Molecular Pathways: Myeloid Complicity in Cancer

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Abstract

Cancer-induced inflammation results in accumulation of myeloid cells. It has become increasingly evident that tumor-dependent factors condition myeloid cells toward an immunosuppressive and pro-tumorigenic phenotype. These myeloid cells include progenitors and progeny of monocytes, granulocytes, macrophages, and dendritic cells. Myeloid cells are not simply bystanders in malignancy or barometers of disease burden. Reflecting their dynamic and plastic nature, myeloid cells manifest a continuum of cellular differentiation and are intimately involved at all stages of neoplastic progression. They can promote tumorigenesis through both immune-dependent and independent mechanisms and can dictate response to therapies. A greater understanding of the inherent plasticity and relationships among myeloid subsets is needed to inform therapeutic targeting. New clinical trials are being designed to modulate the activities of myeloid cells in cancer, which may be essential to maximize the efficacy of both conventional cytotoxic and immune-based therapies for solid tumors.

BACKGROUND

Cancer vaccines are designed to induce tumor-antigen-specific cytolytic T cells, but are rarely effective at eliminating established tumors. This inefficiency potentially reflects a tolerized response and/or a limited endogenous T cell repertoire specific for the non-mutated, self-proteins that represent the majority of targetable tumor antigens. The adoptive transfer of T cells engineered to express high affinity receptors against tumor/self-antigens may, in principle, overcome some of the obstacles faced in engendering an endogenous T cell response (1, 2). However, even when transferred in high numbers, these tailored T cells will

likely encounter multiple mechanisms of cancer-associated immunosuppression that interfere with tumor eradication.

The accumulation of hematopoietic-derived, immunosuppressive cells is now recognized as a primary mechanism employed by tumors to evade elimination by cytotoxic T lymphocytes (3). Cell subsets from both the lymphoid (e.g. regulatory T cells) and myeloid lineages can regulate T lymphocytes; this review focuses on pathways co-opted by tumors that instruct myeloid complicity in cancer progression. In this review, we discuss: 1) the ontogeny of myeloid cells involved in cancer; 2) the pathways initiated by tumors that instruct myeloid accumulation and trafficking; 3) the fate of myeloid cells in malignancy; and 4) the obstacles that must be overcome to successfully translate the targeting of myeloid cells to enhance cancer therapy. We also discuss specific aspects of pancreatic ductal adenocarcinoma (PDA) as a noteworthy example of the challenges presented by this class of cells to effective immune strategies.

The extraordinary plasticity, rapid turnover, and capacity to present antigen to T cells position the myeloid compartment as an attractive focal point for potentiating targeted therapies. However, the heterogeneity and dynamic nature of the myeloid lineage also render its rational targeting a daunting task. A better understanding of the relationships among myeloid progenitors and progeny should help elucidate treatment strategies for solid tumors.

Disrupted myeloid homeostasis: a continuum of cellular differentiation and plasticity

Hematopoiesis represents a dynamic and hierarchical process of cell-fate decisions governed by both intrinsic (e.g., transcription factors) and extrinsic (e.g. cytokines) mechanisms (4). Hematopoietic stem cells in the bone marrow generate phenotypically distinct progenitors that are impaired in the ability to self-renew. In non-pathological settings, immature myeloid cells are largely confined to the bone marrow, have a relatively short half-life and circulate at low frequencies yetretain the capacity torapidly respond to environmental insults. Tumors hijack this homeostatic process by secreting inflammatory mediators that create a state of "emergency hematopoiesis" with preferential expansion of the myeloid, rather than the lymphoid, lineage. Such cancer-conditioned myeloid cells aid and abet chronic inflammation and exacerbate cancer progression.

The cytokine GM-CSF has long been recognized to induce the expansion of immature myeloid cells that promote allograft or transplantable tumor growth by inhibiting T lymphocytes (5–7). These cells have subsequently been termedmyeloid-derived suppressor cells (MDSC), a loosely defined and heterogeneous population of immature myeloid cells with suppressive activity. MDSC are now recognized as a paramount disease-specific tolerance mechanism during both acute and chronic inflammatory conditions. MDSC contribute to immune evasion via suppression of T cell responses (8–12) and also influence tumor remodeling, invasion, metastasis and cancer stemness independent of T cell inhibition (13–15). Thus, MDSC represent a common axis with broad therapeutic application.

MDSC subsets and immunosuppression

There are two subsets of MDSC:monocytic MDSC (Mo-MDSC) and granulocytic MDSC (Gr-MDSC) (16). These subsets can be readily discriminated by distinct phenotypic profiles and morphologies (Table 1). Reagents used in mice to identify the collective population of MDSC (e.g. αGr-1 and αCD11b) do not clearly distinguish between these two subsets so antibodies against additional markers are necessary to study these distinct lineages separately (Table 1). In humans, these subsets can also be distinguished by CD14 and CD15 expression (Tables 1 and 2). It is now clear that both Gr-MDSC and Mo-MDSC are independently suppressive (12, 16, 17). Gr-MDSC have been reported to mediate suppression largely via production of reactive oxygen species (ROS) and Arginase (16). Mo-MDSC have a more expansive suppressive arsenal that could reflect a greater cellular plasticity. This armamentarium includes expression of inducible nitric oxide synthase (iNOS), Arginase (Arg) (16, 18), TGFβ (19), indoleamine-pyrrole 2,3-dioxygenase (IDO) (20), reactive oxygen species (ROS) (21) and factors that induce STAT3 signaling (22). Mo-MDSC may also indirectly promote immunosuppression via the induction of $CD4^+FoxP3^+$ regulatory T cells (Treg) (23). The production of peroxynitrate ($ONOO^-$), a product of NO and superoxide anion (O_2^-), represents another suppressive mechanism associated with MDSC and cancer (24), and may be a result of synergy between the two myeloid subsets *in vivo*. The suppressive activities depend in part on IFNγ signaling (16), but the relative contribution of each subset to immune suppression is context dependent and is heightened within the tumor environment. The finding that IFNγ, a cytokine expressed by effector T cells after antigen encounter, can also induce MDSC-mediated suppression underscores the need to target this population in therapies that depend on T lymphocytes for activity.

