN, proinflammatory cytokines (TNF-α and IL-6), chemokines, and pDC costimulatory molecules, such as CD80, CD86, and HLA-DR (**Fig. 1**).

Recent data are in accordance with the concept that pDCs actively participate in innate and cellular antiviral immune responses. Indeed, immature pDCs are highly recruited to sites of inflammation [2], where they are activated by viruses and subsequently, promote antiviral immunity via various mechanisms, such as secretion of type I IFN with antiviral activities, antigen capture and maturation into potent APCs, and generation of antiviral T lymphocytes in association with LN DCs (for a review, see ref. [3]).

Secreted particularly by activated pDCs, IFN-α is a pleiotropic cytokine with not only antiviral properties, but also antitumoral activities. Notably used for the treatment of different types of cancers, including hematological malignancies and solid tumors, IFN-α has been reported to affect tumor cell proliferation, tumor lymph/angiogenesis, and tumor metastasis. This cytokine is well-known to inhibit tumor lymph/angiogenesis by suppression of endothelial cell motility and survival as a result of inhibition of proangiogenic factor production, such as VEGF, bFGF, and IL-8 [4–6], and induction of apoptosis [7]. McCarty et al. [8] reported that tumor growth, metastasis, and angiogenesis are accelerated in IFN-α/β$R^{-/-}$ mice. With the use of the same model, recent results also showed that TAM density is significantly increased in tumors of IFN-α/

Abbreviations: APC= antigen-presenting cell, ATL=adult T cell leukemia, BDCA=blood DC antigen, BM=bone marrow, BST2=bone marrow stromal cell antigen 2, DAMP=damage-associated molecular pattern, DC=dendritic cell, DC-LAMP=DC-lysosome-associated membrane protein, DCIR=DC immunoreceptor, Foxo3=forkhead box 03, FoxP3=forkhead box P3, GrB=granzyme B, HMGB1=high mobility group box 1, HNSCC=head and neck squamous cell carcinoma, ICOSL=ICOS ligand, IFN-α/β$R^{-/-}$=IFN-α/βR-deficient, ILT7=Ig-like transcript 7, IP-10=IFN-inducible protein 10, IRF7=IFN regulatory factor 7, MM=multiple myeloma, NSCLC=non-small cell lung cancer, ODN=oligonucleotide, pDC=plasmacytoid DC, SDF-1=stromal-derived factor-1, TAM=tumor-associated macrophage, TApDC=tumor-associated plasmacytoid DC, TDLN=tumor-draining LN, TIM-3=T cell membrane protein 3, Treg=T regulatory cell, VIP=vasointestinal peptide

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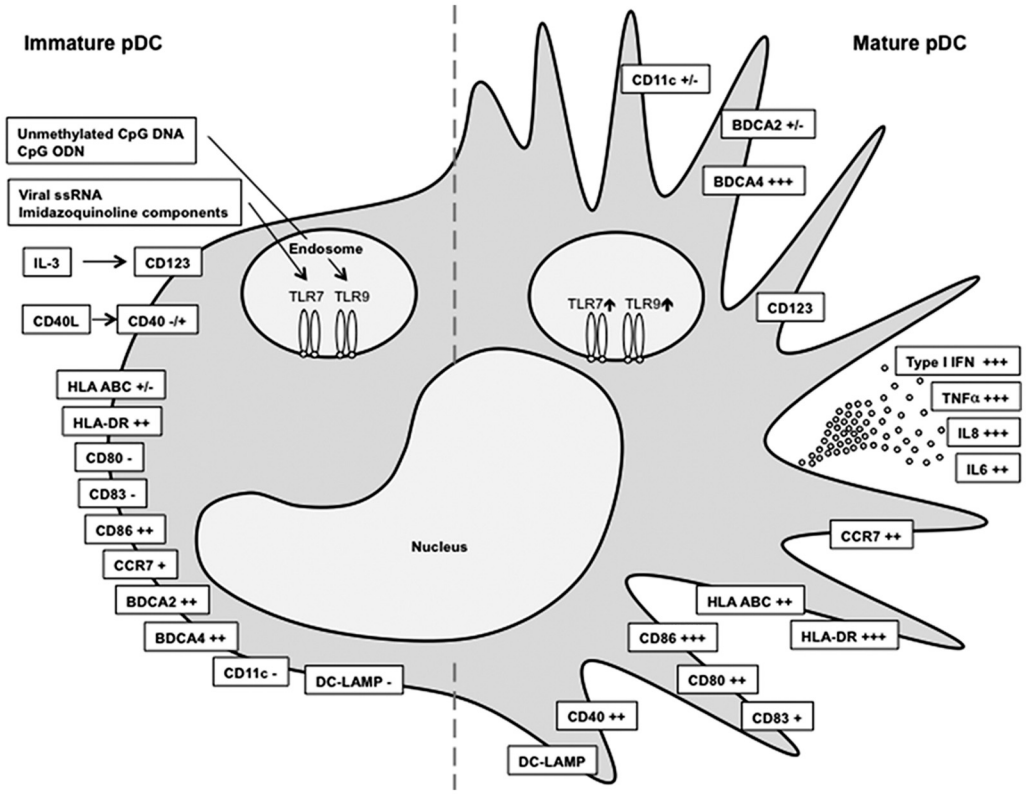

