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Dendritic cells in cancer: the role revisited

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Abstract

Dendritic cells (DCs) with their potent antigen presenting ability are long considered as critical factor in antitumor immunity. Despite high potential in promoting antitumor responses, tumor-associated DCs are largely defective in their functional activity and can contribute to immune suppression in cancer. In recent years existence of immune suppressive regulatory DCs in tumor microenvironment was described. Monocytic myeloid derived suppressor cells (M-MDSCs) can contribute to the pool of tumor associated DCs by differentiating to inflammatory DCs (inf-DCs), which appear to have specific phenotype and is critical component of antitumor response. Here we examine the role of inf-DCs along with other DC subsets in the regulation of immune responses in cancer. These novel data expand our view on the role of DCs in cancer and may provide new targets for immunotherapy.

Keywords

Dendritic cells; inflammation; cancer; immune suppression

Introduction

DCs are professional antigen presenting cells (APCs) that sample the microenvironment and provide antigens and co-stimulatory signals to cells of the adaptive immune system [1]. In steady state, DCs are largely present as an immature and weak APC characterized by the high capacity to capture antigens, low expression of costimulatory molecules and limited secretion of cytokines [2, 3]. Different stimuli associated with bacteria, viruses, and damaged tissues can induce the activation and maturation of DCs. Activated DCs are characterized by a down-regulated antigen capture activity, increased expression of major histocompatibility complex II (MHC class II), costimulatory molecules and C-C chemokine receptor type 7 (CCR7), high ability to produce cytokines and active migration to draining lymph nodes (dLNs). These DCs are potent inducers of T cell responses and are long considered as a critical component of antitumor immunity. However, it is also known that DCs do not effectively function in cancer. Recent advances in the understanding of the

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biology of different populations of DCs allow for better characterization of the nature of tumor associated DCs and provide new avenues for their therapeutic regulation.

Overview of the origin and types of dendritic cells in tumor-bearing hosts

DCs differentiate in bone marrow (BM) via sequential steps involving common myeloid progenitors (CMP) and macrophage/DC progenitors (MDPs). MDPs give rise to common monocyte precursors (cMOP) and common DC precursors (CDPs) [4, 5]. cMOPs then give rise to monocytes [6], which in tissues can differentiate to DCs under certain conditions, such as in cancer [7]. In steady state conditions practically all DCs in tissues differentiate from CDPs [8]. Currently, several subsets of DCs are recognized (Table 1) and the development of each subset of DCs is driven by specific transcriptional factors [9]. E2-2 favors the differentiation of plasmacytoid (pDCs) [10], while Id2 expression drives the differentiation of conventional (cDCs). Among cDCs, CD8 α ⁺ cDC in lymphoid organs and CD103⁺ cDCs in non-lymphoid organs depend on interferon regulatory factor 8 (Irf8) and basic leucine zipper transcriptional factor ATF-like 3 (Batf3) [11, 12], and CD11b⁺ DCs depend on interferon regulatory factor 4 (Irf4) and RbpJ [13, 14]. Zing finger and BTB domain containing 46 (Zbtb46) is selectively expressed by cDC lineages, but not by pDCs, macrophages, or monocytes. Zbtb46 is not necessary for DC development but it might influence DC subset composition [15, 16].

Fms-like tyrosine kinase 3 ligand (Flt3L) and granulocyte-macrophage colony stimulating factor (GM-CSF) are major cytokines involved in DC differentiation. The development of pDCs and cDCs but not monocyte derived DC is dependent on Flt3L and on its receptor Flt3 [17–19]. GM-CSF signaling is required for the development of non-lymphoid tissue-resident DCs in steady state and for the induction of CD8⁺ T cell immunity against particulate antigens. Mice lacking GM-CSF or their receptors showed normal monocyte [20] and lymphoid tissue DC differentiation, but an impaired development of CD103⁺ DCs and CD11b⁺ DCs in intestine, lung and dermis [21]. GM-CSF was also implicated in the acquisition of the capacity to cross-present antigens by cDCs [22, 23].

Plasmacytoid DCs

pDCs is a multifunctional population of BM derived DCs [24, 25] specializing in the production and secretion of type I interferons (IFNs). In mice, pDCs express Siglec-H, B220, Ly6c, and low amount of CD11c along with variable amounts of CD8 α and CD4. In the periphery, mouse pDCs express CC-chemokine receptor 9 (CCR9), LY49Q and SCA1 [26–28]. Human pDCs exhibit plasma cells morphology and express CD4, HLA-DR, CD123, and blood derived cell antigen-2 (BDCA-2), as well as Toll-like receptor (TLR) 7 and 9 within endosomal compartments but not CD11c [26, 28, 29]. Under homeostatic conditions, pDCs are found in small numbers in T cell areas of LNs and spleen, mucosal-associated tissues, thymus and liver. Upon TLR7/9 triggering, pDCs secrete high amount of type I IFN and produce interleukin-12 (IL-12), IL-6, tumor necrosis factor α (TNF- α), and other pro-inflammatory chemokines. pDCs can act as antigen presenting cells, but they are much less efficient than cDCs and depending on the context, antigen presentation by pDCs can induce immunogenic responses or tolerance [28].

