Tumor-altered dendritic cell function: implications for anti-tumor immunity

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INTRODUCTION

Dendritic cells (DC) are key regulators of both innate and adaptive immunity, and the array of immunoregulatory functions exhibited by these cells is dictated by their differentiation, maturation, and activation status. Although a major role for these cells in the induction of immunity to pathogens has long been appreciated, data accumulated over the last several years has demonstrated that DC are also critical regulators of anti-tumor immune responses. However, despite the potential for stimulation of robust anti-tumor immunity by DC, tumor-altered DC function has been observed in many cancer patients and tumor-bearing animals and is often associated with tumor immune escape. Such dysfunction has significant implications for both the induction of natural anti-tumor immune responses as well as the efficacy of immunotherapeutic strategies that target endogenous DC in situ or that employ exogenous DC as part of anti-cancer immunization maneuvers. In this review, the major types of tumor-altered DC function will be described, with emphasis on recent insights into the mechanistic bases for the inhibition of DC differentiation from hematopoietic precursors, the altered programming of DC precursors to differentiate into myeloid-derived suppressor cells or tumor-associated macrophages, the suppression of DC maturation and activation, and the induction of immunoregulatory DC by tumors, tumor-derived factors, and tumor-associated cells within the milieu of the tumor microenvironment. The impact of these tumor-altered cells on the quality of the overall anti-tumor immune response will also be discussed. Finally, this review will also highlight questions concerning tumor-altered DC function that remain unanswered, and it will address factors that have limited advances in the study of this phenomenon in order to focus future research efforts in the field on identifying strategies for interfering with tumor-associated DC dysfunction and improving DC-mediated anti-tumor immunity.

Keywords: dendritic cell, tumor, differentiation, maturation, activation, immunosuppression, tumor microenvironment, immunotherapy

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DC from hematopoietic precursors to effects on the behavior of fully differentiated DC. These effects on DC and their precursors can lead to accumulation in the tumor microenvironment of a variety of cells that include myeloid-derived suppressor cells (MDSC), tumor-associated macrophages (TAM), immature DC, and immunoregulatory myeloid DC (mDC) and plasmacytoid DC (pDC), each of which exhibit distinct phenotypic characteristics (Table 1). The identification of such cells in cancer patients not only has important prognostic value, but it also has significant implications for (1) the induction of natural anti-tumor immune responses and (2) the efficacy of immunotherapeutic strategies that target endogenous DC in situ or that employ exogenous DC as part of immunization maneuvers. Therefore, because of the importance of DC differentiation, maturation, and activation in dictating the immune stimulatory versus inhibitory activities of these cells, interference with any of these processes by factors or cells within the tumor microenvironment may greatly influence the induction and maintenance of anti-tumor immune responses in the host. This review will summarize the current knowledge regarding tumor-altered DC function and its impact on anti-tumor immunity, and it will highlight both recent advances in the field as well as important questions that will need to be answered as efforts are made to improve the quality of DC-mediated anti-tumor immune responses and DC-based cancer immunotherapies in the future.

### Tumor-Altered Differentiation of DC Precursors and Accumulation of Myeloid-Derived Suppressor Cells and Tumor-Associated Macrophages Within Tumors

Dendritic cells are specialized cells that differentiate from both myeloid and lymphoid progenitors before acquiring their unique functions as Ag presenting cells (APC), and a number of studies have described factors derived from both tumors and associated cells within the tumor microenvironment that interfere with DC differentiation from precursors, thereby contributing to a loss of stimulatory APC activity in tumor-bearing hosts. Soluble factors secreted by human renal cell carcinomas and pancreatic cancers, including IL-6 and M-CSF, have been shown to block DC differentiation from CD34 + progenitors and promote lineage commitment toward CD14 + monocytes that express little to no MHC and costimulatory molecules and that fail to induce allogenic T cell proliferation in mixed leukocyte reaction (MLR) assays (25, 26). Similar inhibition of CD34 + precursor cell differentiation into DC has been attributed to tumor-derived VEGF (27), and this blockade of DC differentiation is associated with suppression of NF-κB activity in these cells (28). VEGF has also been implicated in suppressing the differentiation of skin-resident Langerhans cells in a murine fibrosarcoma model (29). In addition to secreting cytokines that inhibit DC differentiation, tumors may also secrete other factors that interfere with the development of different subsets of DC. The gangliosides GD2 and GM3 secreted by human and murine neuroblastoma cell lines have been shown to inhibit differentiation of DC from CD34 + progenitors (30), and human melanomas secrete GM3 and GD3 gangliosides that not only inhibit DC differentiation from monocytic precursors but also induce apoptosis of monocyte-derived DC (31, 32). Likewise, cyclooxygenase-1 (COX-1)- and COX-2-derived prostanooids present in primary tumor-derived supernatants from several freshly isolated human tumor types block DC differentiation as well (33), and the source of these suppressive mediators may be not only tumor cells themselves but also stromal cells within the tumor microenvironment, as stromal cell-derived prostaglandin-E2 (PGE2) was recently shown to inhibit the differentiation of both bone marrow- and monocyte-derived DC (34). Regardless of the mechanism of inhibition, the loss of APC function associated with suppressing DC differentiation may significantly limit the induction of anti-tumor immune responses and contribute greatly to tumor immune escape.

In addition to the inhibitory effects of tumor-derived and tumor-associated factors on DC differentiation that preclude the development of cells with APC function, there is an abundance of data documenting how these factors can also alter the differentiation program of DC precursors and promote the accumulation of immature myeloid cells with immunosuppressive function (Table 2). These MDSC, characterized by expression of CD11b and Gr-1 in mice and a number of cell surface markers in humans (Table 1), are associated with various cancer types and have been recovered at high levels from both tumors and tumor-draining lymph nodes (35–38). Their induction may be driven by a number of factors released by tumors and tumor-associated cells, including VEGF (39), TGFβ (40), IL-1β (41), IL-13 (42), GM-CSF (43), prostaglandins (44), reactive oxygen species (ROS) (45), and components of the complement system (46). Differentiation into

| Table 1 | Phenotypic characteristics of tumor-associated DC and populations derived from DC precursors. |
|---|---|---|
| Cell population | Cell surface markers | Soluble proteins |
| Immature DC | CD11c high, CD80 low, CD86 low, MHC class I low | | |
| Mature/activated mDC | CD11c high, CD80 high, CD83, CD86 high, MHC class II high | IL-12p70 |
| MDSC | CD11b, Gr-1 (mice), CD11b, CD14+/–, CD15, CD33, MHC class II low (humans) | Arginase I, iNOS, ROI, IDO |
| TAM (M2-like) | CD11b, CD14, CD68, CD115, CD163, CD204, CD301, CD312, F4/80 | VEGF, HIF, TGFβ, IL-10, Arginase I, ROI |
| Regulatory mDC | CD11c high, CD40 low, MHC class II low, B7-H1 high, B7-DC high | Arginase I, IL-10, TGFβ |
| pDC | CD11c low, CD19, B220/CD45R, BDCA-4, MHC classI low | IFNα |
| Regulatory pDC | CD11c low, CD19, B220/CD45R, BDCA-4, MHC classII low, ICOS-L | IDO |
Table 2 | Mechanisms of immune suppression by MDSC.  

| Suppressive mediator | Cellular target | Impact on target |
|----------------------|----------------|-----------------|
| Reactive oxygen species | T lymphocytes | ↓ IL-2, ↑ proliferation, ↑ apoptosis |
| IDO | CD8+ T lymphocytes | Anergy |
| | CD4+ T lymphocytes | Induction of Tregs |
| Arginase I | T lymphocytes | ↓ CD3ε chain, ↓ Proliferation, Expansion of Tregs |
| TGFβ | CD4+ T lymphocytes | Induction of Tregs, Anergy |
| | NK cells | ↓ NKG2D, ↓ IFNγ, ↓ Cytotoxicity |
| CCL3, CCL4, CCL5 | CD4+ Tregs | Recruitment to tumor |
| IL-10 | Macrophages | ↓ IL-12 |
| ??? | DC | ↓ Phagocytosis, ↓ Maturation, ↓ Migration, ↓ T cell stimulation |