Figure 1. Maturation of pDCs after exposure to virus or synthetic agonists. TLR stimulation by pathogens or synthetic agonists, such as imidazoquinoline components or CpG ODNs, results in the transcription of genes encoding type I IFN (such as IFN-α, IFN-β, IFN-λ, and IFN-ω), proinflammatory cytokines (TNF-α, IL-6, and IL-8), and costimulatory molecules, such as CD80, CD86, and HLA-DR. CCR7 up-regulation during pDC maturation allows those cells to migrate to local LNs, where they can stimulate adaptive immunity by presenting antigens. Other molecules, such as IL-3 and CD40 ligand, can induce pDC maturation through their ligation to CD123 (IL-3Rα) and CD40, respectively.

$\beta R^{-/-}$ mice compared with WT mice [9]. This result indicates that endogenously produced type I IFNs suppress the generation of TAMs, which are documented as major actors in promoting tumor growth, invasion, and metastasis. Finally, type I IFN was demonstrated to enhance NK cell cytotoxicity against target tumor cells [10] and to act directly on T and B lymphocytes to modulate their activity and/or survival [11, 12].

Given the importance of IFN-α antitumoral activities and pDC capacity to bring together innate and adaptive immune responses, these cells may be important players in the tumor-immune microenvironment as well. Several studies have recently analyzed pDC activation and functions in the tumor setting. pDC infiltration has been reported in several types of cancer—melanoma [13], head and neck cancer [14], ovarian carcinoma [15], and breast cancer [16]. However, their implication in antitumoral response is largely debated. Some studies reported that pDC could limit tumoral progression, particularly by secreting IFN-α. In agreement with this hypothesis, it was demonstrated that stimulated-pDCs from lung cancer induced a cytotoxic T cell response relevant to antitumor immunity [17]. Therapeutic activation of pDC, through their TLR7 or TLR9, was also reported to induce IFN-α production and subsequent tumor regression of melanoma, basal cell carcinoma, and T cell lymphoma [18–20]. In contrast, emerging evidence suggests that pDCs could play a negative regulator role in antitumoral immunity. Indeed, pDCs recruited to the tumor microenvironment often present a nonactivated state and an inadequate functionality characterized by decreased secretion of IFN-α and an incapacity to induce a proper T cell response. This is often associated with the stimulation of a tolerogenic response characterized by Treg expansion. To support this theory, pDC infiltration in the tumor setting of the head and neck, breast, ovarian cancers, and melanoma was recently associated with a poor clinical outcome [21–24].

In the present paper, we present an overview about immunosuppressive/tolerogenic pDCs and summarize current knowledge about the different molecular mechanisms developed by tumor cells to prevent antitumoral pDC immune responses. A better understanding of the mechanisms that regulate pDC function in tumors could aid in the development of more effective cancer vaccines and/or therapies.

IMMUNOSUPPRESSIVE pDC IN THE TUMOR MICROENVIRONMENT

Otorhinolaryngologic cancers

It has been shown that pDCs infiltrate solid tumor tissues and TDLNs of HNSCC. The presence of pDC is significantly associated with decreased survival and intensely compromised proper immune functions in this environment [14, 23]. After exposure to the HNSCC microenvironment, pDCs showed an impaired phenotypical maturation and presented a diminished ability to produce IFN-α in response to CpG ODN [25, 26]. Nevertheless, for unclear reasons, in TDLNs, suppression of CpG-induced IFN-α production is less pronounced than in primary tumor tissues [14].

Respiratory cancers

Data regarding pDCs in the NSCLC microenvironment are quite contradictory. Perrot et al. reported that NSCLC-infiltrat-

ing pDCs express none of the activation markers CD80, CD86, CD83, or CD208/DC-LAMP and undergo only partial up-regulation of CD86 after TLR7 activation. Moreover, even after TLR stimulation, pDCs turn out to only induce a very weak T cell proliferation and IFN-α secretion [27]. Inducing an immunosuppressive environment characterized by an influx of Tregs, pDC recruitment in a mouse model of Lewis lung carcinoma is associated with tumor growth [28]. In contrast to these results, Faith et al. [17] showed that pDC functionality is preserved in human NSCLC draining LN microenvironments. Although pDCs isolated from TDLN show an immature phenotype, they strongly express the TLR9 and respond to CpG ODN by the production of proinflammatory cytokines IFN-α and IL-6 and subsequently induce efficient cytotoxic T cell 1 and Th1 immunity [17]. These authors speculated that functional preservation of TApDCs would be associated with their migration in TDLN, which in turn, may reduce inhibitory signals delivered by tumor cells or stromal components.

