Chapter from the book *Tumor Microenvironment and Myelomonocytic Cells*
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1. Introduction

Cancer immunotherapies have shown considerable promise in pre-clinical studies, but the potency of these interventions has often proved disappointing in vivo. This is in part due to tumor infiltration by myeloid cells, which are usually associated with less favorable clinical outcomes. In the past decade, several distinct subsets of tumor-infiltrating myeloid cells have been described (Movahedi et al., 2010), among which myeloid-derived suppressor cells (MDSC) have been subject to particular scrutiny for exerting a critical role in cancer progression (Bronte, 2009; Gabrilovich and Nagaraj, 2009; Ostrand-Rosenberg and Sinha, 2009; Ribechini et al., 2010). MDSC have been studied intensively in the context of cancer, and the weight of evidence indicates that these cells accumulate in most human cancers and also in experimental animal models with transplanted or spontaneous tumors (Eruslanov et al., 2011; Gabitass et al., 2011; Movahedi et al., 2008; Peranzoni et al., 2010; Raychaudhuri et al., 2011; Youn et al., 2008). MDSC also have significant roles to play in numerous other pathologies, including bacterial infections (Delano et al., 2007), parasitic infections (Brys et al., 2005; Goni et al., 2002), chemotherapy outcomes (Angulo et al., 2000), experimental autoimmunity (Arora et al., 2011; Kerr et al., 2008; Moline-Velazquez et al., 2011; Zhu et al., 2007), inflammatory bowel diseases (Haile et al., 2008), obesity (Xia et al., 2011), transplant rejection (Hock et al., 2011), and stress responses (Makarenkova et al., 2006).

MDSC are a heterogeneous population of myeloid lineage cells that comprises progenitor cells, immature macrophages, immature granulocytes and immature dendritic cells (Gabrilovich and Nagaraj, 2009). MDSC lack specific phenotypic markers of macrophages, dendritic cells and monocytes, but instead exist as two morphologically distinct subsets: monocytic (MO)-MDSC and granulocytic/polymorphonuclear (PMN)-MDSC (Movahedi et al., 2008; Youn et al., 2008). MDSC populations accumulate and become activated in response to various factors released by tumor cells and/or by host cells in the tumor microenvironment, where they suppress both innate and adaptive anti-tumor immunity through a variety of different mechanisms. MDSC are therefore considered to be a major contributor to tumor immune evasion. However, the pro-tumor action of MDSC is not limited to their direct immunosuppressive properties - these cells have also been shown to favor cancer progression by promoting angiogenesis, cancer cell proliferation, invasion, and metastasis. The induction of MDSC by pro-inflammatory mediators and by tumor-derived soluble factors highlights key contributions from chronic inflammation and from the tumor microenvironment to the onset and progression of cancer.
In this chapter, we will review the recent literature on MDSC expansion, their role in cancer progression, their proposed mechanisms of action, and the therapeutic challenges of targeting MDSC. We will focus more specifically on mouse MDSC and their role in melanoma.

2. Origin, distribution and expansion of MDSC

Hematopoietic stem cells give rise to myeloid progenitor and precursor cells in bone marrow. Then these immature myeloid cells (IMC) migrate into peripheral lymphoid organs and differentiate into mature granulocytes, macrophages, or dendritic cells. Various sources of immunological stress, including cancer, inflammation, trauma, and autoimmune disorder, can inhibit the differentiation of IMC and thus promote the expansion of this population. IMC can subsequently become activated by tumor-derived factors and host cytokines which results in the generation of MDSC with potent immunosuppressive potential (Ribechini et al., 2010). In the steady state, IMC primarily reside in the bone marrow, but in pathological settings (cancer being the most well studied), MDSC can be detected in the bone marrow, spleen, blood, tumor, and also in lymph nodes (Haile et al., 2008; Kusmartsev et al., 2005; Serafini et al., 2004; Sinha et al., 2008).