MDSC is associated with hyperactivation of STAT3 (39, 47, 48) and is accompanied by acquisition of a number of immunosuppressive properties. In a murine sarcoma model, MDSC suppression of Ag-specific CD8+ T cell responses required direct cell–cell contact via TCR/MHC class I and was mediated by release of ROS (49–51). Similarly, MDSC lines generated from mice bearing adenocarcinomas exhibit nitric oxide-mediated suppression of IL-2 signaling in activated T cells. In this model, nitric oxide production by MDSC required direct contact with, and IFNγ secretion by, the activated T cell, and this nitric oxide inhibited T cell proliferation and induced T cell apoptosis (52, 53). More recently, the increased production of ROS by MDSC was shown to be the result of upregulated NADPH oxidase activity in these cells in several different murine models and in head and neck cancer patients (54). MDSC have also been shown to induce T cell tolerance through release of indoleamine 2,3-dioxygenase (IDO) and arginase I, enzymes involved in degradation of tryptophan and arginine, respectively (55, 56). In regard to the latter, tumor-derived COX-2 can mediate PGE2 signaling in MDSC, thereby triggering overexpression of arginase I in these cells (57). In both murine models and cancer patients, tumors are enriched in arginase I-producing MDSC, and arginine metabolism in the tumor microenvironment leads to downregulation of CD3ε chain and suppression of proliferative capacity in both CD4+ and CD8+ T cells (58, 59). Interestingly, recent studies evaluating the abundance and activity of MDSC in tumors, tumor-draining lymph nodes, and peripheral blood of cancer patients have shown that both the frequency and arginase I activity of these cells correlates with the clinical stage of the tumor, thus suggesting a critical role for these immunosuppressive cells in disease progression (60, 61).

In addition to the direct tolerization of anti-tumor T lymphocyte responses by MDSC, these cells are also known to induce the development of regulatory T cells (Tregs) that can also suppress T cell activation. In this light, MDSC-derived TGFβ not only suppresses cytolytic activity of T lymphocytes (42), but it has also been demonstrated in the B16 murine melanoma model to promote expansion of CD4+ CD25+ FOXP3+ Tregs in both tumors and tumor-draining lymph nodes (62). Others have reported that tumor-infiltrating MDSC isolated from B16 melanomas also express high levels of the chemokines CCL3, CCL4, and CCL5, the ligands for the CCR5 chemokine receptor that is preferentially expressed on Tregs (63). These results indicate that MDSC likely also play a critical role in recruitment of expanded Tregs into the tumor microenvironment. Additionally, TGFβ-independent MDSC induction of Tregs has been reported in a B cell lymphoma model, where expansion of preexisting natural Tregs required Ag presentation and arginase I activity by MDSC (64). A link between MDSC and Tregs has also recently been reported in a study of breast cancer patients, where the presence of IODO-expressing MDSC correlated with increased infiltration of Tregs into primary tumors and lymph node metastases (56).

The immunosuppressive activities of MDSC extend beyond regulatory effects on T lymphocytes as well. In a murine model of gestation-enhanced metastasis of B16 melanoma, MDSC diminished the number and activity of NK cells (65). Likewise, in both mammary carcinoma and hepatic tumor models, MDSC suppressed NK2G2D expression, IFNγ secretion, and cytotoxic activity by NK cells (66, 67). In the hepatic tumor model, suppression required direct cell–cell contact and was mediated by membrane-bound TGFβ on MDSC, and this interaction caused NK cells to be hyporesponsive to activating stimuli, indicating that they had acquired an anergic phenotype. Similar findings have been reported in patients with hepatocellular carcinoma, where suppression of NK cell activity was dependent on MDSC engagement of the NKP30 receptor on NK cells (68). MDSC have also been shown both to impede the maturation and T cell stimulatory capacity of DC (69) and to engage in cross-talk with macrophages, leading to diminished IL-12 secretion by macrophages and increased IL-10 production by MDSC (70). Such alteration of cytokine secretion patterns has the potential to polarize helper and cytotoxic T cells toward a type 2 response that is less robust in its anti-tumor efficacy.

In addition to shifting the differentiation of myeloid precursors away from DC lineage commitment and promoting development of MDSC, tumors can also drive the differentiation of DC precursors into other immunosuppressive cells of myeloid origin, most notably TAM. Recently, these cells have been shown to descend from both bone marrow-derived and splenic precursors, and some populations are believed to reflect the culmination of MDSC differentiation (71). Importantly, accumulation of TAM, particularly those with an anti-inflammatory M2-like phenotype, correlates with poor prognosis in patients with a variety of cancers (72–76). Th2 cytokines, glucocorticoids, and growth factors present in the tumor milieu are all known to induce M2-like macrophages (77), and tumor-derived IL-10 has specifically been shown to inhibit
DC differentiation from monocytic precursors and to promote the development of TAM from these cells (78). Much like MDSC, these TAM can suppress a variety of immune effectors and promote Treg suppressive functions through production of TGFβ, IL-10, and arginase I (38, 58, 79, 80). They have also been shown to induce T cell apoptosis by upregulating expression of B7-H1 (81), the ligand for the PD-1 receptor on T cells. Taken together, the diverse effects exerted by TAM and MDSC on cells of both the innate and adaptive immune systems contribute greatly to the immunosuppressive nature of the tumor microenvironment, and these phenomena highlight the role that tumor-altered differentiation of DC progenitors into MDSC and TAM plays in promoting tumor immune escape.

**TUMOR-ASSOCIATED SUPPRESSION OF THE MATURATION AND ACTIVATION OF DIFFERENTIATED DC**

In addition to subverting anti-tumor immunity by altering the differentiation of DC precursors and either preventing acquisition of APC function by these cells or inducing their development into immunosuppressive MDSC or TAM, tumors may also interfere with the maturation and activation of fully differentiated DC. While *in vitro* studies have shown that the release of heat-shock proteins by necrotic tumors triggers DAMP-mediated DC maturation (82, 83), and the presence of mature tumor-infiltrating DC correlates with the magnitude of anti-tumor T cell responses and disease prognosis in cancer patients (84, 85), a number of studies have described the accumulation of fully differentiated, yet immature, DC in tumors as well (86–88). Although a lack of mature DC in tumor tissue might reflect tumor-induced death of these cells (31, 32, 89), this phenomenon does not explain the accumulation of immature DC often seen in tumors. In cases where immature DC are recovered from tumors, it is often unclear whether the immature phenotype of these cells reflects a simple failure of tumors to support DC maturation and activation or, alternatively, an active suppression of DC maturation by tumors. One study demonstrated that administration of anti-CD40 Ab to tumor-bearing animals leads to maturation of DC capable of stimulating T cell activation (90), suggesting that the tumor either fails to support DC maturation or that suppression of DC maturation by the tumor is a reversible process. In support of the latter possibility, it has been shown that the maturation of tumor-infiltrating DC is enhanced following dissociation of DC from the tumor and overnight culture *ex vivo*, demonstrating that the tumor had actively limited DC maturation *in vivo* (91). Other studies have revealed that tumor-infiltrating DC are refractory to some maturation stimuli but not others, indicating that tumors can actively suppress DC maturation but that in some cases this suppression can be reversed under appropriate stimulatory conditions (92–94). Interestingly, in a comparative study of melanoma patients exhibiting either progressing or regressing metastases, DC isolated from patients with progressive disease expressed significantly lower levels of costimulatory molecules than those taken from patients with regressing tumors. Furthermore, DC from patients with regressing metastases induced robust T cell proliferation, while DC from patients with progressing metastases induced T cell anergy (95). Collectively, these data suggest that the context in which the tumor is encountered by DC is likely to impact the quality of their maturation, activation, and immunostimulatory capacity, and they emphasize the need to understand the role of tumor-derived factors and the tumor microenvironment in regulating the function of tumor-associated DC.