Urogenital cancers

pDCs were reported to infiltrate tumor and malignant ascites of ovarian cancer patients [15, 29]. The pDC infiltrating ovarian cancers are different from malignant ascites and blood pDCs in terms of distribution, phenotype, innate and adaptive functions, and impact on the patients' outcome, suggesting that malignant ascites do not mirror the microenvironment of the tumor mass [15, 30]. Indeed, the presence of pDCs in ovarian cancer is a prognostic factor associated with early relapse, whereas their presence in ascites is not deleterious for patients. Moreover, the pDC innate response is only strongly inhibited in the tumor mass [15].

pDCs have also been detected in cervical (pre)neoplastic lesions, mainly in the stroma underlying the tumor rather than within the dysplastic/cancerous epithelium [31]. In addition, pDC density is increased significantly in metastatic TDLN compared with metastasis-free TDLN, and this infiltration of pDC is significantly associated with a higher frequency of activated $CD4^+$ and $CD8^+$ $FoxP3^+$ Tregs [32].

Although breast cancer infiltration by pDCs is a rare event, this was found to be an independent prognostic factor for overall survival and relapse-free survival, suggesting a contribution of these cells in tumor progression [22]. Breast TApDCs express a partially activated phenotype and produce very low amounts of IFN-α following activation. Interestingly, TApDCs colocalize with Tregs in breast tumors [16].

Recently, infiltration by tolerogenic pDCs was reported in prostate cancer. Infiltrating pDCs expressed low levels of the costimulating molecules CD80 and CD86 and tolerized autologous T cells. These results were confirmed in an experimental mouse prostate tumor model [33].

Melanoma

Whereas substantially absent in normal skin and naevi, immature pDCs were detected within the lymphoid infiltrate or clustered around blood vessels in primary cutaneous melanoma. Occasionally, pDCs were also observed in close contact with tumor cells, especially in cases of metastasis [13, 34]. Although it is still unclear, pDC recruitment around the tumor is speculated to be related to SDF-1 (also called CXCL12) and CCL20 expression by the peritumoral stromal cells and/or melanoma cells. Circulating pDCs from melanoma patients are immature, but their ex vivo stimulation with a TLR7/8 agonist efficiently up-regulates the expression of their costimulatory molecules, suggesting that circulating pDCs in melanoma patients are functional. Furthermore, blood pDCs efficiently prime naive $CD8^+$ T cells specifically directed against melanoma cells to become IFN-γ-producing cells in vitro [35]. Despite these results, the presence of pDCs in the melanoma microenvironment is independently associated with poor prognosis [21], and although in situ production of IFN-α seems to be sustained by pDCs, the expression of the IFN-α-inducible protein, MxA, is extremely limited in the majority of cases. This suggests that IFN-α is inappropriately secreted or that tumor-dependent mechanisms limit IFN-α activity [34]. Besides, Gerlini et al. [13] showed that in melanoma sentinel LNs, the capability of pDCs to produce IFN-α in situ is impaired and that pDCs express CD86 but neither CD80 nor CD83, suggesting an incomplete activation. Interestingly, regressive melanocytic lesions are characterized by an infiltration of pDCs and high expression of MxA, indicating type I IFN production [36].

Hematological malignancies

Several studies analyzing the distribution of pDCs in MM patients have reported that pDC number is decreased significantly in peripheral blood from MM patients compared with normal donors [37, 38]. Moreover, pDCs purified from MM patients' blood present an altered phenotype characterized by a down-regulation of HLA-DR, HLA-ABC, and several chemokine receptors, including CCR5 and CCR7 [37]. Interestingly, high numbers of pDCs are observed in the BM of MM patients. The authors of this study also demonstrated that in the BM microenvironment, pDCs display a decreased ability to trigger T cell proliferation compared with normal pDC and confer growth, survival, and drug resistance in MM cells [39].

Similar to MM, ATL patients have a significantly reduced number of circulating pDCs compared with control patients. In addition, IFN-α production by circulating pDCs is reduced markedly. Despite these data, the ability of CpG-stimulated pDCs to stimulate allogeneic, naïve $CD4^+$ T cell proliferation is not affected in ATL patients compared with pDCs isolated from healthy controls. Thus, even if circulating pDCs from ATL patients have an altered functionality, as shown by their reduced IFN-α secretion, their allostimulatory function does not seem to be impaired [40].

MECHANISMS IMPLICATED IN THE pDC-DEPENDENT IMMUNOTOLERANCE TO CANCER

As discussed previously, pDCs are important for the generation and maintenance of a proper antitumoral immune response in cancer. Multiple mechanisms used by tumors to escape from immune rejection have been identified in the past years. Several complex immunosuppressive mechanisms, which may act

in concert and often depend on each specific tumor microenvironment, are activated by tumors to counteract effective pDC antitumoral effects. These mechanisms include secretion of immunosuppressive factors, activation of pDC regulatory receptors, expression of immunosuppressive DAMP molecules, and induction of tolerogenic mediator expression by pDCs (**Fig. 2**). Understanding the regulation of these mechanisms might contribute to overcoming the tolerizing conditions imposed by the tumor immunosuppressive microenvironment.