pDCs represent small population of DCs and there is rather limited information about their involvement in antitumor responses. In mouse B16 melanoma, pDCs stimulated with TLR agonists were able to mediate tumor killing by the expression of TNF-related apoptosis-inducing ligand (TRAIL) and granzyme B and C and further exert their antitumor effects via production of type I IFN and subsequent activation of cytotoxic T and NK cells [30–32]. The initiation of immune responses leading to melanoma regression could be linked to the presence of pDC and their production of IFN- α [32]. In an orthotropic murine mammary tumor model, the administration of TLR7 ligands resulted in pDC activation and a potent antitumor effect [33]. The administration of activated pDCs loaded with tumor associated antigens to melanoma patients induced the specific CD4⁺ and CD8⁺ T cells responses [34]

Conventional DCs

In mice, cDCs can be divided into two main subpopulations: CD11b⁻ and CD11b⁺ cells. CD11b⁻DCs include lymphoid tissue CD8 α ⁺CD11b⁻ DCs and non-lymphoid tissue CD103⁺CD11b⁻ DC. Lymphoid-tissue resident CD11c⁺CD8 α ⁺Clec9a/DNGR-1⁺XCR1⁺ DCs and migratory CD11c⁺CD103⁺Clec9a/DNGR-1⁺XCR1⁺DCs are considered the most effective cross-presenting DCs *in vivo* [35]. Contrary to other subsets, Baft3 dependent CD103⁺ and CD8⁺ DCs have specific properties favoring cross-presentation. They limit the antigen degradation by maintaining an alkaline pH in their phagosomes through the production of reactive oxygen species (ROS). A recent study showed that the lectin family member Siglec-G negatively controlled ROS production by inhibiting NOX2 on DC phagosomes. This resulted in an excessive hydrolysis of exogenous antigens which led to a decreased formation of MHC class I-complexes for cross-presentation. Cross presenting CD8⁺ DCs showed lower expression of Siglec-G than CD8⁻ DCs, and Siglec-G deficient mice generated stronger cytotoxic T cells (CTL) than wild type mice [36]. CD11b⁺DCs are an IRF4 dependent subset of lymphoid resident DCs characterized by the expression of CD11c, CD11b, CD172a. CD11b⁺DCs have a dominant role in presenting antigens on MHC class II to CD4⁺ T cells. CD11b⁺DCs, contrary to CD103⁺DCs, induced Th2 cell priming in lung during allergic airway inflammation [37]. However, recently, a subset of migratory CD11b⁺ DCs from lung was also shown to cross-present soluble antigens *in vivo* and to induce cytotoxic T cells in the presence of TLR7 ligand [38]. Contrary to baft3 dependent DCs that cross-present efficiently even in the absence of activation, CD11b⁺ DCs need specific activation to induce cross-presentation.

In human, an equivalent of Baft3 dependent DC subset is characterized by the expression of CD11c, CD141 (BDCA3), Clec9a/DNGR1, and XCR1 [39], and an equivalent of Irf4 dependent DCs is characterized by the expression of CD11c, CD11b, CD1c (BDCA1), and CD172a [40]. These DCs have been described in different human cancer including breast tumors, metastatic melanoma and head neck squamous cell carcinoma. The presence of CD141 DCs correlates with better outcomes in patients with tumors [41].

cDCs are known to infiltrate different tumor tissues and there is evidence that in addition to lymphoid organs the presentation of tumor antigens by DCs can also occur in the tumors. This suggests that DCs in tumor microenvironment (TME) can substantially influence the functions of antitumor T cells [42–45]. Tumor infiltrating DCs capture antigens released

from tumor cells and cross-present antigens to CD8⁺ T cells, driving the expansion of tumor specific CTLs [46–48] (Fig. 1). *Batf3*^{-/-} mice showed a profound reduction in the numbers cross-presenting DC and an impaired antitumor immunity [11, 49] indicating that only *Batf3*⁺ DCs are able to cross-present antigens. *Batf3* independent DC subsets could mediate the antitumor responses under activating conditions [50]. In those experiments *Batf3*-deficient mice were treated with IL-12 to control tumor growth and IL-12 induced a *Batf3*-independent development of CD8⁺DCs. (Fig. 1)

Recently, a rare subset of cross-presenting DCs, MHC⁺CD24⁺F4/80⁻CD103⁺ (DC2), has been identified in some mouse models, as a distinct population from macrophages and from a second subset of DCs (DC1): MHC⁺CD24⁺F4/80⁻CD11b⁺ [51]. Similar DC populations have been found in human tumors, including metastatic melanoma, breast tumors, and head neck squamous cell carcinoma [52]. Human cDCs are represented by CD45⁺ HLA-DR⁺CD14⁻CD11c⁺ and either BDCA1⁺ (counterpart of mouse CD11b⁺DCs) or BDCA3⁺ (counterpart of mouse CD103⁺ DCs). BDCA3⁺ DCs express X-C motif chemokine receptor 1 (XCR1), providing an alternative marker for their characterization. The presence of BDCA3⁺DCs within TME correlated with better clinical outcome [51, 52]. DC2 have unique antigen processing and presentation properties and are dependent on the transcription factors *Irf8*, *Zbtb46* and *Batf3* as well as *Flt3l*. DC1 depends on GM-CSF for their differentiation. DC2 are more potent CTL stimulators within TME than DC1. DC2s traffic tumor antigens in a CCR7-dependent manner to the dLN and this trafficking is critical for the effective anti-tumor CD8⁺ T cell priming [53]. Importantly, tumor infiltrating human CD141⁺DCs, the equivalent of mouse DC2, express detectable amount of CCR7, and the expression of CCR7 correlates with better patients' outcomes. The importance of tumor infiltrating CD103⁺ DCs in regulating antitumor responses has also been demonstrated in two different models of mouse melanoma. These migratory DCs show low expression of lysosomal enzymes, which favor the transport of intact antigens to dLNs and the antigen cross-presentation [54]. Furthermore, efficacy of checkpoint inhibitors relies on the presence of cross-presenting migratory CD103⁺ DCs in TME [48]. The cross-priming DCs induced a measurable CTL response to tumor antigens, which was enhanced by anti-cytotoxic T lymphocyte antigen 4 (CTLA-4) and anti-programmed death-1 (PD-1) mAb therapy [55]. Expanded and activated CD103⁺DCs by injecting FLT3L and poly I:C are shown to be required to enhance anti-tumor responses upon blockade of the checkpoint ligand PD-L1 and BRAF inhibition [54]. The production of type I IFNs within the TME is important for DC function [56]. Tumor-derived DNA can induce the production of type I IFNs by tumor infiltrating DCs via cytosolic DNA-sensing stimulator of IFN genes (STING) pathway [57]. Importantly, type I IFN signaling is particularly required within *Batf3* dependent DCs and is critical for the spontaneous regression of immunogenic mouse transplantable tumors [46].