The relative contribution of each MDSC subset in mediating immunosuppression depends on the disease state and inflammatory milieu, but these capabilities are frequently assessed only *in vitro*. While both subsets have immunosuppressive activity in malignancy, suppressive monocytes (which have an overlapping phenotype with Mo-MDSC, as discussed below) mediate transplantation tolerance (25) and, in some models, have superior suppressive capacity (16). In contrast, some studies in humans indicate Gr-MDSC may be slightly more suppressive (26). Differences in the composition of factors may dictate the specific subset that mediates suppression. Cell number and frequency *in vivo* also bear consideration, as suppression is dependent upon cell ratios. Thus, if Mo-MDSC are superior suppressors *in vitro*, but are less frequent than Gr-MDSC *in vivo*, then targeting the more prevalent population may have greater therapeutic value. Indeed, studies using a genetically engineered mouse model of PDA (27) showed that both Mo-MDSC and Gr-MDSC accumulate during disease progression (12). Specific depletion of Gr-MDSC induced endogenous CD8 T cell activation and infiltration into established PDA resulting in tumor epithelial cell death and stromal remodeling (12). Thus, simply abrogating MDSC can awaken an endogenous cytotoxic T cell response indicating that immune surveillance to cancer may fail, in part, because of a defined subset of cancer-conditioned myeloid cells.

Gr-MDSC depletion in PDA was unexpectedly accompanied by a concomitant rise in Mo-MDSC suggesting homeostatic regulation between these two subsets (12). Gr-MDSC may directly regulate the monocytic population, which could indirectly inhibit the progeny of

Mo-MDSC, including dendritic cells (DC) and macrophages (Figure 1). Alternatively, since both subsets respond to GM-CSF (our unpublished observations) and GM-CSF secretion by PDA is necessary for the establishment of implanted pancreatic tumor cells(10, 11), Gr-MDSC may act as a sink. Thus, the targeted depletion of Gr-MDSC could indirectly increase systemic levels of GM-CSF available to stimulate Mo-MDSC. The finding that the two MDSC subsets are homeostatically regulated during cancer progression (12) has clinical implications. Therapies designed to target one immature population may induce alternative suppressive populations justifying the simultaneous monitoring of these subsets in individual patients.

MDSC ontogeny

Based on the origins of bona fide monocytes, Mo-MDSC likely descend from the macrophage-dendritic cell progenitor that has lost granulocyte potential and gives rise to monocytes, macrophages, and DC (28) (Figure 1). One feature distinguishing Mo-MDSC from normal monocytes in humans is the lower expression of HLA-DR (14, 17), a critical molecule for antigen presentation to T lymphocytes. Exposure of normal circulating monocytes to tumor-conditioned media causes downregulation of HLA-DR (14, 17) and CD14 (29) supporting the contention that Mo-MDSC can be derived from normal circulating monocytes. This transition may be dependent on the activation of the STAT3 pathway (14). The downregulation of CD14 could also make it more difficult to distinguish this population from Gr-MDSC. Intriguingly, $CD14^+HLA-DR^{-/low}$ cells can be isolated from normal blood. These cells can induce the conversion of $CD4^+FoxP3^-$ T cells to $CD4^+FoxP3^+$ Tregvia expression of cell-bound TGFβ and genes involved in retinoic acid metabolism (23). Thus, Mo-MDSC may simply reflect an altered differentiation state of monocytes.

There are two subsets of monocytes at steady state: inflammatory monocytes (IM) and resident monocytes (RM) (30), which differ in expression of Ly6C and the chemokine receptors CCR2 and CX_3CR1 (Table 1, Figure 1). Mo-MDSC are phenotypically similar to the IM subset (Table 1). At steady state, IM are precursors to RM (31), potentially reflecting a less mature and more plastic phenotype. RM patrol the endothelium and have an intrinsic bias to adopt an immunosuppressive macrophage phenotype (M2) reminiscent of tumor-associated macrophages (TAM) (31). In healthy animals, IM circulate and reside in the subscapular space of the spleen where they are poised to rapidly respond and migrate toward sites of inflammation following infection (32). Following extravasation, IM can differentiate into either M1 (anti-tumorigenic, pro-inflammatory) or M2 (pro-tumorigenic, anti-inflammatory) macrophages depending on the context. IM differentiate into M1 macrophages following migration into infected tissues (33). In contrast, elevated levels of T helper 2 (Th2) cytokinesIL-4 and IL-13 can induce IM differentiation toward an M2 phenotype (34), underscoring IM plasticity (Figure 1). Increased accumulation of IM correlates with advanced disease and poor prognosis in PDA patients (35), consistent with an etiological overlap between Mo-MDSC and IM.

Identification of unique markers to distinguish Gr-MDSC from normal granulocytes has proven more elusive. Gr-MDSC may be less dense than normal granulocytes. However,

normal granulocytes can also change in density and become suppressive upon activation (36). Recently, a screening of differential markers on MDSC showed that the expression of extracellular S100 proteins may specifically identify both MDSC subsets in mice (37). Ongoing efforts to identify distinguishing features of human Gr-MDSC could aid in the development of novel therapies. MDSC may include not only immature monocyte and granulocyte progenitors along a continuum of differentiation, but also altered monocytes and granulocytes that have been conditioned by tumor-dependent factors. As the detailed phenotypes of distinct subsets continue to emerge, reconciling the generic term MDSC with the more standard and accepted cell subset classifications may help to unify divergent fields that study myeloid cells in distinct disease contexts and avoid descriptions and experimental conditions that indiscriminately pool various subsets.