MECHANISMS DEVELOPED BY TUMORS TO INDUCE A TOLEROGENIC/ IMMUNOSUPPRESSIVE FUNCTION IN pDC

Tumor microenvironment expression of immunosuppressive mediators

Among the different immunosuppressive mechanisms used by the tumors to induce the dysfunctions of pDCs is their expres-

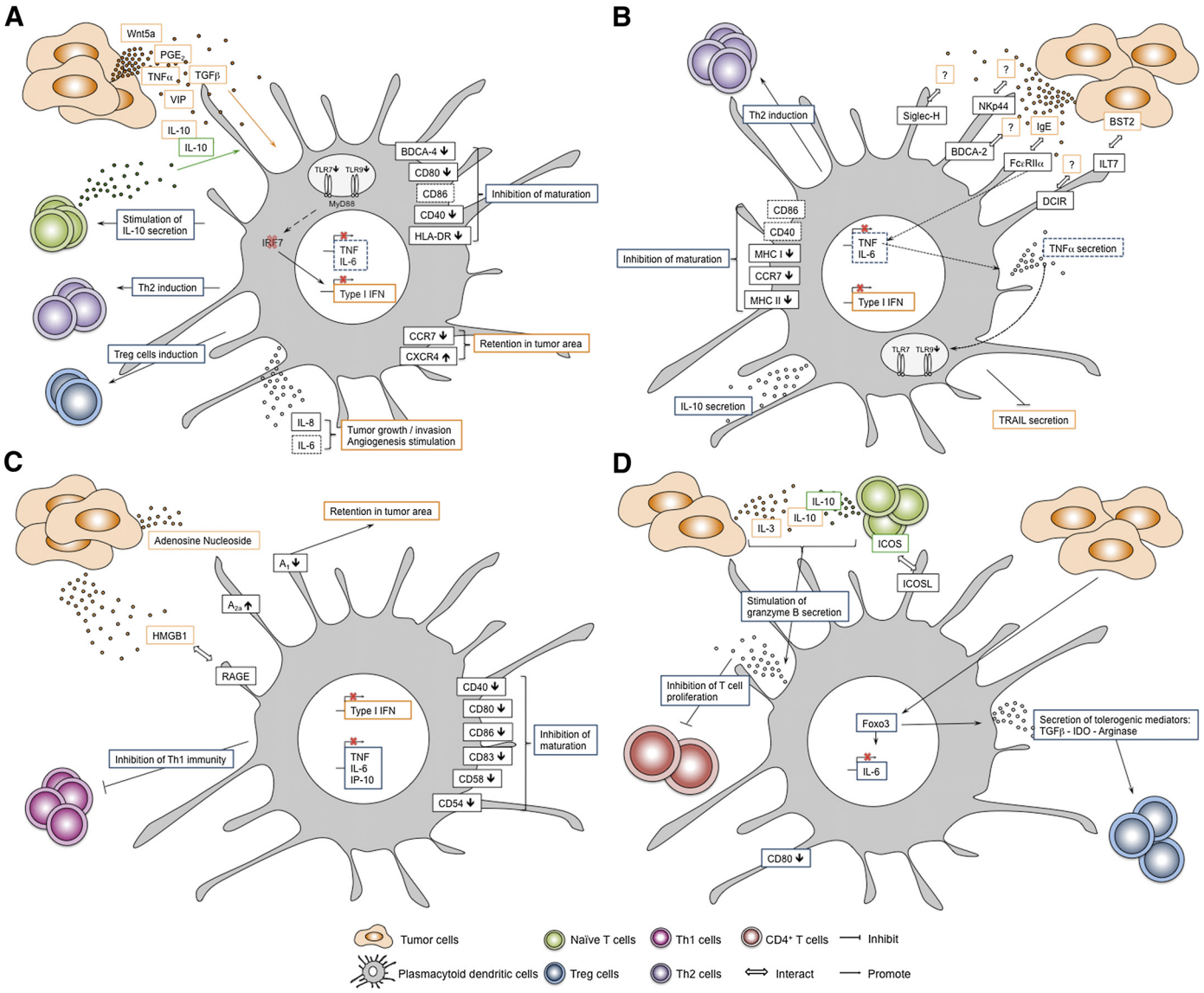

Figure 2. Tumor microenvironment often develops immunosuppressive mechanisms to escape the antitumor activity of pDC. Expression of immunosuppressive mediators (A), activation of pDC regulatory receptors (B), expression of immunosuppressive DAMP molecules (C), and induction of tolerogenic mediators expression by pDC (D) are the main mechanisms developed by cells encountered in the tumor microenvironment. Main pDC-immunosuppressive functions in the tumor setting are: induction of a Th2 response/inhibition of Th1 response, activation of Tregs, proper IL-10 secretion and/or stimulation of IL-10 production by T cells, inhibition of T cell proliferation and inhibition of pDC maturation preventing the development of a proper immune function (blue boxes). Tumors may also benefit from the modifications of chemokine and/or cytokine expression by pDCs. Tumor stimulation of cytokine secretion, such as IL-8, IL-6, and TNF-α, by pDCs can lead to tumor growth, invasion, and/or angiogenesis stimulation. Inhibition of IFN-α and TRAIL secretion by pDCs is another mechanism from which tumors may benefit (orange boxes). Dotted lines (arrows and boxes) represent contradictory results reported in the literature. A_1 and A_{2a}, Adenosine receptors; RAGE, receptor for advanced glycation endproducts.

sion of immunosuppressive mediators. These mediators can be secreted by tumor cells or by immune cells infiltrating the tumor, such as T cells. Immunosuppressive mediators can act on pDCs to inhibit or alter their functional activities, possibly leading to the suppression of IFN-α secretion or to the induction of Tregs to prevent an effective antitumoral response.