The expansion, activation and accumulation of MDSC in peripheral tissues can be driven by multiple factors produced by tumor cells, tumor stromal cells, or by activated T cells. These mediators include prostaglandins; matrix metalloproteinases (MMPs); growth factors such as granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), macrophage colony-stimulating factor (M-CSF), vascular endothelial growth factor (VEGF), stem-cell factor (SCF); cytokines such as transforming growth factor (TGF)-β, tumor necrosis factor (TNF)-α, interferon γ (IFN-γ), IL-1β, IL-4, IL-6, IL-10, IL-12, IL-13; chemokines CCL2, CXCL5, CXCL12; and various other pro-inflammatory molecules including S100A8/9 proteins, toll-like receptor agonists, tumor-derived exosome-associated Hsp72, inflammasome component NLRP3, and complement component C5a (Chalmin et al., 2010; Gabrilovich and Nagaraj, 2009; Ostrand-Rosenberg and Sinha, 2009; Ribechini et al., 2010; van Deventer et al., 2010). These agents either promote MDSC expansion through the JAK2/STAT3 signaling pathway or induce the activation of MDSC via STAT1, STAT6, or through NF-κB-dependent mechanisms (Gabrilovich and Nagaraj, 2009; Kusmartsev and Gabrilovich, 2006).

3. Subsets of murine MDSC

MDSC comprise numerous different types of myeloid precursor cells. In mice, MDSC are characterized by the co-expression of surface markers Gr-1 and CD11b. In healthy mice, cells with this phenotype constitute around 20-30% of cells in bone marrow, approximately 2-4% of cells in the spleen, and fewer still in the lymph nodes (Kusmartsev and Gabrilovich, 2006), although the frequency of these cells can increase dramatically in tumor-bearing mice (Movahedi et al., 2008). Since Gr-1 antibodies can bind to two separate epitopes, Ly6G and Ly6C, it has recently become possible to further delineate MDSC subsets using antibodies that specifically target these distinct antigens. The Ly6G molecule is expressed primarily by granulocytes, whereas Ly6C is highly expressed by monocytes (Fleming et al., 1993; Sunderkotter et al., 2004). Among murine MDSC, the CD11b+Ly6G+Ly6C^low subset (PMN-MDSC), exhibits a polymorphonuclear phenotype, while the CD11b+Ly6G+Ly6C^high subset (MO-MDSC), displays a monocytic phenotype. More recently, some new features of MDSC have emerged that provide further insights into the diversity of these cells. Using a simple
staining strategy, Greifenberg and colleagues were able to divide mouse splenocytes into six distinct sub-populations with regard to their size, granularity, morphology, and relative expression of CD11b and Gr-1 (Greifenberg et al., 2009). Among these various populations, Gr-1\textsubscript{low}CD11b\textsuperscript{high}Ly6C\textsuperscript{high}SSC\textsubscript{low} and Gr-1\textsuperscript{high}CD11b\textsubscript{low} (with ring-shaped nuclei) MDSC possessed suppressive potential. Additionally, Elkabets et al. identified a novel sub-population of murine MDSC that lacks Ly6C expression and predominates during IL-1\beta-induced inflammatory responses. Ly6C\textsuperscript{neg} MDSC and Ly6C\textsuperscript{low} MDSC may constitute separate lineages of MDSC, or could perhaps represent distinct states of differentiation within a single MDSC lineage (Elkabets et al., 2010). In addition to Gr-1 and CD11b, several other surface molecules have been reported to discriminate between sub-populations of MDSC, including the co-stimulatory molecule CD80 (B7.1) (Yang et al., 2006), macrophage marker F4/80 (Huang et al., 2006), the M-CSF receptor (CSF1R/CD115) (Huang et al., 2006), and the α-chain of the receptor for IL-4 and IL-13 (IL-4Ra/CD124) (Gallina et al., 2006). MO-MDSC express higher levels of F4/80, CD115, 7/4, and CCR2 when compared with PMN-MDSC, which suggests a monocytic origin for these cells. However, further studies have demonstrated that, although these additional markers are undoubtedly expressed by MDSC, they do not specifically define a population of immunosuppressive cells (Youn et al., 2008). Indeed, while useful for analytical purposes, the Ly6G and Ly6C antibodies are not essential for identifying MDSC populations by flow-cytometry: differential expression of Gr-1 and F4/80 alone can suffice to distinguish PMN-MDSC (CD11b\textsuperscript{Gr-1\textsuperscript{high}F4/80\textsubscript{low}}) from MO-MDSC (CD11b\textsuperscript{Gr-1\textsuperscript{int}F4/80\textsuperscript{int}}). The use of Ly6G-specific antibodies is therefore only required when attempting to isolate a pure PMN-MDSC subset from a mixed cell population that also includes MO-MDSC (Toh et al., 2011). A summary of MDSC subsets can be found in Table 1.