MDSC trafficking and accumulation: roles of chemokines and cytokines

A complex composition of tumor-dependent factors contributes to the phenotypic and functional heterogeneity of MDSC in distinct malignancies, but some common pathways have emerged (Figure 1). GM-CSF is overexpressed in many malignancies (5–7, 10, 11). Signaling via the GM-CSF receptor induces the proliferation and differentiation of progenitors into MDSC (10) and also promotes the survival of differentiated Gr-MDSC (12). GM-CSF in combination with IL-6 or G-CSF induces expression of the C/EBPβ transcription factor and the subsequent expansion of MDSC (9, 38, 39). Some studies suggest that GM-CSF may preferentially induce Mo-MDSC rather than Gr-MDSC (40–42). However, GM-CSF and IL-6 were recently shown to induce the expression of microRNAs (miR-155 and miR-21) in both MDSC subsets. These microRNAs synergistically promoted the expansion of both Gr-MDSC and Mo-MDSC by modulating key phosphatases, SHIP and PTEN (43), revealing potential targets to interfere with both MDSC populations simultaneously. Of note, GM-CSF in combination with IL-4 induces monocyte differentiation into DC with potent immunostimulatory properties, justifying the use of GM-CSF in vaccines (44). Tumor-derived factors are, however, implicated in inducing tolerogenic DC (Figure 1 and reviewed in (45)). The paradoxical roles for GM-CSF can be ascribed to the *in vivo* context. GM-CSF expression by an irradiated tumor cell vaccine may result in the localized and transient presence of this cytokine in the context of dying tumor cells, where as sustained systemic levels of GM-CSF favors MDSC expansion and survival.

A variety of other factors have been implicated in MDSC generation including S100A8/A9, IL-6, IL-1β, G-CSF, VEGF, PGE$_2$ and TGFβ (3). The S100A8/A9 proteins inhibit DC differentiation, enhance MDSC accumulation and promote pancreatic carcinogenesis in susceptible animals via binding to the receptor for advanced glycation end products (RAGE) (46–48). Another common pathway to induce suppressive granulocytes is mediated by G-CSF (49). Breast cancer cells express G-CSF that expand Gr-MDSC that promote tumor growth and angiogenesis (50). Thus, the observed overexpression of G-CSF by PDA epithelial cells may play a similar role in promoting the preferential accumulation of Gr-MDSC (12).

The chemokine receptor profile is distinct between Mo-MDSC and Gr-MDSC and can be employed to further distinguish these subsets. Since CCR2 is expressed on IM, Mo-MDSC

and TAM but not Gr-MDSC, this pathway may be more exclusive for cells of the macrophage dendritic cell lineage. TGFβ signaling on bone marrow cells favors the induction of MDSC that utilize CCR2 for trafficking into skin tumors (51). CCR2-expressing cells are responsive to the chemokine CCL2, promoting the egress of monocytes from the bone marrow (52) and the infiltration of IM into *Kras*-driven lung tumors (53) and implanted PDA cell lines (35). Mo-MDSC and fibrocytes also express CXCR4 (54) that binds CXCL12, a chemokine that is often overexpressed in cancer. Recently, a CXCR4 antagonist (AMD3100) in combination with anti-PDL1, an antibody that blocks inhibitory signaling in T cells, transiently overcame immunosuppression in an autochthonous mouse model of PDA (55). The proposed mechanism of AMD3100 in this setting was inhibition of stromal fibroblasts, but it is likely that interfering with CXCR4 has many effects *in vivo*, including impacting myeloid progenitor egress from the bone marrow and/or recruitment into tumors.

Like granulocytes, Gr-MDSC express the chemokine receptors CXCR1, CXCR2 and CXCR8. These receptors are involved in the recruitment of Gr-MDSC into neoplasms and are essential for colitis-associated tumor formation (56). CXCR2 blockade inhibited Gr-MDSC migration into a murine rhabdomyosarcoma model and enhanced the therapeutic activity of anti-PD1 (57). Patients with metastatic sarcomas also had elevated levels of the CXCR2 ligand, CXCL8, which was associated with decreased survival (57). Not surprisingly, the ligands for these chemokine receptors (CXCL1, CXCL2, CXCL8) are often expressed by tumor epithelial cells, including PDA (10, 12). The pro-inflammatory cytokineIL-17 is commonly overexpressed in cancer and also is implicated in chronic inflammation and tumor development (58). IL-17 overexpression may mediate some of its tumor-promoting effects by recruiting Gr-MDSC (59) and monocytes (60). Thus, tumors express not only growth factors that induce the proliferation, differentiation and survival of myeloid progenitors, but also chemokines that recruit both MDSC subsets into the evolving cancer.

MDSC fate

The *in vivo* fates of distinct myeloid progenitors have primarily been studied at steady state and in models of acute inflammation or in transplantable tumors, leaving critical knowledge gaps of myeloid progenitor fate in autochthonous cancers that arise in the tissue of origin and progress through a preinvasive state. Genetically engineered mouse models of autochthonous cancer that recapitulate the natural history of nascent human cancer are ideally suited for the identification of the fate of complex myeloid populations in preinvasive, invasive and metastatic disease. Such studies may be particularly relevant for solid tumors in which the tissue resident macrophages that arise during embryogenesis may originally recognize the first mutated epithelial cell. IM can migrate into tumors where they subsequently differentiate into TAM (16, 53, 61, 62). TheTh2-responses that are intimately involved in solid tumors (63) can also drive the local *in situ* proliferation of intratumoral macrophages (64).