The limiting number of DC that can be isolated from tumor-bearing animals and cancer patients and the complex nature of the cell types and soluble proteins present within the tumor microenvironment have made it difficult to gain mechanistic insights into tumor-associated suppression of DC maturation *in vivo*. *Ex vivo* experiments with monocyte-derived and bone marrow-derived DC (BMDC) have been used as an alternative to *in vivo* studies for evaluating the suppression of DC maturation by tumor cells or tumor-conditioned media (96–98). Recent studies using these and similar *ex vivo* models have shown that interference with the HIF-1-induced COX-2/PGE2 and VEGF pathways in colon cancer cells and knockdown of TGFβ expression in hepatocellular carcinoma both restore DC maturation that is otherwise suppressed by these tumors (99, 100). In another system involving a multicellular tumor spheroid three-dimensional model of melanoma, tumor-derived lactic acid was shown to suppress the production of several proinflammatory cytokines, including IL-12, by monocyte-derived DC and to limit the ability of these cells to induce T cell proliferation (101). Importantly, though, because these *ex vivo* systems often require differentiation of DC from progenitors in culture, it is often unclear from these studies whether the effects observed stem from a direct influence of tumors on DC or instead from an indirect action mediated by an influence of tumors on other cells in the culture that have not differentiated into DC. Therefore, to overcome the limitations inherent with studying the influence of tumors on DC function in both *in vivo* and *ex vivo* settings, DC lines that can be maintained as highly pure populations in culture have been generated and are a useful tool for *in vitro* studies aimed at understanding the basic biology of these cells (102–105). Such lines have enabled direct analyses of tumor/DC interactions, and it has recently been shown that melanoma-derived factors suppress the LPS-induced maturation of both the DC2.4 and JAWSII DC lines (106). In a related study, a comparative analysis of multiple murine melanoma cell lines demonstrated that the suppression of DC2.4 costimulatory molecule and proinflammatory cytokine/chemokine expression correlates with the tumorigenicity of the melanoma under study (107), with the highly tumorigenic B16 melanoma exhibiting significantly greater suppression than its poorly tumorigenic, genetically mutated variant D5.1G4. These findings again point to a potentially vital role for tumor/DC interactions in the regulation of overall anti-tumor immunity and tumor outgrowth. It will be interesting to evaluate differences in the profile of immunosuppressive mediators released by these particular melanoma cell lines, as this analysis will identify potential candidate molecules involved in the suppression of DC maturation and activation by this cancer. While concerns have been raised that maneuvers employed to immortalize DC lines may alter the maturation state of these cells and their responsiveness to regulatory factors, many of these lines do exhibit the characteristics of immature DC and are responsive to traditional maturation stimuli (108–110). Therefore, additional studies using these DC lines and other tumor
systems can offer proof-of-principle data that tumors interfere with DC maturation in a straightforward, cost-effective model, and such investigations will provide further mechanistic insight into tumor-associated suppression of DC maturation and activation. Furthermore, observations made in such in vitro systems are likely to inform the design of experiments evaluating the role of tumor-derived factors in the suppression of DC maturation and activation in more physiologically relevant ex vivo and in vivo settings. Collectively, use of these different models will increase our understanding of tumor-induced suppression of DC function, and these insights will suggest immunotherapeutic strategies designed to reverse or prevent this suppression and enhance the immunostimulatory capacity of tumor-associated DC.

**TUMOR-ASSOCIATED INDUCTION OF IMMUNOSUPPRESSIVE REGULATORY DC**

The suppression of DC maturation and activation by tumor cells or factors within the tumor microenvironment has significant implications for the induction of T cell immunity to tumors, as immature DC are poor APC and do not efficiently stimulate T cell activation. There is also now substantial evidence that tumors not only suppress DC maturation but that they can also induce the development of regulatory DC that actively display immunosuppressive activity themselves. In fact, recent studies have demonstrated that progression of ovarian cancer from an immunologically controlled state to metastatic disease is accompanied by a switch in the phenotype and function of tumor-associated DC. Whereas DC isolated from ascites or draining lymph nodes of early-stage tumor-bearing animals elicited robust T cell responses, those isolated from mice with advanced disease induced minimal T cell proliferation and suppressed T cell activation by immunocompetent DC (111, 112). Immunosuppressive DC isolated from late-stage tumor-bearing animals downregulated MHC class II and CD40 expression but significantly upregulated the co-inhibitory molecule B7-H1 and exhibited arginase I activity comparable to that seen in MDSC. These immunosuppressive activities were driven by tumor-derived PGE₂ and TGFβ (112). Other studies have also demonstrated tumor-induced upregulation of DC co-inhibitory molecules, including both B7-H1 and B7-DC (10, 96), as well as tumor-enhanced secretion of arginase I (113, 114) and TGFβ (115) by DC that inhibit T cell effector function and promote Treg development, respectively. In both tumor-bearing mice and prostate cancer patients, the expression of these and other immunoregulatory molecules by tumor-associated DC resulted from elevated expression of FOXO3 (116), a transcription factor recently shown to mitigate DC stimulatory capacity (117). Additionally, inhibition of T cell effector activity by tumor-associated regulatory DC has also been associated with increased IL-10 secretion by these cells. A variety of soluble factors present in colorectal tumor explant cultures, including VEGF and the chemokines CCL2, CXCL1, and CXCL5, were shown to enhance IL-10 production by DC (118, 119). Non-soluble mediators expressed on colorectal carcinoma cells can contribute to this process as well, as IL-10 production by DC was increased following engagement of DC-SIGN by tumor-associated cell surface glycans (120). Likewise, recombinant MUC1 mucas glycosylated in a manner equivalent to those expressed on breast carcinoma cells and natural MUC1 mucins in supernatants of human pancreatic carcinoma cell lines both suppress IL-12 production and promote IL-10 production by monocyte-derived DC, and these regulatory DC are poor stimulators of T cell proliferation and CTL activity but potent inducers of T cell anergy and CD4⁺ Tregs (121, 122). IL-10 production by tumor-associated DC that inhibit anti-tumor T cell responses and promote tumor outgrowth has also been reported to be induced by COX-2/PGE₂ (123, 124). Similarly, in a murine myeloma model, tumor-derived IL-6, IL-10, and TGFβ were all shown to contribute to p38 MAPK signaling-mediated effects on BMDC maturation that led to decreased production of IL-12 and increased production of IL-10 by DC, and these cells elicited poor tumor-specific T₁₁ CTL, and antibody responses (125). Hyperactivation of MAPK signaling similarly inhibited IL-12 production and T₁₁ stimulation by melanoma-altered DC, though these effects were independent of IL-10, TGFβ, VEGF, and PGE₂ in tumor lysates (97). In addition to suppressing the development of T₁₁-like immunity, other studies have shown that melanoma, as well as breast cancer, triggers DC-mediated induction of T₁₁-like responses that promote tumor development (126, 127). Identification of factors produced by these tumors and their role in MAPK hyperactivation in DC will be crucial to developing strategies for skewing anti-tumor T cells toward type 1 responses that are more efficient in mediating tumor rejection.