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<tr>
<th>PMN-MDSC</th>
<th>MO-MDSC</th>
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<td>CD11b\textsuperscript{Ly6G\textsuperscript{Ly6C}\textsuperscript{low}}</td>
<td>CD11b\textsuperscript{Ly6G\textsuperscript{Ly6C}\textsuperscript{high}} Higher expression of F4/80, CCR2 and CD115</td>
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<td>Cell contact-dependent Antigen-specific immunosuppression</td>
<td>Cell contact-independent Antigen-specific and antigen-independent immunosuppression</td>
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<td>Immune suppression via ROS-mediated mechanisms</td>
<td>Immune suppression via arginase and NOS-mediated mechanisms</td>
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<td>Terminally differentiated</td>
<td>Capable in differentiating into macrophages</td>
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Table 1. Main characteristics of two well-accepted MDSC subsets (Movahedi et al., 2008; Youn et al., 2008). It should be noted that some novel sub-populations of MDSC have recently been identified.
4. MDSC in cancer progression

There is ample evidence from the literature that MDSC are associated with tumor progression. Adoptive transfer of MDSC in murine tumor models has been found to significantly promote tumor growth (Balwit et al., 2011; Yang et al., 2004), and administration of MDSC after 5-Fluorouracil (5FU) injection blunted the anti-tumor effect of 5FU in tumor-bearing mice (Vincent et al., 2010). Depletion of Gr-1+ cells in tumor-bearing mice by injection of anti-Gr-1 antibody strikingly inhibited tumor growth, reduced cancer cell dissemination and metastasis, and prolonged survival (Li et al., 2009; Pekarek et al., 1995; Zhang et al., 2009). Treatment of tumor-bearing mice with drugs that target MDSC, such as gemcitabine chemotherapeutic agent, all-trans-retinoic acid, and phosphodiesterase-5 inhibitors led to delayed tumor progression, improved survival, and enhanced efficacy of cancer vaccines and immunotherapies (Kusmartsev et al., 2003; Serafini et al., 2006; Suzuki et al., 2005). Reduction of murine MDSC numbers has also been shown to facilitate the rejection of established metastatic disease after the removal of primary tumors (Sinha et al., 2005).

5. MDSC use multiple mechanisms to suppress T-cell function

MDSC use a variety of different mechanisms to suppress anti-tumor immunity. Multiple lines of evidence indicate that MDSC are potent inhibitors of both antigen-specific and non-specific T-cell activation.

5.1 Arginase

L-arginine is a conditionally essential amino acid and is primarily metabolized by arginases (ARGs) and nitric oxide synthases (NOSs) to produce either L-ornithine and urea, or to provide L-citrulline and nitric oxide (NO) (Bogdan, 2001; Morris, 2002; Wu and Morris, 1998). The suppressive activity of MDSC was initially thought to be associated with the metabolism of L-arginine since depletion of this amino acid is accompanied by marked suppression of T-cell function and proliferation (Bronte et al., 2003; Bronte and Zanovello, 2005; Rodriguez et al., 2005; Rodriguez and Ochoa, 2008). L-arginine deprivation has been reported to induce T-cell dysfunction via two distinct pathways, the first being loss of CD3ζ chain expression by these cells (Ezernitchi et al., 2006; Rodriguez et al., 2004; Rodriguez et al., 2002; Rodriguez et al., 2003a). CD3ζ is a key component of the T-cell receptor (TCR) and contains three immunoreceptor tyrosine-based activation motifs (ITAM) that generate an activation signal in T cells upon antigen recognition (Pitcher and van Oers, 2003). Lack of L-arginine may therefore decrease the propensity for T cells to become activated by down-regulating the CD3ζ signal transduction machinery. Alternatively, shortage of L-arginine may prevent the up-regulation of cell cycle regulators cyclin D3 and cyclin-dependent kinase 4 (CDK4) to arrest T cells in the G0-G1 phase of the cell cycle (Rodriguez et al., 2007). MDSC produce high levels of arginase, which depletes L-arginine in the local microenvironment, and can also uptake excess arginine through the CAT-2B transporter (Rodriguez et al., 2004; Rodriguez et al., 2003b). MDSC may therefore deprive T cells of L-arginine to limit their proliferative potential, as well as decreasing TCR signaling, to induce broad suppression of T-cell function. These mechanisms seem to contribute to the pro-tumor function of MDSC, since injection of the arginase I inhibitor N-hydroxy-nor-l-arginine (Nor-NOHA) in tandem with tumor implantation has been shown to significantly slow the growth of lung carcinoma in a dose-dependent manner. However, inhibition of tumor
growth upon Nor-NOHA treatment was not observed in tumor-laden SCID mice (severe combined immunodeficient animals), suggesting that the anti-tumor effect of arginase inhibition was dependent on lymphocyte function (Rodriguez et al., 2004).