Monocyte-derived inflammatory DCs

Inflammatory DCs (inf-DCs) originate from monocytes [58], as a consequence of inflammation, cancer or infection and largely absent in steady state conditions. Circulating Ly6C^{high} monocytes are considered to be the direct precursors of inf-DCs [5]. In mice, inf-DCs are identified as MHC II⁺CD11b⁺CD11c⁺F4/80⁺Ly6c⁺, and express CD206 CD115/GM-CSFR, Mac-3/CD107b, FcεRI and CD64 as well as *zbtb46*. FcεRI is useful

marker to distinguish inf-DCs from cDCs and macrophages. Several studies have been shown that inf-DCs can activate antigen specific CD4⁺ T cell responses *ex vivo*. Inf-DCs can also cross-present exogenous antigens in different models, including HSV-1 reactivation, EAE and allograft models.

Human inf-DCs have been described in several pathological conditions, including psoriasis and cancer. They express HLA-DR, CD11c, BDCA1, CD1a, FcεRI, CD206, CD172a, CD14 and CD11b. Similar to murine inf-DCs, they express M-CSFR and ZBTB46 [58, 59].

In tumors, Ly6C^{high} monocytes exist as highly suppressive monocytic myeloid-derived suppressor cells (M-MDSC)[60] and can differentiate into tumor associated macrophage and inf-DCs [61]. The cytokines and factors that control the differentiation of monocytes into inf-DCs are not well defined. Elevation of GM-CSF concentration induces accumulation of CD11b⁺MHCII⁺ DCs [62, 63]. Moreover GM-CSF producing B16 melanoma triggers the expansion of CD11b⁺Ly6c^{hi-lo} MHC II⁺ cells similar to CD11b+DCs [64]. A very recent study has shown that monocytes are heterogeneous and contain different precursors giving rise to macrophages and DCs. The differentiation of DCs from PU.1^{hi} Flt3⁺MHCII⁺ monocytes is depending on GM-CSF. Importantly this study supports the view that monocyte derived macrophages and monocyte derived DCs are ontogenically distinct populations [65]. Other key requirements appear to be T cell activation signals [66] and toll like receptor ligands (TLRs) [20].

The presence of inf-DCs correlated with CD8⁺ T cell activation and treatment success in several tumor models. A population of inf-DCs has been identified and characterized in untreated ovary and cancer patients. These inf-DCs have a unique phenotype and are potent stimulator of Th17 cells as compared to macrophages [59]. In a melanoma model, the local treatment with the immunostimulatory agent monosodium urate crystals and *M. segmatis* showed the critical role of inf-DCs in the successful treatment. Intra-tumoral CD11c⁺CD11b⁺Ly6c^{high} cells, that display some characteristics of inf-DC, were found important for the efficacy of anticancer chemotherapy [67–69]. These DCs were efficient in capturing and presenting tumor antigens, and once adoptively transferred into naïve mice they can protect against a challenge with tumor cells [70]. Very recently, it has been shown that a subset of inf-DCs producing TNFα and NO (TIP-DCs) are expanded and activated after adoptive T-cell cell transfer (ACT) [71]. TIP-DCs seem necessary for tumor growth control because of their ability to promote the expansion of anti-tumor T cells and antitumor effect of TNFα and NO. Importantly, antitumor effect of these cells required activation of the CD40-CD40L pathway [71] (Fig. 1). Moreover, tumor of colorectal cancer patients with prolonged disease free survival (DFS) shared a signature comprising activated DCs, CD8⁺T cells and as well as CD40L, NOS and TNF.

DC dysfunction in cancer

Despite the presence of DCs in TME, immune surveillance in cancer ultimately fails and immune therapeutic strategies that rely on cross-presentation have rather limited activity. This suggests that DC function could, among other factors, contribute to immune non-responsiveness in cancer. It was established that tumor associated DCs are defective in their

differentiation and activation and are poor stimulators of immune responses. Recent data provided additional support to this notion and demonstrated novel mechanisms of negative regulation of DC function (Fig 2). Hypoxia, accumulation of adenosine, increased levels of lactate and decreased pH are shown to impair the normal function of DCs. In a breast cancer model, IL-10 has been shown to inhibit IL-12 production by tumor infiltrating CD103⁺CD11b⁻DCs, altering their ability to mount antigen specific T cell responses. The expression of IL-12 and the anti-tumor responses were restored in mice treated with antibody blocking IL-10 receptor (IL-10R) [72]. This was consistent with an earlier study showing that a combination of anti-IL-10R antibody and CpG oligonucleotides restored tumor DC function.