In addition to the regulatory DC activities described above, which are largely associated with conventional mDC, a specialized subset of DC that develop immunosuppressive activity in the context of many tumors is the pDC. IDO-expressing pDC can be induced by tumor-derived PGE₂ (128) and have been recovered from tumor-draining lymph nodes of both melanoma-bearing animals and cancer patients (129). These cells suppress CD8⁺ T cell responses to Ag presented by the pDC themselves as well as to those presented by third-party, non-suppressive APC. In addition to inducing CD8⁺ T cell anergy, IDO production by pDC also promotes the differentiation of CD4⁺ CD25⁺ FOXP3⁺ Tregs (130). Interestingly, pharmacologic blockade of IDO leads to enhanced IL-6 production by pDC that converts tolerogenic CD4⁺ Tregs into T₁₁-like cells, and this conversion correlates with enhanced CD8⁺ T cell activation and anti-tumor immunity (131). CD4⁺ Treg induction by pDC can also be mediated by engagement of ICOS on T cells with ICOS-L on pDC, and ICOS-L⁺ Treg induction by pDC is associated with poor prognosis and disease progression in both breast and ovarian cancer patients (132–134). Tumors can also subvert immunity by regulating pDC production of IFN-α, a type I IFN that functions as a “signal 3” cytokine for CD8⁺ T cell activation (135) and that promotes the survival and Ag retention of CD8a⁺ DC, that cross-prime tumor-specific CD8⁺ T cells (11). In clinical studies, tumor-associated pDC have been isolated from myeloma cell lines via BDCA-4 positive selection of lineage-negative enriched mononuclear cells obtained from patient biopsies. In patients with aggressive breast cancers, these pDC exhibit suppressed IFN-α secretion and are able to sustain CD4⁺ Treg expansion (136), and the suppression of IFN-α production by pDC has been attributed to tumor-derived TGFβ and TNFα mediated-signaling in these cells (137). Finally, pDC isolated from ascites of ovarian carcinoma patients have also been shown to induce CD8⁺ Tregs that secrete high levels of IL-10 and suppress...
Additionally, tumor-associated DC dysfunction may limit the efficacy of immunotherapeutic strategies that rely on the activity of T cells associated with naturally generated immune responses in other experimental animal models and cancer patients (18–24). Additionally, tumor-associated DC dysfunction may limit the efficacy of immunotherapeutic strategies that rely on the activity of DC in situ to stimulate anti-tumor immunity, and it may therefore explain the lack of success observed thus far with many DNA-, peptide-, and protein-based immunization maneuvers that require endogenous DC to process and present tumor Ag to specific T cells (139–143). Even the quality of responses elicited following immunization with previously activated, exogenous DC may be compromised by an influence of tumor-associated factors on DC function. Importantly, though, insights into the mechanistic bases for tumor-associated DC dysfunction have informed the design of novel DC-based cancer immunotherapies, and many of these strategies have enhanced the T cell stimulatory capacity of DC and led to induction of more robust and efficacious anti-tumor immune responses.

Several strategies have been employed to promote DC differentiation from hematopoietic precursors and prevent the accumulation and suppressive activities of tumor-associated cells of myeloid origin. For instance, both IL-4 and IL-13 were shown to prevent renal cell carcinoma-induced blockade of DC differentiation (144). Similarly, administration of the anti-VEGF Ab bevacizumab to patients with lung, breast, and colorectal carcinoma led to a decrease in the frequency of MDSC and enhanced the T cell stimulatory capacity of DC (145). Abrogation of MDSC immunosuppression can also be achieved by exposure of these cells to all-trans retinoic acid, which induces the differentiation of MDSC isolated from a number of murine tumors and renal cell carcinoma patients into mature immunostimulatory DC (146, 147). Others have demonstrated that interference with STAT3-mediated-signaling reverses immune suppression by MDSC and enables differentiation of these cells into mature DC (39, 61). One study also showed that interference with both STAT3 and p38 MAPK signaling pathways in monocyte progenitors further improved the quality of tumor-associated DC, blocking the inhibitory effects of tumor-derived factors on DC differentiation from these progenitors and skewing the IL-12/IL-10 cytokine profile of the resulting DC toward a Th1-promoting phenotype (148). Based on these data, it is not surprising that vaccination with exogenous, STAT3-depleted DC was shown to enhance anti-tumor CD8+ T cell responses and improve control of tumor outgrowth (149).

In addition to strategies that interfere with the development and suppressive activities of tumor-associated myeloid cells, several approaches are being explored for improving the quality of fully differentiated DC in the context of tumors as well. In vivo administration of nanoparticles carrying immunostimulatory miRNA converts endogenous immunosuppressive DC into cells capable of activating robust anti-tumor responses that inhibit progression of established ovarian cancers (150). Moreover, supplementation of stimulatory cytokines whose expression is often suppressed in tumor-associated DC, such as IL-12 and IFNα, can enhance T cell effector function elicited by endogenous DC (151, 152). Significant efforts have also been made to optimize exogenous DC-based cancer immunotherapies. Several studies have investigated various maturation protocols for exogenous DC in order to best promote the immunostimulatory capacity and vaccine efficacy of these cells (153–156). One group has reported that treatment of PBMC-derived immature DC with various combinations of cytokines and inflammatory stimuli, namely LPS + IFNγ, LPS + IFNγ + IL-1β, and LPS + IFNγ + IL-1β + TNFα, results in no discernible difference in DC expression of costimulatory molecules or IL-12 (153). On the other hand, substantial differences in DC maturation have been observed following exposure of

### Table 3 | Induction and suppressive activity of tumor-associated regulatory DC.

| Tumor-derived factor | Regulatory DC activity | Impact on host immunity |
|----------------------|-----------------------|-------------------------|
| TGFβ, PGE2           | ↓ MHC II              | ↓ T cell proliferation  |
|                      | ↓ CD40                | ↓ T cell effector function |
|                      | ↑ B7-H1               |                         |
|                      | ↑ Arginase I          |                         |
| VEGF                 | ↑ IL-10               | ↓ T cell effector function |
| CCL2, CXCL1, CXCL5   |                       |                         |
| Glycans              |                       |                         |
| COX-2/PGE2           |                       |                         |
| MUC1 mucins          | ↓ IL-12               | ↓ T cell proliferation  |
|                      | ↑ IL-10               | ↓ CTL activity          |
| IL-6, IL-10, TGFβ    |                       | ↓ T cell anergy         |
|                      |                       | ↑ CD4+ Tregs            |
| ???                  | ↑ TGFβ                | ↑ CD4+ Tregs            |
| PGE2                 | ↑ IDO by pDC          | CD8+ T cell anergy      |
|                      | ↓ IL6 by pDC          | ↑ CD4+ Tregs            |
| TGFβ, TNFα           | ↑ IFNα by pDC         | CD4+ Tregs              |
|                      |                       | ↑ CD8+ Tregs            |
| ???                  | ↑ ICOS-L by pDC       | ↑ CD4+ Tregs            |