5.2 Nitric oxide

L-arginine is a substrate for inducible nitric oxide synthase (iNOS) which is highly expressed in MDSC. Nitric oxide (NO) production via this pathway is a powerful modulator of inflammation and has been reported to preferentially inhibit Th1-mediated immune responses (Bauer et al., 1997; Sosroseno et al., 2009). NO potently suppresses T-cell activation, proliferation, adhesion, and migration (Bingisser et al., 1998; Bobe et al., 1999; Lejeune et al., 1994; Mazzoni et al., 2002; Medot-Pirenne et al., 1999; Sato et al., 2007). It suppresses T-cell function through blocking the activation of several important signaling molecules in T cells, including Janus-activated kinase 1 (JAK1), JAK3, signal transducer and activator of transcription 5 (STAT5), extracellular signal-regulated kinase (ERK), and AKT (Bingisser et al., 1998; Mazzoni et al., 2002). NO has also been shown to inhibit MHC class II expression and promote T-cell apoptosis (Harari and Liao, 2004; Rivoltini et al., 2002).

5.3 Reactive oxygen species

Reactive oxygen species (ROS) have emerged as a potential key mechanism of MDSC-induced immunosuppression in tumor-bearing hosts. Hyper-production of ROS is an archetypal feature of MDSC in both mouse tumor models and in human cancer patients (Greten et al., 2011; Kusmartsev et al., 2004; Youn et al., 2008). Elevated ROS production by MDSC is mediated primarily by increased NADPH oxidase NOX2 activity (Corzo et al., 2009). In a previous report, lack of NOX2 activity abrogated the ability of MDSC to suppress T-cell responses (Corzo et al., 2009). Arginases and NOS can also contribute to the generation of ROS in MDSC: arginase depletion of L-arginine in the local environment triggers superoxide (O$_2^-$) generation from iNOS (Bronte et al., 2003; Xia et al., 1998). The unstable O$_2^-$ anion can then react with protons in water to generate hydrogen peroxide. ROS appear then to exert a major role in MDSC-mediated T-cell suppression (Kusmartsev et al., 2008; Markiewski et al., 2008; Nagaraj et al., 2007) and have been implicated in the inhibition of antigen-specific CD8$^+$ T-cell responses in tumor-bearing mice (Kusmartsev et al., 2004). ROS are also thought to play a direct role in inducing apoptosis of activated T cells by decreasing Bcl-2 expression (Hildeman et al., 2003). Accordingly, inhibition of ROS production in MDSC by the addition of ROS scavengers can reverse MDSC-mediated immune suppression and rescues IFN-γ production (Kusmartsev et al., 2004; Kusmartsev et al., 2008).

5.4 Peroxynitrite

Peroxynitrite (ONOO$^-$) is a reactive nitrogen-oxide species (RNOS) formed from the reaction between NO and O$_2^-$ (Squadrito and Pryor, 1995). A major action of peroxynitrite is the modification of proteins by oxidation or nitration of the amino acids tyrosine, cysteine, methionine, and tryptophan (Gabrilovich and Nagaraj, 2009). MDSC are copious producers of peroxynitrite, and increased levels of this species are associated with tumor progression (Cobbs et al., 2003; Ekmeckioglu et al., 2000; Nakamura et al., 2006). Hyper-production of peroxynitrite during direct contact with T cells allows MDSC to induce nitration of tyrosine residues in the TCR and CD8 co-receptor, leading to decreased conformational flexibility of the TCR chains and impaired interactions with MHC, thus inhibiting antigen-specific,
cytotoxic T-cell responses (Nagaraj et al., 2007). Peroxynitrite-driven nitration of tyrosine residues in human lymphocytes is also able to promote apoptotic cell death by inhibiting activation-induced tyrosine phosphorylation in these cells (Brito et al., 1999).