Tumor-derived PGE_2 and TGF-β have been shown to act in synergy to inhibit IFN-α and TNF production in activated pDCs. Indeed, in the presence of these two immunosuppressive soluble factors secreted by cancer cells (PGE_2) or tumor-infiltrating leukocytes (TGF-β), TLR7 and TLR9 ligands fail to induce pDC-mediated IFN and TNF expression. This impairment of pDC function could be explained by a down-regulation of these TLRs after their exposure to the cancer microenvironment [26]. Another possible mechanism is that the effects of PGE_2 on pDCs might occur downstream of TLR9 or TLR7, as the inhibition of IFN-α secretion was associated with IRF7 mRNA suppression in TLR9-stimulated pDCs [41]. Moreover, PGE_2 and TGF-β-treated pDCs display an altered CCR7 expression, which reduces their ability to migrate to TDLN, to present antigen, and to prime T cells in those areas [42].

Likewise, PGE_2-stimulated pDCs could be beneficial for tumors. Through IL-6 and IL-8 secretion, PGE_2-stimulated pDCs could indirectly promote tumor cell proliferation [43–45], migration, and invasion [46, 47] and stimulate angiogenesis [48–50]. Accordingly, pDCs infiltrating malignant ascites from ovarian cancer have been shown to induce angiogenesis in vivo through production of TNF-α and IL-8 [51].

In addition to TGF-β and PGE_2, IL-10 has been shown to exert immunosuppressive effects on pDCs in mice and human inflammatory conditions. For example, Beckebaum et al. [52] showed that increased IL-10 levels in serum induce a substantial reduction in circulating pDCs in hepatocellular carcinoma patients and significantly reduce the expression of HLA-DR, CD80, and CD86 on pDCs, revealing the induction of an immature phenotype.

TNF-α, in association with TGF-β, was also reported to alter pDC functions, notably in the ovarian cancer context. Indeed, tumor-derived TNF-α and TGF-β alter pDC functionality through the deregulation of their maturation status and their secretion of IFN-α, TNF-α, IL-6, MIP-1β, and RANTES, all decreased in TApDCs compared with the pDCs found in ascites or blood. Moreover, TApDCs induce IL-10 production from allogeneic naïve $CD4^+$ T cells, suggesting the existence of a paracrine-immunosuppressive loop [15]. Interestingly, even if pDCs are attracted to malignant ascites through the action of SDF-1 (CXCL12), they present normal, functional activities, such as the production of high amounts of type I IFN in response to the TLR ligand; they also present immunosuppressive properties, such as the induction of $CCR7^+$ $CD8^+$ Tregs, which suppress tumor antigen-specific T cells through IL-10 production [53]. Furthermore, SDF-1 (CXCL12) expression was also reported to protect pDCs from apoptosis affected by TAM-derived IL-10 through CXCR4 expression [30].

Several studies demonstrated that pDCs express vasoactive intestinal peptide receptor-1 and -2, the receptors for VIP. Interestingly, after culturing pDCs with VIP, pDC secretion of IFN-α and expression of BDCA4 and MHC I are impaired. When VIP is present during pDC activation, the potential of CpG ODN-activated pDCs to induce proliferation of allogeneic $CD4^+$ T cells is also altered. Furthermore, VIP-primed pDCs support a Th2 cytokine profile by inducing IL-4 rather than IFN-γ production by $CD4^+$ T cells [54]. As VIP is expressed in various cancers, it might be hypothesized that tumors take advantage of this expression to alter pDC antitumoral immune functions.

Finally, the expression of Wnt5a, the prototypic representative of the so-called noncanonical, wingless-homologous ligands, by cancer cells, such as melanoma cells [55], was reported to inhibit up-regulation of CD80 and CD86 on naïve human pDCs and IFN-α secretion by pDCs after their stimulation by CpG ODNs [56].

Tumor activation of pDC regulatory receptors

Several lines of evidence show that some cancer cells express ligands recognizing pDC regulatory receptors, suggesting that tumor activation of pDC regulatory receptors is probably another mechanism developed by the tumor to escape antitumoral immune responses.