M-CSF (CSF1) is highly expressed by both normal and mutated epithelial cells. This cytokine promotes macrophage development (65, 66) and myeloid cell recruitment into

tumors. M-CSF correlates with poor prognosis in many cancers (reviewed in (67)) and can induce monocyte differentiation into macrophages that are highly phagocytic and have low MHC class II expression, indicative of minimal antigen presentation (68). M-CSF induces polarization of human CD14^{+} blood monocytes to secrete the immunosuppressive cytokine IL-10 and not IL-12 (69), a secretory phenotype of M2 macrophages that may subvert tumor immunity. However, the complex chronic inflammatory milieu associated with autochthonous tumors *in vivo* generates much more complex populations. Tissue macrophages can also express the dendritic cell marker CD11c, hampering the ability to distinguish these distinct cell lineages in tumors (70). Despite this, M-CSF has been implicated in the differentiation of inflammatory DC from IM (71). This subset of DC, also referred to as Tip-DC (TNFα- and iNOS-producing), accumulates in the spleen during infection (72) and inflammatory diseases and promotes T cell immunity.

Although the evidence is derived from studies across various model systems, Mo-MDSC/IM may also differentiate into mature myeloid cells that promote tumor immunity, indicating that depletion of some myeloid populations during other forms of therapy may not be beneficial. This notion is supported by recent findings that CCR2^{+} IM rapidly migrate into transplantable tumors in response to chemotherapy and serve as functional antigen presenting cells to promote tumor immunity (73). In addition, low-dose irradiation of tumors induced the accumulation of iNOS[+] macrophages that contributed to the effectiveness of adoptive T cell therapy (74). These results reveal a less well-appreciated salutary role for myeloid cells in cancer and indicate that simply removing myeloid populations, particularly if performed in combination with cytotoxic therapies that induce immunogenic cell death, may be self-defeating. Alternatively, in other studies, tumor-infiltrating myeloid cells can impart resistance to chemotherapy (75–77) and support cancer stemness (14, 15). Thus, in some settings, depletion of TAM can also have therapeutic benefit.

IM can also give rise to fibrocytes, an unusual class of hematopoietic-derived fibroinflammatory cells with properties of both myeloid cells and fibroblasts (Table 1, Figure 1 and reviewed in (78)). Fibrocytes express the activated fibroblast marker α-smooth muscle actin, extracellular matrix components including collagen, as well as the pan-hematopoietic marker CD45. In some settings, fibrocytes can serve as potent antigen presenting cells due to the expression of HLA-DR and costimulatory molecules (79, 80). On the other hand, an immunosuppressive population of fibrocytesis increased in the circulation of sarcoma patients (20). In this setting, the fibrocytes mediate T cell suppression by expression of IDO, an immunomodulatory enzyme that degrades tryptophan. The collective properties of fibrocytes raise the question of whether they overlap with at least a fraction of fibroblasts that contribute to immune suppression in solid tumor models (55, 81). CD14^{+} monocytes derived from healthy donors will differentiate into fibrocytes after exposure to IL-4 (20), TGFβ as well as PDGF. Immunosuppressive fibrocytesin humans can co-express the granulocytic markers CD15 and CD66b (20), suggesting a potential lineage relationship with granulocytes. The extraordinary plasticity of the Mo-MDSC makes it enticing to speculate that Mo-MDSC could differentiate into Gr-MDSC in settings of pathology. However, in the accepted steady-state developmental pathways for monocytes and granulocytes each subset arises from distinct progenitors (Figure 1).

CLINICAL-TRANSLATIONAL ADVANCES

Both subsets of MDSC are expanded systemically in a number of human malignancies (Table 2). Targeting myeloid cells in malignancy represents an active and expanding area of investigation that should lay the groundwork for defining the safety and efficacy of such strategies either alone or in combination with immunotherapy and/or chemotherapy (Table 3). Promising strategies to date have been identified almost exclusively in implantable tumor models and it is important to keep in mind that successful approaches in this setting may have limited clinical benefit in patients. This disconnect is highlighted in PDA in which the desmoplastic stroma of the autochthonous disease contributes to inordinately high interstitial fluid pressures that compress blood vessels and create a biophysical barrier to drug penetration – findings that are not recapitulated in implantable tumors (82). In this regard, the observation that targeted depletion of a single MDSC subset in autochthonous PDA activates cytotoxic T cells and tumor cell death is encouraging and warrants further study into how best to translate such myeloid-targeted therapies (12). The concern that targeting one population can have unforeseen consequences on other suppressive subsets may be especially relevant for therapies designed to target the myeloid lineage in which many of the progenitors are in a transitory state and may be susceptible to an overlapping spectrum of homeostatic and/or tumor-derived modulators.

There are presently a number of strategies under investigation to target MDSC in malignancy (Table 3 and reviewed in (3)). Such strategies include interfering with suppressive activity (PDE-5 inhibitors, COX-2 inhibitors, and the synthetic triterpenoid CDDO-Me); inhibiting induction from progenitors (Zoledronic acid, sunitinib); inhibiting egress from the bone marrow and recruitment into tumors (blockade of CCR2 or CSF/ CSF1R); and modulating differentiation (ATRA, anti-CD40). Cytotoxic drugs including gemcitabine or 5-FUhave been suggested to transiently target proliferating populations of MDSC (83), yet such non-specific approaches have limitations; these chemotherapies can also eliminate effector T cells that may be induced to proliferate and may additionally cause a rebound expansion of myeloid progenitors.