### 5.5 Cysteine

Recent work has demonstrated that murine MDSC block T-cell activation by depleting cysteine from the local microenvironment (Srivastava et al., 2010). Cysteine is an essential amino acid required for T-cell activation, differentiation and proliferation. Cells generate cysteine through two distinct pathways: the cystathionase enzyme can convert intracellular methionine into cysteine (Gout et al., 2001; Ishii et al., 2004), or alternatively, the plasma membrane cysteine transporter \( x_c^- \) can import the oxidized form of the acid (cystine) from the extracellular environment. Imported cystine can then be reduced to form cysteine (Arner and Holmgren, 2000; Mansoor et al., 1992). Since T cells lack both cystathionase and an intact \( x_c^- \) transporter, they are unable to generate cysteine independently. Under homeostatic conditions, antigen-presenting cells (APC) provide cysteine to T cells by importing cystine, converting it to cysteine, and then exporting the cysteine through their plasma membrane ASC transporters (Sato et al., 1987; Angelini et al., 2002). Like T cells, MDSC lack cystathionase and depend on extracellular cysteine for the synthesis of cysteine, but they lack the APC-expressed ASC transporter required to export cysteine. This results in MDSC readily importing cystine at a rate similar to that of macrophages and DC, but they do not export cysteine. This action depletes the environment of cysteine and results in the inhibition of T-cell activation and function (Srivastava et al., 2010).

### 5.6 Alternative immunosuppressive mechanisms

Alternative pathways have been identified through which MDSC might exert their suppressive functions. The immunoregulatory cytokine TGF-\( \beta \) has been implicated in MDSC function. It is regulated in MDSC by IL-13 and CD4\(^+\) CD1d-restricted T cells. Blocking IL-13 or TGF-\( \beta \) limited tumor incidence in murine transplanted tumor models (Fichtner-Feigl et al., 2008; Terabe et al., 2003). MDSC also have the ability to systemically down-regulate CD62L (L-selectin) on T cells in tumor-bearing mice. This action impairs naïve CD4\(^+\) and CD8\(^+\) T-cell homing to lymph nodes. Therefore, these T cells are not able to be activated by tumor antigens (Hanson et al., 2009). Down-regulation of CD62L was not due to general T-cell activation and could even be observed in tumor-free mice that exhibited high numbers of MDSC, (a common profile in aged animals). MDSC also constitutively express a disintegrin and metalloproteinase domain 17 (ADAM17, also known as TACE/TNF-\( \alpha \)-converting enzyme) on their cell surface, thus allowing the proteolytic cleavage and shedding of the ectodomain of CD62L (Hanson et al., 2009).

Various reports have demonstrated that MDSC can induce the differentiation of regulatory T cells (Treg) in tumor-bearing hosts and indirectly promote immune suppression (Gabrilovich and Nagaraj, 2009). Treg induction can occur through diverse pathways that depend on the tumor model in use (Bianchi et al., 2011). In a mouse lymphoma model, induction of Treg is dependent on arginase and is independent of TGF-\( \beta \) (Serafini et al., 2008), but in murine ovarian cancer, cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) expression on MDSC can mediate Treg induction (Yang et al., 2006). Another study by Huang et al. using several murine transplanted tumor models showed that IL-10 and IFN-\( \gamma \),
but not NO, were important factors in MDSC-mediated Treg development (Huang et al., 2006). A more recent study has also reported that the immune stimulatory receptor CD40 on MDSC is required to induce tumor-specific Treg expansion in a mouse colon cancer model (Pan et al., 2010).

5.7 Suppressive mechanisms differ between MDSC subsets

In addition to morphological and phenotypic distinctions, PMN-MDSC and MO-MDSC also exert their suppressive activity by different mechanisms. MO-MDSC express high levels of NO and low levels of ROS, and they effectively suppress T-cell function in both antigen-dependent and independent manners without requiring cell-cell contact. Primarily, MO-MDSC inhibit T-cell function through NOS-mediated mechanisms since NOS inhibitors are able to block this suppressive effect. This pathway is IFN-γ/STAT1-dependent (Movahedi et al., 2008; Youn et al., 2008). In contrast, PMN-MDSC produce high levels of ROS but only nominal amounts of NO, indicating that ROS are the primary mediators of their suppressive functions (Movahedi et al., 2008; Youn et al., 2008). PMN-MDSC generally require antigen-specific interactions with T cells to mediate suppression (Nagaraj et al., 2007), although it has also been reported that PMN-MDSC do not require direct MHC I presentation to exert inhibitory effects (Movahedi et al., 2008). In most tumor models, PMN-MDSC are the main MDSC subset to be expanded in the peripheral lymphoid organs (Youn et al., 2008), while the MO-MDSC population possesses more potent inhibitory activity (Dolcetti et al., 2010; Movahedi et al., 2008; Nausch et al., 2008; Priceman et al., 2010).