Expression of BST2, a type II transmembrane protein, also known as tetherin, by several cancers [57–59], may be one of the potential mechanisms for impairment of pDC type I IFN secretion in the tumor microenvironment. Indeed, the interaction between the inhibitory receptor ILT7, naturally expressed by pDCs [60], and its ligand BST2, suppresses the secretion of IFN-α and TNF-α, and the transcription of type I IFN subtypes, including IFN-α1, IFN-α4, IFN-α8, and IFN-β, and IL-6 [59, 61]. TLR7 and TLR9 responses are inhibited after BST2 ligation to ILT7 on activated pDCs [62]. Nevertheless, ILT7 cross-linking does not affect the surface maturation markers CD80 and CD86 of pDC [59, 61, 62]. Some cancer cells, such as melanoma, renal cell carcinoma, and lung cancer cells, constitutively express BST2, whereas others need stimulation by cytokines, likely produced by surrounding stromal cells and/or immune cells in the tumor microenvironment, to express this molecule. Mechanisms for BST2 constitutive expression are heterogeneous among cancer cells, but activation of NF-κB appears to be one of the mechanisms frequently involved [59].

Cross-linking of a variety of ITAM receptors expressed by pDCs can negatively control TLR signaling. For example, engagement of the pDC receptor BDCA2 and high-affinity FcR for IgE, which all associate with the γ-chain of the FcϵRI adaptor, exerts negative effects on the IFN response to TLR activation [63–69]. Actually, autocrine secretion of TNF-α, resulting from IgE FcϵRI-dependent activation, was reported to reduce TLR9 expression in pDCs and their ability to mount an IFN-α response when subsequently activated with CpG ODN [64]. Indeed, neutralization of TNF-α activity after pDC incubation with an anti-IgE antibody completely reverses the suppression of TLR9 inhibition. This result corroborates the studies from Labidi-Galy et al. [15] showing that TNF-α alters pDC functionality, notably through their secretion of IFN-α and confirms that TNF-α is an immunosuppressive regulator of pDC. In addition, FcϵRI aggregation on pDC not only reduces the amounts of IFN-α secreted by preactivated pDCs but also impairs the surface expression of MHC I, II, and CD40 [66, 67], enhances pDC stimulation of a Th2-type immune response [66], and induces the production of IL-10 by pDCs [65, 66].

IgE expression by plasma cells was detected in Barrett's esophagus, an intermediate step in the progression from reflux esophagitis to esophageal adenocarcinoma [70]. This suggests that the production of IgE in a preneoplastic environment [71] could counter pDC functionality, thus favoring the development of cancer in those areas. The use of an anti-BDCA2 antibody was reported to inhibit TLR9-activated pDC from expressing the CD40, CD86, and CCR7 maturation markers and from producing IFN-α and IFN-β, and IL-6 [69]. Moreover, BDCA2 ligation polarizes the Th1 response into Th2 by TLR9-triggered pDCs [68]. Another important effect of anti-BDCA2 mAb on pDCs is that they abolish the secretion of soluble TRAIL by activated pDCs and subsequent TRAIL-dependent killing of target cells [72]. The existence of natural BDCA2 ligands and their possible expression in the tumor microenvironment remain unknown. Similarly, activation of the human receptor NKp44 and the mouse Siglec-H, associated with an ITAM-containing adaptor protein, reduces type I IFN production by pDCs [73, 74]. Interestingly, NKp44 ligands are ubiquitously and variably expressed in several cell lines derived from solid tumors [75]. Thus, expression of NKp44 ligands by tumor cells may provide a mechanism for inhibition of TApDCs.

Triggering of DCIR, a pDC regulatory receptor containing an immunoreceptor tyrosine-based inhibitory motif, leaves up-regulation of costimulatory molecule expression (CD40, CD86, CD80) unaffected, while it induces a significant inhibition of TLR9-mediated IFN-α production. Moreover, DCIR is internalized into pDC after triggering by its ligand through a clathrin-dependent mechanism [63].

Tumor expression of immunosuppressive DAMP molecules

DAMPs, like the HMGB1 protein or nucleosides, such as adenosine, were shown to suppress pDC antitumoral immune function. HMGB1 is a nonhistone nuclear protein that can serve as an extracellular alarmin. Indeed, this protein is a crucial cytokine with key functions required to alert and mobilize the immune response during infection and tissue damage. Although primarily located in the cell nucleus, HMGB1 can be released into the extracellular space from necrotic cells [76, 77] or actively secreted by activated macrophages [78], mature DCs [79], and NK cells [80] in response to injury, infection, and other inflammatory stimuli. Then, HMGB1 can have activating and chemotactic effects on APCs and mononuclear cells [81].

HMGB1 has also been proposed to be a crucial mediator in the pathogenesis of many diseases, including cancer. Recent data reported that HMGB1, which is highly expressed in various human tumors [82–84] and actively secreted by inflammatory cells, would suppress pDC cytokine secretion (IFN-α, IL-6, TNF-α, IP-10) and maturation in response to TLR9 agonists. In addition, HMGB1 prevents the up-regulation of costimulatory and adhesion molecules (CD40, CD80, CD83, CD86, CD54, and CD58) on pDCs and suppresses their ability to drive the generation of IFN-γ-secreting T cells [85]. Recent findings show that TIM-3, expressed on pDCs isolated from tumor-bearing mice, strongly suppressed production of IFN-β and IL-12 by these pDCs. TIM-3, on tumor-associated DCs, was reported to interact with HMGB1. This interaction suppressed the transport of nucleic acids into endosomal vesicles, attenuating the antitumor efficacy of DNA vaccines and cytotoxic chemotherapy by interfering with HMGB1-mediated activation of nucleic acid-sensing systems [86].