MDSC are being monitored in some recent clinical trials and may serve as a prognostic indicator of patient response to therapy. In metastatic melanoma, a specific inhibitor of mutant B-raf^{V600E} (Vemurafenib) reduced the frequency of both Gr-MDSC and Mo-MDSC in the blood (17), with the changes in the monocytic subset correlating more closely with response to therapy. The effects of this inhibitor on MDSC may be due to simply lowering the tumor burden, thereby indirectly impacting overall inflammatory cytokine levels presumed critical for MDSC maintenance. Alternatively, this therapy may change the quality of the cytokine milieu produced by individual melanoma cells (i.e. GM-CSF, IL-1β, IL-6, TNFα, VEGF, FLT3-L, TGFβ) (17, 38, 39, 84). Anti-CTLA-4 (Ipilimumab), an immunotherapy designed to activate T cells, also reduced Mo-MDSC frequency in melanoma patients, correlating with improved response (85).

Several clinical trials targeting the CSF/CSF1R pathway are underway. Inhibiting this pathway yielded striking results in an autochthonous murine glioblastoma model (86). A selective inhibitor of CSF1 reduced TAM number and increased CD8 T cell infiltration in a

transgenic breast cancer model (87). Surprisingly, radiotherapy induced prostate tumors to increase CSF1 levels, resulting in the enhanced recruitment of myeloid progenitors that undesirably contributed to tumor growth (88). Thus, in some therapeutic settings, interfering with myeloid recruitment may be beneficial. While preclinical results are encouraging, the heterogeneity of myeloid cells in tumors may present additional obstacles to TAM depletion via CSF1, as not all TAM populations are susceptible to this pathway.

Inducing MDSC differentiation toward a functional state that is stimulatory to T cells is conceptually more feasible than sustained depletion of a population that is constantly being regenerated. An agonistic antibody to the CD40 costimulatory protein has demonstrated some recent success in patients with PDA (89). The therapeutic effect of anti-CD40 was reproduced in the genetically engineered *KPC* mouse model where it induced systemic activation of myeloid cells, infiltration into tumors, acquisition of an M1 tumoricidal phenotype and tumor cell death resulting in stromal involution. Surprisingly, the observed tumor responses appeared to be independent of T cells (89), suggesting that modulating the myeloid lineage may be sufficient to generate at least transient anti-tumor activity. Another approach to induce myeloid differentiation is an antibody that targets phosphatidylserine exposed on the surface of apoptotic tumor cells (Bavituximab). Phosphatidylserine signals to macrophages and DC and induces inhibitory cytokine production there by thwarting immune surveillance. Inhibiting phosphatidylserine signaling via antibody blockade can modulate TAM to have therapeutic activity in preclinical studies (90).

Zoledronic acid (ZA), an aminobisphosphonate, has been shown to inhibit myelopoeisis including the generation of $CD11b^+Gr-1^+$ myeloid cells, decrease tumor growth and improve survival in a transplantable PDA model (91). This compound was recently evaluated as aneoadjuvantin PDA patients with resectable disease. However, ZA did not decrease the percentage of Gr-MDSC in the blood or marrow nor did it improve overall survival (92). The potential impact of ZA on other cell subsets in the blood including IM or Mo-MDSC that also accumulate systemically in PDA patients (35, 93) remains unanswered. Further assessment of MDSC isolated from clinical tumor specimens should advance our understanding of the relative contribution of MDSC subsets in distinct malignancies. This approach was recently described in head and neck cancer (94). Cell percentages alone can be misleading due to fluctuations in other cell populations and measuring MDSC numbers whenever possible will be informative in ways frequencies will not.

CCL2, another candidate target, is produced at high levels by transformed epithelial cells, which may reflect a dependence of tumor growth on recruitment of myeloid cells through paracrine signaling (33, 35, 83). CCL2 induces the mobilization of bone marrow progenitor cells into the blood as well as the recruitment of IM and/or Mo-MDSC where they differentiate into macrophages or DC following extravasation. Thus, targeting CCR2 could potentially: 1) inhibit the egress of progenitors from the bone marrow into circulation; 2) prevent the extravasation and accumulation of myeloid cells into neoplasms; and/or 3) disruptadialogue between tumor epithelial cells and tumor macrophages. CCL2/CCR2 blockade decreased IM and TAM and increased survival in a transplantable PDA model (92). The percentage of IM in the blood correlates directly with lymph node metastases and inversely with PDA patient survival (92), suggesting that circulating IM may be a cellular

surrogate for disease burden. Additionally, CCL2 levels in patients with renal cell carcinoma correlate with MDSC levels and response to immunotherapy (83). ACCR2 inhibitor is currently in Phase 1b clinical trials in combination with FOLFIRINOX in patients with borderline resectable and locally advanced PDA. However, if the therapeutic activity of inhibiting CCL2 depends upon a stimulated endogenous T cell response, combining CCL2/ CCR2 blockade with concurrent cytotoxic therapy may inadvertently mitigate efficacy. On the other hand, if the effects are T cell independent, as was found with anti-CD40, then it may have some therapeutic benefit.

CONCLUSIONS

Targeting immunosuppression may be required, though perhaps not sufficient, to stimulate anti-tumor immunity. Among the clinical conundrums that immediately arise is how best to integrate the targeting of suppressive myeloid cells with cytotoxic regimens. Combining these modalities may be self-defeating, as cytotoxic drugs will also target the effector T cells that are induced to proliferate - one of the major mechanisms immune therapies are designed to promote. Additionally, if we are to benefit from the immunogenic cell death that cytotoxic chemotherapies can induce, and which requires myeloid cells for antigen presentation and T-cell activation, then the indiscriminate and global depletion of myeloid cells will be counterproductive. The successful clinical translation of treatment regimens that incorporate myeloid-centric, cytotoxic, and T cell reagents will require rational preclinical modeling with studious attention to the sequencing, frequency and dosing of the various components. We are currently exploring strategies to target specific immunosuppressive cells and/or their inhibitory mediators as a means to enhance adoptive T cell-based technologies and provide the patient with a *de novo* anti-tumor T cell response. Although clinical reagents are still in development, eventual Gr-MDSC depletion, in concert with a method to modulate cells of the mononuclear-phagocyte system to become competent antigen presenting cells, may provide the most favorable context for both endogenous and adoptively transferred T cells to achieve substantive and long-term clinical benefit.