???, unidentified factor(s).
immature DC to a mixture of various other inflammatory mediator/cytokine cocktails. Stimulation with lipid A and IFNγ resulted in significantly higher DC expression of costimulatory molecules and IL-12 than stimulation with a combination of TNF-α, IFN-α, IFN-γ, IL-1β, and poly(I:C) or a combination of TNF-α, IFN-γ, IL-1β, and CL097 (156). Still others have evaluated DC maturation following exposure to tumor lysates. PBMC-derived DC treated with lysates from heat-shocked melanoma cells exhibited robust maturation and immunostimulatory capacity, as these cells were capable of cross-presenting melanoma-associated Ag and inducing anti-tumor CD8+ T cell responses (154). Importantly, heat-shocking of melanoma cells induced membrane translocation of CRT and expression of HMGB1, and the maturation of immunostimulatory DC in this study was dependent on their recognition of these tumor-derived “danger signals.” It has also recently been shown that TNFα can augment tumor lysate-induced DC maturation (155). In addition to investigating strategies for optimal induction of DC maturation, many researchers have employed strategies to block the suppressive effects of tumor-derived factors on exogenous DC. In this light, DC genetically engineered to secrete a VEGF/vascular permeability factor decoy receptor that neutralizes soluble VEGF and precludes signaling in DC resulted in increased expression of costimulatory molecules and proinflammatory cytokines/chemokines by DC and improved CTL activity and anti-tumor immune control in a murine colon cancer model (157). Similar improvements in the efficacy of an exogenous DC vaccine were observed following neutralization of tumor-derived TGFβ (158). Alternatively, other approaches for enhancing exogenous DC-induced anti-tumor immune responses aim at blocking either the immunosuppressive mediators expressed by tumor-altered DC or the targets of these mediators expressed on other immune cells. In a murine model of breast cancer, siRNA-mediated silencing of IDO in vaccinating DC enhanced the ability of these cells to stimulate
T cell proliferation and CTL effector function, decreased the induction of CD4^+ CD25^+ FOXP3^+ Tregs, and led to enhanced control of tumor outgrowth (159). Similarly, immunization with IL-10-deficient DC conferred enhanced protective and therapeutic immunity against a murine hepatocellular carcinoma (160). Furthermore, DC genetically engineered to interfere with immunomodulatory receptors expressed on endogenous immune cells, such as CTLA-4 on effector T cells and GITR on Tregs, can enhance the overall immunogenicity of these cells as well (161, 162). Improved anti-tumor immunity has also been observed for a DC-based vaccine administered in combination with anti-CTLA-4 Ab and Treg-depleting anti-CD25 Ab (163). Likewise, administration of neutralizing Ab that interferes with the B7-H1/PD-1 pathway improved the efficacy of a DC/tumor fusion vaccine in multiple myeloma patients (164). Finally, it is also possible to improve the efficacy of both exogenous and endogenous DC-based vaccines by transducing DC either ex vivo or in vivo with viral vectors that encode immunostimulatory molecules. A number of studies have reported improved anti-tumor immunity when this approach was used to drive expression of CD80/CD86 costimulatory molecules (165, 166) or IL-12 (167) by DC. Collectively, these strategies highlight the advances made in tumor immunotherapy as our understanding of tumor immune suppression and evasion has evolved over the last several years. As additional insights into tumor-altered DC function are gained, optimization of these current immunotherapies and development of novel strategies for enhancing anti-tumor immune responses will further improve the efficacy of DC-based cancer vaccines.

CONCLUSION

Tumor immunosurveillance is now a well-documented phenomenon whereby host immune cells and effector molecules function to recognize and eradicate developing tumors in the body. At the heart of this process are DC, innate immune cells that function to acquire tumor Ag through phagocytosis, activate adaptive immunity against these specific tumor Ag, and recruit immune effectors to the site of the tumor for immunologic destruction of these transformed cells. However, one of the hallmarks of cancer growth is immune evasion, and tumor cells may evolve a number of escape mechanisms during their progression that subvert immunosurveillance. A significant contributor to tumor immune evasion is the alteration of DC function by tumors and associated factors present in the tumor microenvironment. As discussed, such alteration of DC function may include effects on the differentiation of DC from bone marrow-derived precursors, suppression of the maturation and activation of already differentiated DC, and the induction of immunosuppressive regulatory DC that inhibit antitumor immune responses. Over the last several years, significant efforts have been made to gain mechanistic insights into these processes of tumor-altered DC function. These findings have in turn led to the development of several immunotherapeutic strategies for improving the function of tumor-associated DC. Still, much remains to be learned about the processes by which tumors impact the function of DC and how such altered DC influence the quality of other immune effectors. As this field moves forward, it will be important to increase our understanding of factors that contribute to tumor recognition by DC and to identify additional tumor-associated DAMPs and inflammatory stimuli that promote optimal maturation and activation of immunostimulatory DC. Additionally, a better understanding of how tumor microenvironmental factors impact the quality of DC differentiation, maturation, and activation will suggest new possibilities for interfering with the suppression of these processes by tumors. Such knowledge will enable the optimization of current, and the development of novel, DC-based immunotherapies that aim to improve the quality and outcome of host anti-tumor immune responses.

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REFERENCES

1. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature (1998) 392:245–52. doi:10.1038/32588
2. Albert ML, Jegathesan M, Darnell RB. Dendritic cell maturation is required for the cross-presentation of CD8+ T cells. Nat Immunol (2001) 2:1010–7. doi:10.1038/nij722
3. Hawiger D, Inaba K, Dorsett Y, Guo M, Mahnke K, Rivera M, et al. Dendritic cells induce peripheral T cell unresponsiveness under steady state conditions in vivo. J Exp Med (2001) 194:769–80. doi:10.1084/jem.194.6.769
4. Dhodapkar MV, Steinman RM, Krasovsky I, Munz C, Bhardwaj N. Antigen-specific inhibition of effector T cell function in humans after injection of immature dendritic cells. J Exp Med (2001) 193:233–8. doi:10.1084/jem.193.2.233
5. Probst HC, Lagnel J, Kollias G, Van den Broek M. Inducible transgenic mice reveal resting dendritic cells as potent inducers of CD8+ T cell tolerance. Immunity (2003) 18:713–20. doi:10.1016/S1074-1045(03)00120-1
6. Probst HC, McCoy K, Okazaki T, Ionjo T, Van den Broek M. Maturing dendritic cells induce peripheral CD8+ T cell tolerance through PD-1 and CTLA-4. Nat Immunol (2005) 6:280–6. doi:10.1038/ni1165
7. Matzinger P. Tolerance, danger, and the extended family. Annu Rev Immunol (1994) 12:991–1045. doi:10.1146/annurev.immunol.12.1.991
8. Janeway CA, Medzhitov R. Innate immune recognition. Annu Rev Immunol (2002) 20:197–216. doi:10.1146/annurev.immunol.20.083001.084359
9. Joffre O, Noel MA, Sporri R, Reis e Sousa C. Inflammatory signals in dendritic cell activation and the induction of adaptive immunity. Immunity Rev (2009) 227:234–47. doi:10.1111/j.1600-065X.2008.00718.x
10. McDonnell AM, Prosser AC, Van Bruggen L, Robinson BWS, Currie AJ. CD8αβ γδ + DC are not the sole subset cross-presenting cell-associated tumor antigens from a solid tumor. Eur J Immunol (2010) 40:1617–27. doi:10.1002/eji.200940153
11. Lorenzi S, Mattei F, Sistig A, Bracci L, Spadaro F, Sanchez M, et al. Type I IFNs control antigen retention and survival of CD8αβ(+) dendritic cells after uptake of tumor apoptotic cells leading to cross-priming. J Immunol (2011) 186:5142–50. doi:10.4049/jimmunol.1004163
12. Tel J, Schreibelt G, Sittig SP, Mathian TSM, Buschow SI, Cruz LI, et al. Human plasmacytoid dendritic cells efficiently cross-present exogenous Ags to CD8+ T cells despite lower Ag uptake than myeloid dendritic cell subsets. Blood (2013) 121:1459–67. doi:10.1182/blood-2012-06-435644
13. Speiser DE, Miranda R, Zakarian A, Bachmann MF, McCaffery K, Odermatt B, et al. Self-antigens expressed by solid tumors do not efficiently stimulate naive or activated T cells: implications for immunotherapy. J Exp Med (1997) 186:645–53. doi:10.1084/jem.186.5.645

14. Hermans IF, Daish A, Yang J, Ritchie DS, Ronesch F. Antigen expressed on tumor cells fails to elicit an immune response, even in the presence of increased numbers of tumor-specific cytotoxic T lymphocyte precursors. Cancer Res (1998) 58:3909–17.

15. Ochsnein BF, Kloener P, Karrer U, Ludewig B, Pericin M, Hengartner H, et al. Immune surveillance against a solid tumor fails because of immunologic ignorance. Proc Natl Acad Sci U S A (1999) 96:2233–8. doi:10.1073/pnas.96.5.2233

16. Ochsnein BF, Sterro S, Odermatt B, Pericin M, Karrer U, Hermans I, et al. Roles of tumor localization, second signals and cross priming in cytotoxic T-cell induction. Nature (2001) 411:1058–64. doi:10.1038/35082583

17. Spiotto MT, Yu P, Rowley DA, Nishimura MI, Meredith SC, Gajewski TF, et al. Increasing tumor antigen expression overcomes “ignorance” to solid tumors via crosspresentation by bone marrow-derived stromal cells. Immunity (2002) 17:757–47. doi:10.1016/S1074-7613(02)00404-0

18. Radajo S, Saito M, Scheidt F, Koneru M, Vukmanovic S, Frey AB. CD8+ tumor-infiltrating T cells are deficient in perforin-mediated cytolytic activity due to defective microtubule-organizing center mobilization and lytic granule exocytosis. J Immunol (2001) 167:5042–51.