The expression of inhibitory molecules, such as PD-L1, contributes to the altered functionality of DCs in tumors or dLNs. It was recently shown that CD103⁺DCs from tumor dLNs expressed higher PD-L1 than DCs from non dLNs, and blockade of PD-L1 and PD-1 mitigated DC dysfunction. This resulted in increased production of TNF α , IL-12, IL-1 β , and enhanced T cell-stimulatory capacity by DCs [54].

Emerging evidences also suggested the presence of DCs in TME with an impaired antigen cross-presentation [43–45]. This resulted in altered activation and maintenance of antitumor immunity, thus supporting tumor progression. Abnormal accumulation of lipids in DCs is one of the major mechanisms contributing to DC dysfunction [73]. DCs isolated from several tumor models and cancer patients, showed an impaired antigen cross-presentation caused by the accumulation of lipids in DCs [73–76]. Consistent with these findings, the accumulation of lipid droplets was found to be responsible for the inability of DCs to induce antitumor T cell responses in ovarian cancer [77]. These DCs exhibited endoplasmic reticulum (ER) stress and robust activation of an ER stress response factor spliced X-box-binding protein 1, which induced triglyceride biosynthesis leading to abnormal lipid accumulation. Other recent studies confirmed that dysfunction of DCs in radiation-induced thymic lymphoma and mesothelioma was mainly due to lipid accumulation [78, 79] (Fig. 2).

Recently, new evidence suggested that tumors can convert tumor-infiltrating DCs into immunosuppressive regulatory cells. A population of inf-DCs with a suppressive phenotype was described in TME of different transplantable and autochthonous models of ovarian cancer [80]. These DCs produce tumor promoting IL-6 and immunosuppressive galectin-1. These cells are characterized by the expression of CD11c, MHCII, Dngr1, and Zbt46. They also co-expressed inf-DCs markers: Fc ϵ RI, CD11b and CCR7. Tumor infiltrating DCs overexpressed several genes that regulated their immune-suppressive functions. STAT3 was found to be activated in tumor DCs and induces S100A9, which prevents full maturation of DCs by blocking their responsiveness to local danger signals. DCs can overexpress FOXO3 transcription factor, which drives the expression of indolamine 2,3-dioxygenase (IDO), arginase, and TGF- β and suppress the expression of costimulatory molecules. The unremitting expression of special AT-rich sequence-binding protein 1 was found to drive a genome wide transcriptional programs that transforms DCs into high producers of IL-6 and galectin-1 [80]. It is important to note that the high level of tumor associated DCs was rather unique feature of this particular tumor model as most solid tumor models have much smaller proportion of DCs.

In the TME, pDCs also tend to be tolerogenic, favoring tumor progression. The recruitment of pDCs to several tumors, such as ovarian, head and neck, breast and primary melanoma, is often associated with poor prognosis [29, 30]. Many studies have shown that tumor associated pDCs are immature and are defective in the production of type I IFN. pDCs can induce Treg (through IDO or inducible T cell co-stimulator ligand (ICOSL)[81] and ICOSL expression on pDCs seems to correlate with breast cancer progression by a mechanism involving the induction of IL-10 producing Tregs. Moreover a high proportion of OX40L and ICOSL-expressing pDCs correlated with the frequency of IL-5, 10, and 13 producing T cells in melanoma, and Th2-promoting pDC were associated with the progression of melanoma.

Conclusions

Successful immune therapy of cancer relies on the ability of DCs to act as antigen presenting cells to T cells. The reduced efficacy of DC based cancer therapies is mainly due to suppressive TME and insufficient functionality of DCs. Recent studies have demonstrated that not all myeloid cells within TME have suppressive/regulatory functions. CD103⁺ DCs are able to induce anti-tumor responses. Moreover, it appears that highly suppressive M-MDSC may differentiate under some circumstances to inf-DCs with anti-tumor functions. This is a novel concept that revisits the role of M-MDSC within TME, which are known to give rise to suppressive tumor associated macrophages. Whether, this dichotomy is a general phenomenon or depends on specifics of TME remains to be elucidated. However, it is clear that the suppressive TME is one of the main factors responsible for the failure of cell based therapies. Thus, it will be critical to define strategies to enhance the differentiation and function of inf-DCs and simultaneously reduce the suppressive functions of other immune cells within TME. The main challenge for upcoming years is not only to better understand molecular mechanisms regulating transition of DCs to regulatory cells, but also develop methods for selective activation of DCs in TME.

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References

1. Shortman K, Naik SH. Steady-state and inflammatory dendritic-cell development. *Nat Rev Immunol.* 2007; 7(1):19–30. [PubMed: 17170756]
2. Trombetta ES, Mellman I. Cell biology of antigen processing in vitro and in vivo. *Annu Rev Immunol.* 2005; 23:975–1028. [PubMed: 15771591]
3. Steinman RM, Hawiger D, Nussenzweig MC. Tolerogenic dendritic cells. *Annu Rev Immunol.* 2003; 21:685–711. [PubMed: 12615891]
4. Fogg DK, et al. A clonogenic bone marrow progenitor specific for macrophages and dendritic cells. *Science.* 2006; 311(5757):83–7. [PubMed: 16322423]