As HMGB1 was also reported to promote the recruitment of inflammatory cells to damaged tissues by forming a heterocomplex with SDF-1 (CXCL12) [87], we could postulate that HMGB1 expression could favor SDF-1 (CXCL12) recruitment of pDCs, thus amplifying the development of a tolerogenic milieu in the tumor. In addition, HMGB1 protects CXCL12 from degradation, which could maintain the recruitment of pDCs by prolonging CXCL12 chemoattractant effects [88]. Interestingly, it was proposed that the biological activities of HMGB1 vary depending on the post-translational redox modifications of its cysteine residues [89, 90]. An oxidized environment has been proposed to inactivate HMGB1 and thus, to restrict its action. In contrast, a reduced environment might contribute to maintaining HMGB1 bioactivity. Carta et al. [91] speculated that HMGB1 inactivation by the oxidative stress encountered in the tumor microenvironment is delayed because of the creation of an antioxidant milieu by stressed or necrotic cells, thus prolonging the extracellular lifespan and activity of HMGB1. The local redox state of HMGB1 in the tumor microenvironment also regulates autophagy and apoptosis in cancer cells and modifies the sensitivity of tumors to anti-cancer treatments [92].

Moreover, adenosine, a nucleoside released in tissue during inflammation and tissue damage, was reported to recruit immature pDCs to inflammatory sites via A_1, a membrane-bound adenosine receptor. In contrast, upon maturation, pDCs down-regulate the A_1R, resulting in a loss of migratory function. pDC maturation was also associated with an up-regulation of A_{2a}, another adenosine receptor; this up-regulation was associated with reduced production of IL-6, IL-12, and IFN-α [93].

TOLEROGENIC/IMMUNOSUPPRESSIVE FUNCTIONS OF pDC IN THE TUMOR SETTING

Tumor induction of tolerogenic mediator expression by pDC

A population of TApDCs was identified in human and mouse prostate cancers showing tolerogenic activities by inducing suppressive activity in tumor-specific T cells. These cells express many genes associated with immune suppression and produce high levels of TGF-β, which may result in the induction of T cell-suppressive activity. *Foxo3* was defined as a novel signaling molecule critically implicated in the tolerogenic programming of human and transgenic adenocarcinoma of mouse prostate TApDCs. Indeed, silencing of *Foxo3* expression by using small interfering RNA ablates the immunosuppressive activities of human and murine TApDCs. *Foxo3* silencing was associated with a diminished expression of tolerogenic mediators, including IDO, which suppresses T cell function through catabolizing tryptophan, arginase, as well as TGF-β. In addition, this silencing up-regulates the expression of IL-6 and CD80, consistent with enhancing antitumoral immunity. High expression of *Foxo3* was also detected in TApDCs from other mouse tumor models, including B16 mela-

noma and orthotopic renal tumors. *Foxo3* expression by TApDCs in these models also induces T cell tolerance [33]. Another population of pDC-expressing IDO has been identified in human melanoma sentinel nodes [94]. IDO was also reported to be a key immunosuppressive factor, conferring T cell-suppressor activity on pDCs, which facilitates tumor progression in skin chronically exposed to carcinogens [95]. Furthermore, a tolerogenic role for IDO^+ pDC has been observed in mouse melanomas TDLNs. Indeed, these IDO^+ pDCs are capable of mediating active immunosuppression in vitro and of creating profound local T cell anergy in vivo through the direct activation of mature Tregs [96]. Virally stimulated pDC-expressing IDO can also induce the differentiation of naïve $CD4^+$ T cells into Tregs with suppressive function, confirming that authentic human pDCs can be induced to express IDO
[97, 98].

Tumors may regulate the expression of other tolerogenic mediators on TApDCs. For example, ICOSL might be expressed by TApDCs and could interact with ICOS expressed on naïve $CD4^+$ T cells to induce their differentiation in IL-10-producing T cells. Indeed, stimulated pDCs up-regulate the expression of ICOSL, which leads to the generation of IL-10-producing Tregs [99]. A recent report shows that the expansion and suppressive function of $FoxP3^+$ ICOS Tregs in an ovarian cancer environment is strictly dependent on ICOSL costimulation provided by tumor pDCs. Moreover, pDC and $ICOS^+$ $FoxP3^+$ Tregs were found to be strong predictors for disease progression in ovarian cancer patients, indicating an essential role for pDCs and ICOSL in $FoxP3^+$ Treg-mediated immunosuppression in ovarian cancer [100].

There is also evidence for the expression of GrB in human pDCs [101, 102]. Even though the classical function of this serine protease is to induce apoptosis in target cells, evidence is growing that apart from their cytotoxic functions, granzymes may also have alternative effects, such as immunosuppression [20, 103, 104]. To support this hypothesis, recent reports showed that GrB can be a tolerogenic mediator when secreted by pDCs. Indeed, pDCs activated by IL-3 and IL-10 are an abundant source of GrB and may produce GrB in amounts that exceed GrB produced by classical cytotoxic lymphocytes. GrB^+ pDCs potently suppress $CD4^+$ T cell proliferation [101]. As the cytokines mainly responsible for the induction of GrB in pDCs—IL-3 and IL-10—can be expressed in the environment of neoplastic diseases, it can be hypothesized that GrB could be secreted into the tumor environment by pDCs and suppress the expansion of tumor-specific T cells.