19. Blohen U, Roth E, Brommer K, Dumreise T, Rosenthal FM, Pircher H. Lack of effector cell function and altered tetramer binding of tumor-infiltrating lymphocytes. J Immunol (2002) 169:5522–30.

20. Anichini A, Scarito A, Molla A, Parmiani G, Mortarini D. Differentiation of CD8+ T cells from tumor-invaded and tumor-free lymph nodes of melanoma patients: role of common gamma-chain cytokines. J Immunol (2003) 171:2134–41.

21. Mortarini R, Piris A, Maurisch A, Molla A, Bersani I, Bono A, et al. Lack of terminally differentiated tumor-specific CD8+ T cells at tumor site in spite of anti-tumor immunity to self-antigens in human metastatic melanoma. Cancer Res (2003) 63:2335–45.

22. Zappelius A, Batard P, Rabogodoy V, Boley G, Lie D, Lejeune F, et al. Effector function of human tumor-specific CD8 T cells in melanoma lesions: a state of local functional tolerance. Cancer Res (2004) 64:2865–73. doi:10.1158/0008-5472.CAN-03-3066

23. Whiteside TL. Down-regulation of zeta-chain expression in T cells: a biomarker of prognosis in cancer? Cancer Immunol Immunother (2004) 53:865–78. doi:10.1007/s00262-004-0521-0

24. Hargadon KM, Brinkman CC, Faienza K, Odermatt B, et al. Tumor-induced CD11b+Gr-1+ myeloid cells suppress T cell function and altered tetramer binding of tumor-infiltrating Langerhans cells in tumor-bearing animals is the result of defective maturation from hemopoietic progenitors. J Immunol (1998) 161:1842–51.

25. Shirun GV, Shirun MR, Bykovskia S, Shogan J, Lotze MT, Barksdale EM. Neuroblastoma-derived gangliosides inhibit dendritic cell generation and function. Cancer Res (2001) 61:363–9.

26. Péguet-Navarro J, Sportouch M, Popa I, Berthier O, Schmitt D, Portoulakian J. Gangliosides from human melanoma tumors impair dendritic cell differentiation from monocytes and induce their apoptosis. J Immunol (2000) 165:3488–94.

27. Bennaceur K, Popa I, Chapman JA, Migdal G, Péguet-Navarro J, Touraine J-L, et al. Different mechanisms are involved in apoptosis induced by melanoma gangliosides on human monocyte-derived dendritic cells. Glycobiology (2009) 19:576–82. doi:10.1093/glycob/cwp105

28. Sombroek CC, Stam AGM, Massey AJ, Loughheed SM, Schakel MDG, Meijer CJLM, et al. Prostanoids play a major role in the primary tumor-induced inhibition of dendritic cell differentiation. J Immunol (2002) 168:4335–43.

29. Stock A, Booth S, Cerrudolo V, Prosperatigranin E2 suppresses the differentiation of retinoic acid-producing dendritic cells in mice and humans. J Exp Med (2011) 208:761–73. doi:10.1084/jem.20101967

30. Almand B, Clark II, Nikitina E, Van Beynen J, English NR, Knight SC, et al. Increased production of immature myeloid cells in cancer patients: a mechanism of immunosuppression in cancer. J Immunol (2001) 166:678–89.

31. Watanabe S, Deguchi K, Zheng R, Tamai H, Wang L-X, Cohen PA, et al. Tumor-induced CD11b+Gr-1+ myeloid cells suppress T cell sensitization in tumor-draining lymph nodes. J Immunol (2008) 181:3291–300.

32. Ostrand-Rosenberg S, Sinha P. Myeloid-derived suppressor cells: linking inflammation and cancer. J Immunol (2009) 182:4499–506. doi:10.4049/jimmunol.0802740

33. Gabrilovich DI, Ostrand-Rosenberg S, Sinha P, Muller SE, Bhowmick NA, Bronte V, et al. Tumor expressed indoleamine 2,3 dioxygenase 1 regulate macrophages: effects on tumor immunity and regulatory T cell function. J Immunol (2010) 184:6253–68. doi:10.4049/jimmunol.2010007

34. Fox PD, Cabrello C, Bronte V, Chappell DB, Apolloni E, Cabrelle A, Wang M, Hwu P, et al. Unopposed production of granulocyte-macrophage colony-stimulating factor by tumors inhibits CD8+ T cell responses by dysregulating antigen-presenting cell maturation. J Immunol (2004) 172:654–64.

35. Obertinger S, Sengutav M, Edwards RP, Odunsi K, Myoshiy K, Kalininski P, PEGE(2)-driven induction and maintenance of cancer-associated myeloid-suppressed dendritic cell populations. Immunol Invest (2012) 41:635–7. doi:10.3109/00916724.2011.695417

36. Jayaraman P, Parikh F, Lopez-Rivera E, Haislemichael Y, Clark A, Ma G, et al. Tumor-expressed indolamine 2,3 dioxygenase 1 regulates tumor-induced myeloid-derived suppressor cells through modulation of vascular endothelial growth factor release. J Immunol (2013) 182:3656–65. doi:10.4049/jimmunol.1300053

37. Hsieh C-C, Chou H-S, Yang H-R, Lin F, Bhatt S, Qin J, et al. The role of complement component C3 in differentiation of...
myeloid-derived suppressor cells. Blood (2013) 121:1760–8. doi:10.1182/blood-2012-06-440214

47. Cheng P, Corzo CA, Lutete N, Yu B, Nagaraj S, Bui MM, et al. Inhibition of dendritic cell differentiation and accumulation of myeloid-derived suppressor cells in cancer is regulated by S100A9 protein. J Exp Med (2008) 205:2235–49. doi:10.1084/jem.20080132

48. Mace TA, Ameen Z, Collins A, Spitzer JH, Apolloni E, Serizawa SE, Mair M, Young GS, et al. Mechanism regulating myeloid-derived suppressor cells promote cross-tolerance in B-cell lymphoma by expanding regulatory T cells. Cancer (2008) 68:5439–49. doi:10.1158/0008-5472.CAN-07-6621

49. Gabrilovich DI, Mace TA, Ameen Z, Collins A, Spitzer JH, Apolloni E, Serizawa SE, Mair M, Young GS, et al. Mechanism regulating myeloid-derived suppressor cells promote cross-tolerance in B-cell lymphoma by expanding regulatory T cells. Cancer (2008) 68:5439–49. doi:10.1158/0008-5472.CAN-07-6621

50. Kusmartsev S, Sotomayor E, Gabrilovich DI, Velders MP, et al. Myeloid suppressed suppressor cells in a STAT3-dependent manner. Cancer Res (2013) 73:3007–18. doi:10.1158/0008-5472.CAN-12-4601

51. Kusmartsev S, Nagaraj S, Gabrilovich DI, Tumor-associated CD8+ T cell response is mediated by immature myeloid cells. J Immunol (2001) 166:5398–406.

52. Apolloni E, Bronte V, Mazzoni A, Serafini F, Cabrello A, Segal DM, et al. Immortalized myeloid suppressor cells trigger apoptosis in antigen-activated T lymphocytes. J Immunol (2000) 165:6725–30.

53. Mazzoni A, Bronte V, Visintin A, Spitzer JH, Apolloni E, Serafini P, et al. Myeloid suppressor lines inhibit T cell responses by an NO-dependent mechanism. J Immunol (2002) 168:689–95.