Acknowledgments

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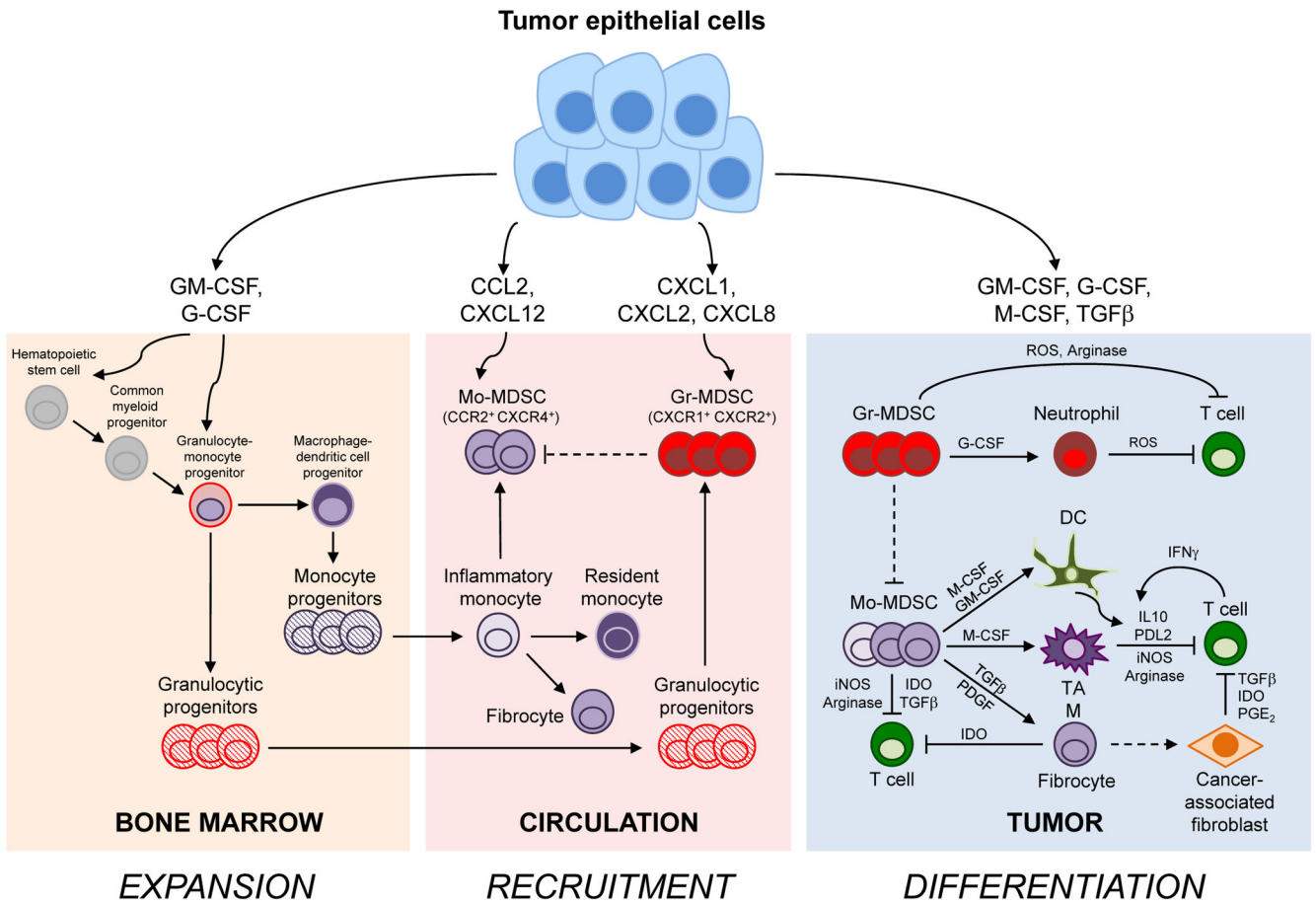
Figure 1.
Tumor epithelial cells induce the expansion, recruitment and differentiation of monocytic and granulocytic cells with distinct ontogenies, plasticities and fates. A simplified diagram of the cellular pathways instructing myeloid cell complicity in cancer is presented. *Expansion*: In normal physiology, bone marrow mesenchymal cells express low and localized concentrations of cytokines, including GM-CSF, G-CSF, SCF, and Flt3L, to regulate hematopoiesis (not shown). Carcinoma cells overexpress many of these same factors resulting in elevated and sustained systemic levels and the subsequent expansion of immature myeloid cells. This chronic inflammation induces myeloid cell egress from the bone marrow and extramedullary hematopoiesis. The pleiotropic cytokine, GM-CSF, can signal to myeloid cells at various stages of differentiation with distinct cellular outcomes. GM-CSF induces the proliferation and differentiation of hematopoietic stem cells and their progenitors into MDSC that can accumulate in pathology. G-CSF, another myelopoietic cytokine, promotes granulocyte maturation and can induce the accumulation of Gr-MDSC. GM-CSF and to a lesser extent, G-CSF, can promote Gr-MDSC survival but not proliferation. S100 proteins, VEGF and IL-6 also contribute to MDSC expansion (not shown), perhaps in synergy with GM-CSF.
Recruitment: Solid tumors secrete chemokines that attract myeloid cells. The chemokines CXCL1, CXCL2 and CXCL8 recruit Gr-MDSC. Both normal and tumor epithelium express CCL2 to attract inflammatory monocytes and Mo-MDSC. Inflammatory monocytes give

rise to resident monocytes. In cancer, tumor-derived factors can convert normal monocytes into Mo-MDSC or suppressive fibrocytes. Tumors also express CXCL12 to recruit CXCR4+ inflammatory monocytes, Mo-MDSC and fibrocytes. CXCR4 is also expressed on T cells and may inhibit T cell accumulation within the tumor bed (not shown).