Murine splenic MDSC have also been shown to differ from their tumor-derived counterparts with regards to T-cell suppression. Tumor MDSC can potently suppress T-cell proliferation in both antigen-specific and non-specific manner, whereas splenic MDSC are comparatively weak suppressor cells and exert only antigen-specific T-cell inhibition. This functional difference is suggested to be due to the different suppressive mechanisms used by splenic and tumor MDSC. Splenic MDSC suppress T cells through ROS production. In contrast, at the tumor site, MDSC, as a result of the effect of hypoxia via HIF-1α, dramatically up-regulate inos and arg1 expression and therefore acquire the ability to inhibit antigen-nonspecific T-cell functions (Corzo et al., 2010).

6. Mechanisms by which MDSC disrupt innate immunity

In addition to T-cell suppression, MDSC restrict innate responses via their interactions with macrophages, NK cells, and NKT cells to further impair anti-tumor immunity. Cross-talk between MDSC and macrophages results in increased MDSC production of the type 2 cytokine IL-10, and decreased macrophage production of type 1 cytokine IL-12, which skews tumor immunity towards a tumor-promoting type 2 response (Sinha et al., 2007).

The role of MDSC in regulating NK-cell function remains controversial. Some studies have shown that MDSC impair NK-cell development, IFN-γ production and cytotoxicity against tumor cells. This suppression is mediated by membrane-bound TGF-β1 and through down-modulation of NKG2D (the primary activating receptor for NK cells) (Elkabets et al., 2010; Li et al., 2009; Liu et al., 2007; Suzuki et al., 2005). However, in a separate mouse study, MO-MDSC isolated from RMA-S tumor-bearing mice failed to suppress NK-cell function, and instead elicited high production of IFN-γ by these cells. These effects partially depended on
the interaction of NKG2D on NK cells with ligand RAE-1 on MDSC. Following activation, the NK cells eliminated the MDSC (Nausch et al., 2008).

7. Non-immunosuppressive pro-tumor functions

MDSC support for tumor growth does not depend solely on immunosuppression – these cells also promote tumor progression by augmenting blood vessel development and enhancing tumor-cell invasion and metastasis. In a murine colorectal cancer model (MC26), tumors co-injected with MDSC from mice bearing large tumors exhibited increased vascular density and maturation, as well as decreased necrosis (Yang et al., 2004). Tumor growth was markedly facilitated when co-injected with tumor-derived MDSC, but not when co-injected with MDSC from normal mice. This increased vasculature was attributed to the production of MMP9, a critical mediator of tumor angiogenesis, vasculogenesis, and metastasis. MDSC-derived MMP9 was shown to increase the bioavailability of VEGF in tumors and promote tumor angiogenesis and vascular stability. Accordingly, selective deletion of MMP9 in MDSC completely abolished their tumor-promoting activity (Yang et al., 2004). In a separate study using the mouse MT1A2 mammary cancer model, it was demonstrated that bone marrow-derived CD11b+ myelomonocytic cells significantly contributed to tumor vasculogenesis by producing MMP9 (Ahn and Brown, 2008). Various other MMPs, including MMP14, MMP13, and MMP2, were also found to be highly expressed in tumor-resident MDSC (Yang et al., 2008). These MDSC were recruited to the invasive front of mammary carcinomas with conditional deletion of the type II TGF-β receptor gene. The MDSC infiltrate directly facilitated tumor invasion and metastasis through enhanced MMP and TGF-β production (Yang et al., 2008). Furthermore, Bv8 protein (also known as prokineticin 2, or Prok2) has also been reported to contribute to MDSC-dependent tumor angiogenesis. Transplantation of tumor cells in mice resulted in significant up-regulation of Bv8 in MDSC, while treatment with neutralizing anti-Bv8 antibodies suppressed tumor angiogenesis and inhibited tumor growth (Shojaei et al., 2007). In addition, murine tumor-associated MDSC were shown to confer tumor resistance to anti-angiogenic therapy (anti-VEGF antibody) that was mediated by G-CSF and depended on Bv8 expression. Combining anti-VEGF treatment with anti-Gr-1, anti-G-CSF, or anti-Bv8 antibody inhibited growth of refractory tumors more effectively that anti-VEGF therapy alone. Anti-G-CSF treatment robustly reduced MDSC frequency in refractory tumors, decreased Bv8 levels, and inhibited tumor angiogenesis (Shojaei et al., 2009). The tumor microenvironment has also been proposed to support MDSC shape change and expression of endothelial markers such as VEGFR2 and VE-Cadherin (Yang et al., 2004), which may allow MDSC to differentiate locally and directly incorporate into the tumor endothelium to contribute to vascular development.