5. Merad M, et al. The dendritic cell lineage: ontogeny and function of dendritic cells and their subsets in the steady state and the inflamed setting. *Annu Rev Immunol.* 2013; 31:563–604. [PubMed: 23516985]
6. Hettinger J, et al. Origin of monocytes and macrophages in a committed progenitor. *Nat Immunol.* 2013; 14(8):821–30. [PubMed: 23812096]
7. Segura E, Amigorena S. Identification of human inflammatory dendritic cells. *Oncoimmunology.* 2013; 2(5):e23851. [PubMed: 23762786]
8. Onai N, et al. Identification of clonogenic common Flt3+M-CSFR+ plasmacytoid and conventional dendritic cell progenitors in mouse bone marrow. *Nat Immunol.* 2007; 8(11):1207–16. [PubMed: 17922016]
9. Murphy TL, et al. Transcriptional Control of Dendritic Cell Development. *Annu Rev Immunol.* 2016; 34:93–119. [PubMed: 26735697]
10. Cisse B, et al. Transcription factor E2-2 is an essential and specific regulator of plasmacytoid dendritic cell development. *Cell.* 2008; 135(1):37–48. [PubMed: 18854153]
11. Hildner K, et al. Batf3 deficiency reveals a critical role for CD8alpha+ dendritic cells in cytotoxic T cell immunity. *Science.* 2008; 322(5904):1097–100. [PubMed: 19008445]
12. Hacker C, et al. Transcriptional profiling identifies Id2 function in dendritic cell development. *Nat Immunol.* 2003; 4(4):380–6. [PubMed: 12598895]
13. Schlitzer A, et al. IRF4 transcription factor-dependent CD11b+ dendritic cells in human and mouse control mucosal IL-17 cytokine responses. *Immunity.* 2013; 38(5):970–83. [PubMed: 23706669]
14. Persson EK, et al. IRF4 transcription-factor-dependent CD103(+)CD11b(+) dendritic cells drive mucosal T helper 17 cell differentiation. *Immunity.* 2013; 38(5):958–69. [PubMed: 23664832]
15. Meredith MM, et al. Expression of the zinc finger transcription factor zDC (Zbtb46, Btbd4) defines the classical dendritic cell lineage. *J Exp Med.* 2012; 209(6):1153–65. [PubMed: 22615130]
16. Satpathy AT, et al. Zbtb46 expression distinguishes classical dendritic cells and their committed progenitors from other immune lineages. *J Exp Med.* 2012; 209(6):1135–52. [PubMed: 22615127]
17. Karsunky H, et al. Flt3 ligand regulates dendritic cell development from Flt3+ lymphoid and myeloid-committed progenitors to Flt3+ dendritic cells in vivo. *J Exp Med.* 2003; 198(2):305–13. [PubMed: 12874263]
18. McKenna HJ, et al. Mice lacking flt3 ligand have deficient hematopoiesis affecting hematopoietic progenitor cells, dendritic cells, and natural killer cells. *Blood.* 2000; 95(11):3489–97. [PubMed: 10828034]
19. Waskow C, et al. The receptor tyrosine kinase Flt3 is required for dendritic cell development in peripheral lymphoid tissues. *Nat Immunol.* 2008; 9(6):676–83. [PubMed: 18469816]
20. Greter M, et al. GM-CSF controls nonlymphoid tissue dendritic cell homeostasis but is dispensable for the differentiation of inflammatory dendritic cells. *Immunity.* 2012; 36(6):1031–46. [PubMed: 22749353]
21. Bogunovic M, et al. Origin of the lamina propria dendritic cell network. *Immunity.* 2009; 31(3):513–25. [PubMed: 19733489]
22. Zhan Y, et al. GM-CSF increases cross-presentation and CD103 expression by mouse CD8(+) spleen dendritic cells. *Eur J Immunol.* 2011; 41(9):2585–95. [PubMed: 21660938]
23. Sathe P, et al. The acquisition of antigen cross-presentation function by newly formed dendritic cells. *J Immunol.* 2011; 186(9):5184–92. [PubMed: 21422244]
24. Liu K, et al. In vivo analysis of dendritic cell development and homeostasis. *Science.* 2009; 324(5925):392–7. [PubMed: 19286519]
25. Naik SH, et al. Development of plasmacytoid and conventional dendritic cell subtypes from single precursor cells derived in vitro and in vivo. *Nat Immunol.* 2007; 8(11):1217–26. [PubMed: 17922015]
26. Swiecki M, Colonna M. Accumulation of plasmacytoid DC: Roles in disease pathogenesis and targets for immunotherapy. *Eur J Immunol.* 2010; 40(8):2094–8. [PubMed: 20853492]
27. Swiecki M, Colonna M. Unraveling the functions of plasmacytoid dendritic cells during viral infections, autoimmunity, and tolerance. *Immunol Rev.* 2010; 234(1):142–62. [PubMed: 20193017]