54. Corzo CA, Cotter MJ, Cheng P, Cheng F, Kusmartsev S, Sotomayor E, et al. Mechanism regulating reactive oxygen species in tumor-induced myeloid-derived suppressor cells. J Immunol (2009) 182:5693–701. doi:10.4049/jimmunol.0900092

55. Rodriguez PC, Ochoa AC. Arginine regulation by myeloid derived suppressor cells and tolerance in cancer: mechanisms and therapeutic perspectives. Immunol Rev (2008) 222:180–91. doi:10.1111/j.1600-065x.2008.0068.x

56. Diaz-Montero CM, Salem ML, Nishimura MI, Garrett-Mayer E, Cole DJ, Montero AI. Increased circulating myeloid-derived suppressor cells correlate with clinical cancer stage, metastatic tumor burden, and doxorubicin-cyclophosphamide chemotherapy. Cancer Immunol Immunother (2009) 58:49–59. doi:10.1007/s00262-008-0525-4

57. Vasey-Dundjel D, Pan D, Zeng Q, Gorboumov M, Albesiano E, Fu J, et al. STAT3 regulates arginase-1 in myeloid-derived suppressor cells from cancer patients. J Clin Invest (2013) 123:1580–9. doi:10.1172/JCI60803

58. Ghiringhelli F, Puig PE, Reux S, Parcellier A, Schmitt E, Solary E, et al. Tumor cells convert immature myeloid dendritic cells into TGF-beta-secreting cells inducing CD4+CD25+ regulatory T cell proliferation. J Exp Med (2005) 202:919–29. doi:10.1084/jem.20050463

59. Kumatle C, Cotter MJ, Cheng F, Kusmartsev S, Sotomayor E, et al. Inhibition of dendritic myeloid-derived suppressor cells mediate CRC5-dependent recruitment of regulatory T cells favoring tumor growth. J Immunol (2012) 189:5602–11. doi:10.4049/jimmunol.1201018

60. Serafini P, Mgebbof N, Neoh K, Borrello I. Myeloid-derived suppressor cells promote cross-tolerance in B-cell lymphoma by expanding regulatory T cells. Cancer Res (2008) 68:5439–49. doi:10.1158/0008-5472.CAN-07-6621

61. Mauti LA, Le Bitoux M-A, Baumer K, Stehle J-C, Golshayan D, Provero P, et al. Myeloid-derived suppressor cells are implicated in regulating permissiveness for tumor metastasis during mouse gestation. J Clin Invest (2011) 121:2794–807. doi:10.1172/JCI41936

62. Li H, Han Y, Guo Q, Zhang M, Cao X. Cancer-expanded myeloid-derived suppressor cells induce anergy of NK cells through membrane-bound TGF-beta 1. J Immunol (2009) 182:240–9.

63. Schweder J, Ribeiro VSG, Dinarello CA, Osttrand-Rosenberg S, Di Santo JP, Apte CP, Skov RL, et al. Inhibition of dendritic myeloid-derived suppressor cells increases CD8+ T cell responses to cancer antigens. Cancer Immunol Immunother (2011) 60:2491–6. doi:10.1007/s00262-011-0949-0

64. Hoechst B, Voigtlander T, Ormandy L, Gamrekelsashvili L, Zhao F, Wedemeyer H, et al. Myeloid-derived suppressor cells inhibit natural killer cells in patients with hematocellular carcinoma via the NKG2D receptor. Hepatology (2009) 50:799–807. doi:10.1002/hep.23054

65. Poschke I, Mao Y, Adamson L, Salazar-Onfray F, Masucci G, Kiessling R. Myeloid-derived suppressor cells impair the quality of dendritic cell vaccines. Cancer Immunol Immunother (2012) 61:827–38. doi:10.1007/s00262-011-1143-y

66. Simha P, Clements VK, Bensett SK, Albeda SM, Osttrand-Rosenberg S. Cross-talk between myeloid-derived suppressor cells and macrophages subverts tumor immunity toward a type 2 response. J Immunol (2007) 179:977–83.

67. Cortes-Retamozo V, Etzrodt M, Newton A, Rauch PJ, Chudnovsky A, Berger C, et al. Origins of tumor-associated macrophages and neutrophils. Proc Natl Acad Sci U S A (2012) 109:2491–6. doi:10.1073/pnas.1113744109

68. Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mielecik B, Lagorce-Pages C, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science (2006) 313:1960–4. doi:10.1126/science.1129139

69. Steidl C, Lee T, Shah SP, Farinha P, Han G, Nayar T, et al. Tumor-associated macrophages and survival in classic Hodgkin’s lymphoma. N Engl J Med (2010) 362:875–85. doi:10.1056/NEJMoa0905680

70. DeNardo DG, Brennan DJ, Rex-hopaj E, Ruffel B, Shiao SL, Maden DF, et al. Leukocyte complexity predicts breast cancer survival and functionally regulates response to chemotherapy. Cancer Discov (2011) 1:54–67. doi:10.1158/2159-8274.CD-10-0028

71. Kurahara H, Shinch H, Matakai Y, Maemura K, Noma H, Kubo F, et al. Significance of M2-polarized tumor-associated macrophages in pancreatic cancer. J Surg Res (2011) 167:e211–9. doi:10.1016/j.jss.2009.05.026

72. Zhang BC, Gao J, Wang J, Rao ZG, Wang BC, Gao JJ. Tumor-associated macrophages infiltration is associated with peritumoral lymphangiogenesis and poor prognosis in lung adenocarcinoma. Med Oncol (2011) 28:1447–52. doi:10.1007/s12032-010-9638-5

73. Schoupe E, De Baetselier P, Van Ginderachter JA, Sarukhan A. Instruction of myeloid cells by the tumor microenvironment: open questions on the dynamics and plasticity of different tumor-associated myeloid cell populations. Oncocimunol (2012) 1:1135–45. doi:10.4161/onci.21566

74. Allavena P, Pimonti L, Longoni D, Bernascioni S, Stoppacciaro A, Ramaciotti L, et al. IL-10 prevents the differentiation of monocytes to dendritic cells but promotes their maturation to macrophages. Eur J Immunol (1998) 28:659–69.
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II molecules. *J Immunol* (1997) 158:2723–30.

103. Winzler C, Rovere P, Rescigno M, Granucci F, Penna G, Adorini L, et al. Maturation stages of mouse dendritic cells in growth factor-dependent long-term cultures. *J Exp Med* (1997) 185:317–28. doi:10.1084/jem.185.2.317

104. van Helden SFG, Van Leeuwen FN, Fidgell CG. Human and murine model cells for dendritic cell biology evaluated. *Immunol Lett* (2008) 117:191–7. doi:10.1016/j.imlet.2008.02.003

105. Fuertes Marraco SA, Grosjean F, Duval A, Rosa M, Lavanchy C, Ashok D, et al. Novel murine dendritic cell lines: a powerful auxiliary tool for dendritic cell research. *Front Immunol* (2012) 3:331. doi:10.3389/fimmu.2012.00331

106. Hargadon KM, Arzato YT, Forrest OA, Harte CM. Melanoma-associated suppression of the dendritic cell line DC2.4 and JAWSII. *Am J Immunol* (2012) 8:179–90.

107. Hargadon KM, Forrest OA, Reddy PR. Suppression of the maturation and activation of the dendritic cell line DC2.4 by melanoma-derived factors. *Cell Immunol* (2012) 272:275–82. doi:10.1016/j.cellimm.2011.10.003

108. He T, Tang C, Xu S, Moyana T, Xiang J. Interferon gamma stimulates cellular maturation of dendritic cell line DC2.4 leading to induction of efficient cytotoxic T cell responses and antimicrobial immunity. *Cell Immunol* (2007) 4:105–11.