Although MDSC up-regulation of proteases seems to be the primary route by which these cells promote tumor metastasis, a recent study by Boutte and colleagues also highlighted the importance of down-regulating protease inhibitors in tumor dissemination (Boutte et al., 2011). In this study of transplanted tumors, neutrophilic granule protein (NGP: a cathepsin B inhibitor), was down-regulated in MDSC from metastatic tumor-bearing mice compared with non-metastatic controls. Up-regulation of NGP in tumors delayed primary tumor growth and greatly reduced tumor vasculature, invasiveness, and metastasis.

MDSC have been further implicated in pre-metastatic niche formation in the lungs of tumor-bearing mice. The concept of the pre-metastatic niche arises from the observation that many tumors have a pre-disposition to metastasize certain organs. Various leukocyte populations
and secreted inflammatory factors have been shown to “prepare” distal organs for metastatic cells (Hiratsuka et al., 2006; Kaplan et al., 2006; Kaplan et al., 2005), and MDSC can infiltrate the lungs of tumor-bearing mice before the arrival of tumor cells. These MDSC create a proliferative and immunosuppressive lung environment that is permissive for the growth of metastatic tumor cells. Pre-metastatic lungs with elevated MDSC have increased levels of basic fibroblast growth factor (bFGF), insulin growth factor 1 (IGF1), IL-4, IL-5, IL-9, IL-10, and MMP9, whereas IFN-γ is down-regulated in these lungs. Up-regulation of MMP9 in pulmonary MDSC drives abnormal vasculature development in the pre-metastatic lung (Yan et al., 2010), while myeloid cell-derived S100A8 and S100A9 predispose the lung microenvironment towards eventual tumor metastasis (Hiratsuka et al., 2006).

Finally, our own data reveal that PMN-MDSC promote melanoma cell proliferation by secreting soluble factors while also supporting cancer cell dissemination and metastasis by inducing epithelial-mesenchymal transition (Toh et al., 2011). These novel MDSC functions are discussed in more detail in the subsequent sections.

8. Melanoma and the immune system

Malignant melanoma is one of the most immunogenic forms of cancer and hundreds of immunotherapeutic trials have been conducted in melanoma patients to date. Substantial knowledge has been accumulated on the immunosuppressive pathways at work in melanoma and the role played by MDSC in disease progression. Tumor infiltrating lymphocytes (TIL) have been correlated with better prognoses and improved five-year survival rates (Day et al., 1981), and TIL isolated from melanoma patients are able to lyse MHC-matched allogeneic tumors (Degiovanni et al., 1988; Oble et al., 2009). However, the prognostic value of TIL is only valid in the early stages of melanoma, since TIL numbers in thick lesions do not predict clinical outcomes. Many melanoma-associated antigens are non-mutated proteins that contribute to melanin synthesis, such as MelanA/MART-1, tyrosinase related protein (TRP)-1, TRP-2, gp100 and tyrosinase (Kawakami, 2000). Unfortunately, there has been only limited success in vaccinating patients with these antigens (Linette et al., 2005). Large numbers of MelanA/MART-1 specific T cells have been found in the blood and tumors of melanoma patients (Salcedo et al., 2006), but only the circulating T cells were able to produce IFN-γ and granzyme B upon antigen stimulation (Zippelius et al., 2004). These data indicate potent local immunosuppression at the tumor site which is most likely driven by immune cells recruited into the tumor itself. Accordingly, lymphocyte depletion has been shown to be effective method of enhancing adoptive T-cell transfer therapy in melanoma patients (Hershkovitz et al., 2010). In a clinical trial to test the efficacy of adoptive T-cell transfer in combination with lympho-depletion (non-myeloablative chemotherapy; NMC), better objective responses and complete remission could be achieved when NMC was combined with total body irradiation or high dose irradiation alone (Dudley et al., 2008). These findings suggest that tumor-induced immunosuppression does not arise from lymphocytes alone but also from myeloid cells.