28. Swiecki M, Colonna M. The multifaceted biology of plasmacytoid dendritic cells. *Nat Rev Immunol.* 2015; 15(8):471–85. [PubMed: 26160613]
29. Saadeh D, Kurban M, Abbas O. Plasmacytoid dendritic cell role in cutaneous malignancies. *J Dermatol Sci.* 2016; 83(1):3–9. [PubMed: 27236509]
30. Lombardi VC, Khaiboullina SF, Rizvanov AA. Plasmacytoid dendritic cells, a role in neoplastic prevention and progression. *Eur J Clin Invest.* 2015; 45(Suppl 1):1–8.
31. Salio M, et al. Plasmacytoid dendritic cells prime IFN-gamma-secreting melanoma-specific CD8 lymphocytes and are found in primary melanoma lesions. *Eur J Immunol.* 2003; 33(4):1052–62. [PubMed: 12672071]
32. Wenzel J, et al. Type I interferon-associated recruitment of cytotoxic lymphocytes: a common mechanism in regressive melanocytic lesions. *Am J Clin Pathol.* 2005; 124(1):37–48. [PubMed: 15923172]
33. Le, Mercier I., et al. Tumor promotion by intratumoral plasmacytoid dendritic cells is reversed by TLR7 ligand treatment. *Cancer Res.* 2013; 73(15):4629–40. [PubMed: 23722543]
34. Tel J, et al. Natural human plasmacytoid dendritic cells induce antigen-specific T-cell responses in melanoma patients. *Cancer Res.* 2013; 73(3):1063–75. [PubMed: 23345163]
35. Schraml BU, Reis e Sousa C. Defining dendritic cells. *Curr Opin Immunol.* 2015; 32:13–20. [PubMed: 25553392]
- *36. Ding Y, et al. The lectin Siglec-G inhibits dendritic cell cross-presentation by impairing MHC class I-peptide complex formation. *Nat Immunol.* 2016; 17(10):1167–75. This paper describes novel role of Siglec-G in regulation of cross-presentation by DCs in cancer. [PubMed: 27548433]
37. Plantinga M, et al. Conventional and monocyte-derived CD11b(+) dendritic cells initiate and maintain T helper 2 cell-mediated immunity to house dust mite allergen. *Immunity.* 2013; 38(2):322–35. [PubMed: 23352232]
38. Desch AN, et al. Dendritic cell subsets require cis-activation for cytotoxic CD8 T-cell induction. *Nat Commun.* 2014; 5:4674. [PubMed: 25135627]
39. Poulin LF, et al. Characterization of human DNGR-1+ BDCA3+ leukocytes as putative equivalents of mouse CD8alpha+ dendritic cells. *J Exp Med.* 2010; 207(6):1261–71. [PubMed: 20479117]
40. Segura E, Amigorena S. Cross-Presentation in Mouse and Human Dendritic Cells. *Adv Immunol.* 2015; 127:1–31. [PubMed: 26073982]
41. Sluijter BJ, et al. Arming the Melanoma Sentinel Lymph Node through Local Administration of CpG-B and GM-CSF: Recruitment and Activation of BDCA3/CD141(+) Dendritic Cells and Enhanced Cross-Presentation. *Cancer Immunol Res.* 2015; 3(5):495–505. [PubMed: 25633713]
42. Liu Y, Cao X. Intratumoral dendritic cells in the anti-tumor immune response. *Cell Mol Immunol.* 2015; 12(4):387–90. [PubMed: 25597333]
43. Zong J, et al. Tumor-derived factors modulating dendritic cell function. *Cancer Immunol Immunother.* 2016; 65(7):821–33. [PubMed: 26984847]
44. Conejo-Garcia JR, Rutkowski MR, Cubillos-Ruiz JR. State-of-the-art of regulatory dendritic cells in cancer. *Pharmacol Ther.* 2016; 164:97–104. [PubMed: 27118338]
45. Tang M, Diao J, Catral MS. Molecular mechanisms involved in dendritic cell dysfunction in cancer. *Cell Mol Life Sci.* 2016
46. Diamond MS, et al. Type I interferon is selectively required by dendritic cells for immune rejection of tumors. *J Exp Med.* 2011; 208(10):1989–2003. [PubMed: 21930769]
47. Fuertes MB, et al. Host type I IFN signals are required for antitumor CD8+ T cell responses through CD8{alpha}+ dendritic cells. *J Exp Med.* 2011; 208(10):2005–16. [PubMed: 21930765]
- **48. Sanchez-Paulete AR, et al. Cancer Immunotherapy with Immunomodulatory Anti-CD137 and Anti-PD-1 Monoclonal Antibodies Requires BATF3-Dependent Dendritic Cells. *Cancer Discov.* 2016; 6(1):71–9. This paper provides a direct evidence of critical role of cross-presenting DCs in regulation of antitumor effect of check-point inhibitors in cancer. [PubMed: 26493961]
49. Spranger S, Bao R, Gajewski TF. Melanoma-intrinsic beta-catenin signalling prevents anti-tumour immunity. *Nature.* 2015; 523(7559):231–5. [PubMed: 25970248]
50. Tussiwand R, et al. Compensatory dendritic cell development mediated by BATF-IRF interactions. *Nature.* 2012; 490(7421):502–7. [PubMed: 22992524]