109. Rhule A, Rase B, Smith JR, Shepherd DM. Toll-like receptor ligand-induced activation of murine DC2.4 cells is attenuated by Panax notoginseng. *J Ethnopharmacol* (2008) 116:179–86. doi:10.1016/j.eur.2007.11.019

110. Jiang X, Shen C, Rey-Ladino J, Yu H, Brunham RC. Characterization of murine dendritic cell line JAWS II and primary bone marrow-derived dendritic cells in Chlamydia muridarum antigen presentation and induction of protective immunity. * Infect Immun* (2008) 76:2392–401. doi:10.1128/IAI.01584-07

111. Krempski J, Karyampudi L, Behrens MD, Erskine CL, Hartmann L, Dong H, et al. Tumor-infiltrating programmed death receptor-1+ dendritic...
with advanced melanoma. PloS ONE (2011) 7:e48424. doi:10.1371/journal.pone.0048424

Menetrier-Caux C, Thomachot MC, Alberti L, Montmain G, Blay JY. IL-4 prevents the blockade of dendritic cell differentiation induced by tumor cells. Cancer Res (2003) 63:3096–104.

Osada T, Chong G, Tanisk R, Hong T, Spector N, Kumar R, et al. The effect of anti-VEGF therapy on immature myeloid cell and dendritic cells in cancer patients. Cancer Immunol Immunother (2008) 57:1115–24. doi:10.1007/s00262-007-0441-x

Kusmartsev S, Cheng F, Yu B, Nefedova Y, Sotomayor E, Lux R, et al. All-trans-retinoic acid eliminates immature myeloid cells from tumor-bearing mice and improves the effect of vaccination. Cancer Res (2003) 63:4441–9.

Kusmartsev S, Su Z, Heiser A, Dannull J, Eruslanov E, Kübler R, et al. All-trans-retinoic acid exhibits anti-tumor activity and resistance to cancer with high Th1-inducing ability. Eur J Immunol (2009) 39:299–407. doi:10.1007/ CJII.0b013e3181e1773

Aguilera R, Safdie C, Tittarelli A, González FE, Ramírez M, Reyes D, et al. Heat-shock induction of tumor-derived danger signals mediates rapid monocyte differentiation into clinically effective dendritic cells. Clin Cancer Res (2011) 17:2474–83. doi:10.1158/1078-0432.CCR-10-2384

Iwata-Kajihara T, Sumimoto H, Kawamura N, Ueda R, Takahashi T, Mizuguchi H, et al. Enhanced cancer immunotherapy using STAT3-depleted dendritic cells with high Th1-inducing ability and resistance to cancer cell-derived inhibitory factors. J Immunol (2011) 187:27–36. doi:10.4049/jimmunol.1002067

Cubillos-Ruz JR, Baird JR, Tesone AI, Rutkowski MR, Scarlett UK, Camposeo-Iacobz AI, et al. Reprogramming tumor-associated dendritic cells in vivo using miRNA mimics triggers protective immunity against ovarian cancer. Cancer Res (2012) 72:1683–93. doi:10.1158/0008-5472.CAN-11-3160

Ferguson AR, Nichols LA, Zarling AL, Thompson ED, Brinkman CC, Hargadon KM, et al. Strategies and challenges in eliciting immunity to melanoma. Immunol Rev (2008) 222:28–42. doi:10.1111/j.1600-065X.2008.00620.x

Hong S, Qian J, Li H, Yang J, Lu Y, Zheng Y, et al. CpG or IFN-α are more potent adjuvants than GM-CSF to promote anti-tumor immunity following idiosyncratic vaccine in multiple myeloma. Cancer Immunol Immunother (2012) 61:561–71. doi:10.1007/s00262-011-1123-2

Han TH, Jin P, Ren J, Sleazak S, Marincola FM, Stonecek DF. Evaluation of 3 clinical dendritic cell maturation protocols containing lipopolysaccharide and interferon-gamma. J Immunother (1999) (2009) 32:399–407. doi:10.1007/CJII.0b013e3181e1773

Aguijera R, Safdie C, Tittarelli A, González FE, Ramírez M, Reyes D, et al. Heat-shock induction of tumor-derived danger signals mediates rapid monocyte differentiation into clinically effective dendritic cells. Clin Cancer Res (2011) 17:2474–83. doi:10.1158/1078-0432.CCR-10-2384

Mivea S, Nishida H, Tanazawa Y, Takata M, Takeuchi A, Yamamoto N, et al. TNF-α and tumor lysis promote the maturation of dendritic cells for immunotherapy for advanced malignant bone and soft tissue tumors. PLoS ONE (2012) 7:e52926. doi:10.1371/journal.pone.0052926

Massa C, Seliger B. Fast dendritic cells stimulated with alternative maturation mixtures induce polyfunctional and long-lasting activation of innate and adaptive effector cells with tumor-killing capabilities. J Immunol (2013) 190:3328–37. doi:10.4049/jimmunol.1200204

Sugiyama M, Kakeji Y, Tsujitani S, Harada Y, Onimaru M, Yoshida K, et al. Antagonism of VECF by genetically engineered dendritic cells is essential to induce antitumor immunity against malignant ascites. Mol Cancer Ther (2011) 10:540–9. doi:10.1158/1535-7163.MCT-10-0479

Zhang M, Berndt BE, Chen J-J, Kao JY. Expression of a soluble TGF-beta receptor by tumor cells enhances dendritic cell/tumor fusion vaccine efficacy. J Immunol (2008) 181:3690–7.

Zheng X, Koropatkine J, Chen D, Velenosi T, Ling H, Zhang X, et al. Silencing IDO in dendritic cells: a novel approach to enhance cancer immunotherapy in a murine breast cancer model. Int J Cancer (2013) 132:967–77. doi:10.1002/ijc.27710

Chen Y-X, Man K, Ling GS, Chen Y, Sun B-S, Cheng Q, et al. A crucial role for dendritic cell (DC) IL-10 in inhibiting successful DC-based immunotherapy: superior antitumor immunity against hepatocellular carcinoma evoked by DC devoid of IL-10. J Immunol (2007) 179:609–15.

Boczkowski D, Lee J, Pruitt S, Nair S. Dendritic cells engineered to secrete anti-GITR antibodies are effective adjuvants to dendritic cell-based immunotherapy. Cancer Gene Ther (2009) 16:900–11. doi:10.1038/sj.gene.3602941

Pruitt SK, Boczkowski D, De Rosa N, Haley NR, Morris MA, Tyler DS, et al. Enhancement of anti-tumor immunity through local modulation of CTLA-4 and GITR by dendritic cells. Eur J Immunol (2011) 41:3553–63. doi:10.1002/eji.201141383

Saha A, Chatterjee SK. Combination of CTL-associated antigen-4 blockade and depletion of CD25 regulatory T cells enhance tumour immunity of dendritic cell-based vaccine in a mouse model of colon cancer. Scand J Immunol (2010) 71:70–82. doi:10.1111/j.1365-3083.2009.0235x.x

Rosenblatt J, Glotzbecker B, Mills H, Vaisir T, Tzachanis D, Levine JD, et al. PD-1 blockade by CT-011, anti-PD-1 antibody, enhances ex vivo T-cell responses to autologous dendritic cell/myeloma fusion vaccine. J Immunother (1991) (2011) 34:409–18. doi:10.1097/01.IIM.0b013e31821ca6ce

Oertli D, Marti WR, Zachaj P, Noppen C, Kocher T, Padovan E, et al. Rapid induction of specific cytotoxic T lymphocytes against melanoma-associated antigens by a recombinant vaccinia virus vector expressing multiple immunodominant epitopes and costimulatory molecules in vivo. Hum Gene Ther (2002) 13:569–75. doi:10.1089/1043034252808956

Adaminat A, Rosenthal R, Weber WP, Frey DM, Vielh CT, Bolli M, et al. Intramural immunization with a vaccinia virus encoding multiple antigenic epitopes and costimulatory molecules in metastatic melanoma. Mol Ther (2010) 18:651–9. doi:10.1038/mct.2009.275

Tatsuki T, Takehara T, Yamaguchi S, Sasakawa A, Miyagi T, Jinushi M, et al. Injection of IL-12 gene-transduced dendritic cells into mouse liver tumor lesions activates both innate and acquired immunity. Gene Ther (2007) 14:863–71. doi:10.1038/sj.gt.3302941

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