Differentiation: After extravasation into normal tissues, monocytes differentiate into macrophages or DC that can either promote immunity or induce T cell tolerance depending on the context. The complex tumor inflammatory milieu instructs myeloid cells to become immunosuppressive Mo-MDSC, macrophages, DC and fibroblasts. Granulocytes and Gr-MDSC may differentiate into neutrophils following extravasation. M-CSF and Th2 cytokines (IL-4, IL13) promote conversion of monocytes into immunosuppressive macrophages; TAM may also be derived from tissue resident macrophages and or resident monocytes. Monocytes can be induced to form regulatory DC by Th2 cytokines or suppressive fibrocytes by TGFβ and PDGF. Fibrocytes may also seed the cancer-associated fibroblast populations with suppressive activity. IFNγ secretion, a hallmark of cytotoxic T cells, also activates a negative-feedback loop that mitigates T cell responses.

Mo-MDSC, monocytic myeloid-derived suppressor cells; Gr-MDSC, granulocytic myeloid-derived suppressor cells; DC, dendritic cell; TAM, tumor-associated macrophage; GM-CSF, granulocyte-macrophage colony-stimulating factor; G-CSF, granulocyte colony-stimulating factor; M-CSF, macrophage colony-stimulating factor; TGFβ, transforming growth factor beta; PGE_2, prostaglandin E2; ROS, reactive oxygen species; IDO, indoleamine 2,3-dioxygenase; PDGF, platelet-derived growth factor; iNOS, inducible nitric oxide synthase; PDL2, programmed death ligand 2.

Table 1

Immunosuppressive myeloid progenitors in cancer

A. Mouse

Name	Ontogeny	Phenotype	Other Markers	Progeny
Gr-MDSC	Granulocytic	$CD45^+$ $CD11b^+$ $Gr1^{high}$ $Ly6C^{int}$	$Ly6G^+$ $CXCR1^+$ $CXCR2^+$	Potentially Neutrophils
Mo-MDSC	Monocytic	$CD45^+$ $CD11b^+$ $Gr1^{int}$ $Ly6C^{high}$	$CCR2^+$ $CD115^+$ $CD62L^+$ $CX_3CR1^{-/low}$	High plasticity: Macrophages, Dendritic cells, resident monocytes, fibrocytes
Inflammatory monocytes	Monocytic	$CD45^+$ $CD11b^+$ $Gr1^{int}$ $Ly6C^{high}$	$CCR2^+$ $CD115^+$ $CD62L^+$ $CX_3CR1^{-/low}$	High plasticity: Macrophages, Dendritic cells, Resident monocytes, Fibrocytes, Mo-MDSC
Resident monocytes[1]	Monocytic	$CD45^+$ $CD11b^+$ $Ly6C^-$ CX_3CR1^{high}	$CCR2^-$ $CD62L^-$	M2 Macrophages
Fibrocytes	Monocytic	$CD45^+$ $CD34^+$ $Collagen^+$	$CXCR1^+$ $CXCR4^+$	Fibroblasts

B. Human

Name	Ontogeny	Phenotype	Other Markers	Progeny
Gr-MDSC	Granulocytic	$CD45^+$ Lin^- $CD11b^+$ $CD14^-$ $HLA\text{-}DR^{low}CD33^+$	$CD15^+$ $CD66b^+$	Potentially Neutrophils
Mo-MDSC	Monocytic	$CD45^+$ Lin^- $CD11b^+CD14^+$ $HLA\text{-}DR^{low}$ $CD33^+$	$CD66b^+$	High plasticity: Macrophages, Dendritic cells, resident monocytes, fibrocytes
Inflammatory monocytes	Monocytic	$CD45^+$ Lin^- $CD14^+$ $HLA\text{-}DR^+$ $CD33^+$	$CCR2^+$ $CD115^+$ $CD62L^+$ $CD15^{-/low}$ $CXCR4^+$	High plasticity: Macrophages, Dendritic cells, Resident monocytes, Fibrocytes, Mo-MDSC
Resident monocytes[1]	Monocytic	$CD45^+$ $CD14^+$ $CD16^+$ CX_3CR1^{high} $CD33^{dim}$	$CD115^+$ $CD62L^-$ $CXCR4^+$	M2 Macrophage
Fibrocytes	Monocytic	$CD45^+$ $CD34^+$ $Collagen^+$	$\alpha\text{-}SMA^+$ $HLA\text{-}DR^+$ $CXCR1^+$ $CXCR4^+$	Fibroblasts

[1] Although resident monocytes (RM) have not been well studied during cancer, during other inflammatory contexts, RM have a propensity to differentiate into wound-healing (M2) macrophages after extravasation and thus may contribute to macrophage heterogeneity in solid tumors.