51. Broz ML, et al. Dissecting the tumor myeloid compartment reveals rare activating antigen-presenting cells critical for T cell immunity. *Cancer Cell*. 2014; 26(5):638–52. [PubMed: 25446897]
52. Broz ML, Krummel MF. The emerging understanding of myeloid cells as partners and targets in tumor rejection. *Cancer Immunol Res*. 2015; 3(4):313–9. [PubMed: 25847968]
- *53. Roberts EW, et al. Critical Role for CD103(+)/CD141(+) Dendritic Cells Bearing CCR7 for Tumor Antigen Trafficking and Priming of T Cell Immunity in Melanoma. *Cancer Cell*. 2016; 30(2):324–36. Important supporting evidence of critical role of cross-presenting DCs in cancer. [PubMed: 27424807]
- **54. Salmon H, et al. Expansion and Activation of CD103(+) Dendritic Cell Progenitors at the Tumor Site Enhances Tumor Responses to Therapeutic PD-L1 and BRAF Inhibition. *Immunity*. 2016; 44(4):924–38. Important observation of involvement of cross-presenting DCs in antitumor effect of PD-L1. [PubMed: 27096321]
55. Gubin MM, et al. Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens. *Nature*. 2014; 515(7528):577–81. [PubMed: 25428507]
56. Zitvogel L, et al. Type I interferons in anticancer immunity. *Nat Rev Immunol*. 2015; 15(7):405–14. [PubMed: 26027717]
57. Corrales L, et al. The host STING pathway at the interface of cancer and immunity. *J Clin Invest*. 2016; 126(7):2404–11. [PubMed: 27367184]
58. Segura E, Amigorena S. Inflammatory dendritic cells in mice and humans. *Trends Immunol*. 2013; 34(9):440–5. [PubMed: 23831267]
59. Segura E, et al. Human inflammatory dendritic cells induce Th17 cell differentiation. *Immunity*. 2013; 38(2):336–48. [PubMed: 23352235]
60. Kumar V, et al. The Nature of Myeloid-Derived Suppressor Cells in the Tumor Microenvironment. *Trends Immunol*. 2016
61. Kumar V, et al. CD45 Phosphatase Inhibits STAT3 Transcription Factor Activity in Myeloid Cells and Promotes Tumor-Associated Macrophage Differentiation. *Immunity*. 2016; 44(2):303–15. [PubMed: 26885857]
62. Daro E, et al. Polyethylene glycol-modified GM-CSF expands CD11b(high)CD11c(high) but not CD11b(low)CD11c(high) murine dendritic cells in vivo: a comparative analysis with Flt3 ligand. *J Immunol*. 2000; 165(1):49–58. [PubMed: 10861034]
63. Mach N, et al. Differences in dendritic cells stimulated in vivo by tumors engineered to secrete granulocyte-macrophage colony-stimulating factor or Flt3-ligand. *Cancer Res*. 2000; 60(12):3239–46. [PubMed: 10866317]
64. Dranoff G, et al. Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony-stimulating factor stimulates potent, specific, and long-lasting anti-tumor immunity. *Proc Natl Acad Sci U S A*. 1993; 90(8):3539–43. [PubMed: 8097319]
65. Menezes S, et al. The Heterogeneity of Ly6Chi Monocytes Controls Their Differentiation into iNOS+ Macrophages or Monocyte-Derived Dendritic Cells. *Immunity*. 2016; 45(6):1205–1218. [PubMed: 28002729]
66. De, Trez C., et al. iNOS-producing inflammatory dendritic cells constitute the major infected cell type during the chronic Leishmania major infection phase of C57BL/6 resistant mice. *PLoS Pathog*. 2009; 5(6):e1000494. [PubMed: 19557162]
67. Kuhn S, Yang J, Ronchese F. Monocyte-Derived Dendritic Cells Are Essential for CD8(+) T Cell Activation and Antitumor Responses After Local Immunotherapy. *Front Immunol*. 2015; 6:584. [PubMed: 26635798]
68. Kuhn S, et al. Increased numbers of monocyte-derived dendritic cells during successful tumor immunotherapy with immune-activating agents. *J Immunol*. 2013; 191(4):1984–92. [PubMed: 23858033]
69. Rich FJ, et al. Induction of T cell responses and recruitment of an inflammatory dendritic cell subset following tumor immunotherapy with *Mycobacterium smegmatis*. *Cancer Immunol Immunother*. 2012; 61(12):2333–42. [PubMed: 22714285]
70. Ma Y, et al. Anticancer chemotherapy-induced intratumoral recruitment and differentiation of antigen-presenting cells. *Immunity*. 2013; 38(4):729–41. [PubMed: 23562161]

- *71. Marigo I, et al. T Cell Cancer Therapy Requires CD40-CD40L Activation of Tumor Necrosis Factor and Inducible Nitric-Oxide-Synthase-Producing Dendritic Cells. *Cancer Cell*. 2016; 30(3): 377–90. Important description of antitumor effect of TIP DCs. [PubMed: 27622331]
72. Ruffell B, et al. Macrophage IL-10 blocks CD8+ T cell-dependent responses to chemotherapy by suppressing IL-12 expression in intratumoral dendritic cells. *Cancer Cell*. 2014; 26(5):623–37. [PubMed: 25446896]
73. Herber DL, et al. Lipid accumulation and dendritic cell dysfunction in cancer. *Nat Med*. 2010; 16(8):880–6. [PubMed: 20622859]
74. Ramakrishnan R, et al. Oxidized lipids block antigen cross-presentation by dendritic cells in cancer. *J Immunol*. 2014; 192(6):2920–31. [PubMed: 24554775]
75. Mohammadyani D, et al. Molecular speciation and dynamics of oxidized triacylglycerols in lipid droplets: Mass spectrometry and coarse-grained simulations. *Free Radic Biol Med*. 2014; 76:53–60. [PubMed: 25110833]
76. Tyurin VA, et al. Mass-spectrometric characterization of peroxidized and hydrolyzed lipids in plasma and dendritic cells of tumor-bearing animals. *Biochem Biophys Res Commun*. 2011; 413(1):149–53. [PubMed: 21872574]
- **77. Cubillos-Ruiz JR, et al. ER Stress Sensor XBP1 Controls Anti-tumor Immunity by Disrupting Dendritic Cell Homeostasis. *Cell*. 2015; 161(7):1527–38. This study directly linked defective cross-presenting activity of tumor associated DCs with ER stress sensor sXBP1 suggesting novel therapeutic opportunities. [PubMed: 26073941]
78. Gao F, et al. Radiation-driven lipid accumulation and dendritic cell dysfunction in cancer. *Sci Rep*. 2015; 5:9613. [PubMed: 25923834]
79. Gardner JK, et al. Mesothelioma tumor cells modulate dendritic cell lipid content, phenotype and function. *PLoS One*. 2015; 10(4):e0123563. [PubMed: 25886502]
- **80. Tesone AJ, et al. Satb1 Overexpression Drives Tumor-Promoting Activities in Cancer-Associated Dendritic Cells. *Cell Rep*. 2016; 14(7):1774–86. Characterization of the population of inflammatory DCs with immune suppressive activity in tumor microenvironment and identification of Satb1 transcriptional regulator responsible for this effect. [PubMed: 26876172]
81. Ito T, et al. Plasmacytoid dendritic cells prime IL-10-producing T regulatory cells by inducible costimulator ligand. *J Exp Med*. 2007; 204(1):105–15. [PubMed: 17200410]