THERAPEUTIC APPROACHES AND CONCLUDING REMARKS

An effective recruitment of pDCs is observed in numerous cancers, but it clearly appears that this is not sufficient for an effective antitumoral response. In addition, in melanoma, breast, and HSNCC cancer, pDC infiltration is associated with a poor prognosis. pDC functionality strongly depends on the tumor microenvironment. Indeed, tumor cells or cells composing the tumor microenvironment have developed numerous mechanisms to alter pDC functionality, making them suppressive or tolerogenic.

Although results concerning the relation between IFN-α expression by TApDCs and their immune status are contradictory in the literature, it appears that suppression of IFN-α expression is a common feature of immunosuppressive pDCs in the tumor setting. Thus, several therapeutic protocols have been developed in cancers to stimulate pDC production of IFN-α. Imiquimod, a TLR7 agonist, has notably been used in cancer therapy because of its antitumoral action associated with the activation of NF-κB, which leads to the induction of proinflammatory cytokines such as IFN-α. Tumors that respond well to treatment with Imiquimod include basal cell carcinomas, melanoma, and cutaneous T cell lymphomas (for a review, see ref. [105]). A direct implication of pDCs in the antitumoral actions of TLR7 agonists has been described in several reports. Imiquimod was reported to induce pDC recruitment in skin cancers and lymphoma and to stimulate pDC production of IFN-α and subsequent IFN-α-inducible genes [19]. In a mouse model of melanoma, Imiquimod treatment leads to CCL2-dependent recruitment of pDCs that were fully equipped to produce type I IFN, which matured the pDCs into cytolytic cells and triggered the expression of TRAIL and GrB that directly eliminates tumor cells [20]. Imiquimod-activated pDCs were also reported to lyse human melanoma and T cell leukemia cell lines in a TRAIL-dependent fashion [106, 107].

Similar to TLR7 agonists, TLR9 agonists directly activate innate immune effectors to secrete IFN-α and proinflammatory cytokines, such as TNF-α and IL-6. Use of TLR9 agonists, such as CpG ODN, has shown promising results in clinical trials, including tumor vaccine models [108–111]. TLR9 agonists were active not only as monotherapy but also in combination with traditional anticancer therapies. Interestingly, it was recently shown that CpG ODN restores the ability of BM-pDCs from MM patients to trigger allogeneic and autologous T cell proliferation. Furthermore, CpG enhances IFN-α secretion from MM patient BM-pDCs, up-regulates TLR9 expression in MM BM-pDCs, and reduces pDC growth-promoting activity in MM [39]. In a mouse model of melanoma, TLR9-activated pDCs were also reported to initiate the orchestration of an immune cascade involving the sequential activation of NK cells, DCs, and $CD8^+$ T cells, ultimately leading to an effective and systemic antitumoral immunity [112].

Although CpG ODN and TLR7 agonists may facilitate tumor regression, the frequent limited responsiveness of pDC to TLR stimulation after their exposure to tumor microenvironments may prevent the beneficial actions of these agonists in cancer treatment [28]. As notably reported in HNSCC, TApDCs can exhibit a reduced capacity to produce IFN-α upon simulation with CpG ODN because of a down-regulation of TLR expression, showing that pDC stimulation by CpG ODN in the tumor environment may not always restore pDC functionality [26, 42]. Because of the frequent limited TLR expression by pDCs and the functional deficiency of pDCs in the tumor setting, effective antitumoral immunotherapy targeting pDCs may require prior restoration of their full function. As the inhibition of the proper antitumoral pDC function seems to be dependent on the presence of immunosuppressive ligands or on the expression of inhibitory molecules by the tumor microenvironment, a strategy of shifting the balance of factors to counteract the inhibitory function on

pDCs may be effective in restoring the antitumoral immune response. A potential therapeutic approach using CpG ODN or TLR7 agonists may benefit from combined therapy with specific molecules, directly depending on the tumor microenvironment to restore pDC functionality. For example, it may be possible to restore the effective tumor response through treatment with agents that promote an antitumoral response, such as anti-IL10 and COX inhibitors. Vicari et al. [113] provided evidence that the administration of an antibody against IL-10R potentiates the efficacy of CpG ODN by reversing IL-10-induced immunosuppression. Strategies that block inhibitory pathways in TApDCs, such as those initiated by *Foxo3*, may also enhance antitumoral immunity and prevent the immunosuppression observed in the microenvironment of many types of tumors [33].

Many questions about the regulation of pDC functionality in the tumor microenvironment remain to be answered. As IFN-α is a key molecule controlling cancer development, a better comprehension of the mechanisms used by the tumor to modify pDC functionality is an essential step for the development of more effective cancer vaccines and/or immunotherapies.

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DISCLOSURES

The authors declare that they have no conflict of interest.

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