Table 2

Immature myeloid progenitors in representative human cancers

Cancer	Subset	Putative suppressive mechanism	Phenotype	Other Markers	Ref
Metastatic melanoma	Mo-MDSC	--	CD45+ Lin− CD14+ HLA-DR−/low	CD15dimCD66b− CD33+ Arg− CD16−/low	(17)
Metastatic melanoma	Gr-MDSC	--	CD45+ Lin− CD14− CD15+ CD33+ HLA-DR−/low	Arg+ CD16−/low	(17)
Metastatic Melanoma	Mo-MDSC	TGFβ	CD45+ CD14− HLA-DR−/low	--	(19)
Metastatic Melanoma	Mo-MDSC	PGE2 STAT3 COX2 Superoxide	CD14+ HLA-DR−/low	--	(22)
Metastatic Melanoma	Mo-MDSC	STAT3 Arg	CD45+ HLA-DR−/low CD14−	CD80+CD83+ CD209+	(95)
Metastatic Melanoma	Mo-MDSC	--	HLA-DR−/low CD86low	--	(96)
Pediatric sarcomas	Fibrocytes	IDO	CD14− CD66b+ CD33int/low CD15+ CD123− CD11c+	α-SMA+ Collagen+ CXCR1+ MMP9+ TSLPR+ CD127+	(20)
Non-small-cell lung cancer	Mo-MDSC	ROS	CD11b+ CD14+ HLA-DR−/low	CD86low CD16low	(21)
Non-small-cell lung cancer	Gr-MDSC	Arg1 iNOS suppression of CD3ζ	CD11b+ CD14− CD15+ CD33+	SSChigh IL-4R+ IFNγR+	(97)
Glioblastoma	Mo-MDSC	TGFβ IL-10 B7H1	Lin− HLA-DR−/low CD33+	B7H1+	(29)

Cancer	Subset	Putative suppressive mechanism	Phenotype	Other Markers	Ref
Renal cell carcinoma	Gr-MDSC	Low arginine: ornithine ratio in plasma correlated with decreased CD3ζ	CD11b+ CD15+ CD14− CD33+	--	(98)
Colorectal cancer	Gr-MDSC	--	Lin−/low HLA-DR− CD11b+ CD33+	CD14− CD13+ CD117+ CD39+ SSС high	(99)
GI malignancies[1]	Mo-MDSC	--	CD14+ HLA-DR−/low	--	(26)
GI malignancies[1]	Gr-MDSC	--	CD15+ CD14− CD11b+ CD33+	--	(26)
Metastatic pancreas, colon, & breast adenocarcinomas	Gr-MDSC	H_2O_2-mediated suppression of CD3ζ	CD15+ CD11b+ Low density	--	(36)
Ovarian cancer	Mo-MDSC	--	CD11b+ CD14+ CD33+ CXCR4+	MDSC correlated w/PGE$_2$ and CXCL12 in ascites	(54)
Numerous solid tumors	--	--	CD11b+ Lin−/Lo HLA-DR− CD33+	--	(100)
Multiple myeloma	Mo-MDSC	PDE5 NOS2 Arg	CD14+ cells	--	(101)
Hepatocellular carcinoma	--	--	Lin−/low CD33+ HLA-DR−	COX-2, MMP-13, and VEGF correlated with MDSC	(102)
Hepatocellular carcinoma	Gr-MDSC	--	CD11b+CD14− HLA-DR−CD33+	--	(103)
GM-CSF induced MDSC from normal blood	Gr-MDSC	NOS TGFβ, NADPH oxidase Arg	CD11b+ HLA-DR−/low CD33+	CD66b+	(38)

[1] Colorectal, pancreatic, hepatocellular, and gastric cancer.

Table 3

Clinical trials targeting myeloid cells in cancer

Cancer	Therapy	Target	Effect	MDSC Phenotype	Ref
Metastatic melanoma	Vemurafenib*1*	B-RAF inhibitor	Decrease in Gr-MDSC and Mo-MDSC	Mo-MDSC: CD14+ HLA-DR−/low Gr-MDSC: CD14− CD66b+ Arg+	(17)
Metastatic melanoma	Ipilimumab*1*	Anti – CTLA-4	Lower Mo-MDSC frequency correlated with a more positive response to immunotherapy	Mo-MDSC: Lin− CD14+ HLA-DR−/low	(85)
RCC	ATRA + IL-2	Various	Decreased MDSC frequency in blood	Lin− HLA-DR− CD33+	(104)
RCC	Sunitinib (Tyrosine kinase inhibitor)	VEGF PDGF c-kit CSF1R	Reduced MDSC and Treg; Impact on distinct MDSC populations is not clear: as CD33 is expressed on both subsets	Mo-MDSC: CD33+ HLA-DR− Gr-MDSC: CD14− CD15+	(105)
RCC	Sunitinib (Tyrosine kinase inhibitor)	VEGF PDGF c-kit CSF1R	Mo-MDSC decreased in frequency following treatment	Lin− HLA-DR−/low CD14+	(106)
HNSCC	25-hydroxyvitamin D3	Pleiotropic effects	Decreased CD34+ cells; increased HLA-DR, increased IL-12 and IFNγ in plasma, improved T-cell blasts	CD34+	(107)
SCLC	ATRA + Vaccine	Numerous targets	Decreased MDSC frequency ~2-fold in blood	Lin− HLA-DR− CD33+ *or* CD11b+ CD14− CD33+	(108)
PDA	Zoledronic acid	Farnesyl pyrophosphate synthase	No effect on Gr-MDSC in blood at doses studied	CD45+ Lin− CD11b+ CD33+ CD15+	(92)

Cancer	Therapy	Target	Effect	MDSC Phenotype	Ref
PDA	CDDO-Me[2] + gemcitabine	iNOS COX2 NFκB	No change in % MDSC; increased T cell proliferation	Lin⁻ HLA-DR⁻/low CD33⁺ or Lin⁻ CD14⁻ CD11b⁺ CD33⁺	(109)
Multiple Myeloma[3]	PDE5 inhibitor (tadalafil)	iNOS Arg	Decreased iNOS, Arg, ROS, & nitrotyrosine; increased TCRζ expression and IFNγ	CD14⁺ IL-4R⁺	(110)

[1]These therapies were not designed to target MDSC but did decrease MDSC frequency that correlated with disease response suggesting that MDSC number can be surrogate marker for therapeutic response.

[2]CDDO-Me:synthetic triterpenoid C-28 methyl ester of 2-cyano-3,12-dioxooleana-1,9,-dien-28-oic acid

[3]Case study