Key points

- All major subsets of DCs are critical component of anti-tumor immunity;
- DCs in TME show defects in differentiation, activation and functions;
- The presence inf-DCs with anti-tumor functions is a distinctive feature of TME;
- Inf-DC could differentiate from suppressive M-MDSC;
- Therapeutic interventions can improve the functionality of DCs in tumor bearing hosts

Figure 1. DC subsets in tumor microenvironment

(A) DCs arise from macrophage/DC progenitors (MDPs) in the bone marrow. MDPs give rise to the common monocyte progenitors (cMOPs) and to the common DC precursors CDPs. CDPs further differentiate into plasmacytoid DCs (pDCs) and pre-cDCs. pDCs terminally differentiated in the bone marrow, pre-cDCs become fully differentiated in conventional DCs (cDCs) in periphery. (B) In lymphoid and non-lymphoid tissues in tumor-free hosts, cDCs differentiate into CD11b⁺, CD103⁺ and CD8⁺ subsets. cMOPs give rise to monocyte, which can differentiate in macrophages and small number of DCs. (C) In tumor microenvironment (TME), pre-DC differentiate to two rare populations of DCs: CD11b⁺DCs (DC1) and CD103⁺DCs (DC2). Monocytes reach the TME and differentiate to macrophages and inflammatory DCs (Inf-DCs). pDCs are also found infiltrated in the TME. (D) DC2s and Inf-DCs migrate to dLNs in a CCR7 dependent manner and cross-present tumor antigens to CD8 T cells, starting antitumor responses.

Figure 2. Mechanisms of DC dysfunction in cancer

(A) Hypoxia, adenosine, lactic acid, low pH impair the ability of DCs to stimulate T cell responses. (B) IL-10 induces the differentiation of tolerogenic DCs, characterized by a low expression of costimulatory and MHC molecules, and high production of IL-10. (C) IL-10 produced by TAM inhibits the production of IL-12 by CD103⁺DCs, resulting in an impaired T cell activation. (D) The expression of PD-L1 on CD103⁺DCs in TME contributes to their dysfunction. (E) Tumor derived factors drive the differentiation of immunosuppressive inf-DC. They produce tumor promoting IL-6 and immunosuppressive galectin-1. (F) Abnormal accumulation of lipids in DCs impairs their ability to cross-present tumor antigens. (G) pDCs produce low amount of type I IFN but show higher expression of OX40L and ICOSL. The expression of these markers is associated with the production of IL-5, IL-10, and IL-13 by T cells. (H) IDO producing pDCs induce the differentiation of Treg cells in TME.

Table 1

DC subsets and their basic functions

Subsets	Transcriptional factor	Mouse	Human	Functions
pDC	E2-2	CD11c, Ly6C, B220, Siglec-H	CD4, HLA-DR, CD123, BDCA2, TLR7 AND 9	Type I IFN production, tumor killing, antigen presentation,
Lymphoid tissues Resident cDC	Irf4, Rbpj	CD11b, CD11c, CD172a	CD11b, CD11c, CD172a, CD1c	Antigen presentation, induction of Th2 T cell responses
Lymphoid tissues Resident cDCs	Batf3, Irf8	CD11c, CD8 α , Clec9a/ DNGR1, XCR1	CD11c, CD141, Clec9a/DNGR1, XCR1	Antigen cross presentation, induction of anti-tumor responses
Non Lymphoid tissues Migratory cDC	Batf3, Irf8	CD11c, CD103, Clec9a/ DNGR1, XCR1	CD11c, CD141 (BDCA3), Clec9a/ DNGR1, XCR1	Antigen cross presentation, migration, induction of anti-tumor responses
Inflammatory DCs		MHC II, CD11b, F4/80, Ly6c, CD206, CD115/GM-CSFR, Mac-3/CD107b Fc ϵ RI, CD64	HLA-DR, CD11c, CD1c (BDCA1), CD1a, Fc ϵ RI, CD206, CD172a, CD14, CD11b	Antigen presentation, migration, induction of anti-tumor responses, production of TNF and NO, tumor rejection
Intratumoral DCs (DC2)	Batf3, Irf8, Zbtb46	MHCII, CD11c, CD24, CD103	CD45, HLA-DR, CD141 (BDCA3)	Antigen cross presentation, induction of anti-tumor responses, production of IL- 12, tumor rejection,
Intratumoral (DC1)	Irf4	MHCII, CD24, CD11b	CD45, HLA-DR, CD1c (BDCA1)	Unknown

The characterization is based on the most recent transcriptional factors and phenotypic markers used to distinguish different population of myeloid cells in TME and periphery.