To study the complex interactions between tumors and the immune system, investigators are progressively turning to transgenic mice that develop spontaneous tumors and replicate human cancers more closely than transplanted tumor models. RETAAD mice are transgenic for the activated RET oncogene which is specifically expressed in melanocytes of the skin and eyes, leading to spontaneous skin tumors and primary uveal melanomas that are
clinically detectable by four to eight weeks of age. While exophthalmos eventually presents in adult RETAAD mice, microscopic eye tumors can be detected as early as ten days after birth, and cancer cells disseminate from the primary eye tumor throughout the body within three weeks (Eyles et al., 2010; Kato et al., 1998). Disseminated RETAAD cancer cells remain dormant for months before developing into cutaneous and visceral metastases, and the stepwise evolution of melanoma in these mice closely mimics the histopathology and natural history of human cancers (Eskelin et al., 2000; Kato et al., 1998; Kato et al., 2004). The RETAAD melanoma model is therefore particularly suitable for dissecting the role of host immune cells in metastatic processes.

Similar to human melanoma patients, tumors in RETAAD mice grow despite the induction of a broad melanoma-specific CD8$^+$ T-cell response (Lengagne et al., 2008). It is surprising then that cutaneous tumor cell lines derived from RETAAD mice are still recognized by tumor-specific T cells, indicating that they are indeed antigenic (Lengagne et al., 2008). Functionally active, melanoma-specific, memory T lymphocytes can be detected at the early stages of melanoma progression, in the absence of clinically visible cutaneous tumors (Lengagne et al., 2008; Umansky et al., 2008). However, tumor progression continues despite the presence of these antigen-specific CD8$^+$ T cells, suggesting that potent suppressive mechanisms shield the developing tumor from immune destruction.

Even though a pathological role for Treg cells has been implicated in several tumor models, depletion of Treg in RET transgenic melanoma mice neither delayed nor inhibited tumor development (Kimpfler et al., 2009). In RET mice, intra-tumoral dendritic cell (DC) numbers correlated with tumor size, and DC from mice with macroscopic tumors secreted...
significantly less IL-12p70, increased quantities of IL-10, and were impaired in their ability to activate T cells. The tolerogenic properties of these DC were mediated by IL-6, VEGF, and TGF-\(\beta\), secreted in the tumor microenvironment (Zhao et al., 2009). Interestingly, in a separate study, IL-6 ablation in RET mice also reduced the incidence and size of tumors (von Felbert et al., 2005). Relative aggression of cutaneous tumors in RET mice correlated with numbers of tumor-infiltrating CD11b\(^+\)Gr1\(^{low}\) macrophages that displayed an M2-like, pro-tumor phenotype, characterized by high transcript levels of il10, arginase I, mgl1, fizz1, and ccl2. Tumor- and spleen-derived macrophages in these mice were able to potentiate inhibition of T-cell function. Surprisingly, depletion of T cells from RET mice resulted in the switching of these macrophages towards a M1, anti-tumor phenotype, characterized by secretion of IL-12. In the absence of T cells, macrophages in RET mice also displayed reduced ability to support tumor growth (Lengagne et al., 2011). In our own laboratory, we have further observed that the microenvironment of RETAAD cutaneous tumors supports only limited infiltration of CD4\(^+\) and CD8\(^+\) T cells compared with transplanted B16 tumors (Hong et al., 2011).

9. MDSC in melanoma

We have observed that CD11b\(^+\)Gr1\(^{high}\) PMN-MDSC are increased in the spleen and blood of RETAAD mice during tumor progression. PMN-MDSC, but not MO-MDSC, preferentially accumulate in the primary tumor compared with metastases, which is due to the expression of CXCL1, CXCL2 and CXCL5 (chemotactic mediators specific for PMN-MDSC) in the primary tumor, but not in metastases. PMN-MDSC notably affect two primary aspects of tumor progression – tumor growth and metastasis. In RETAAD mice, depletion of PMN-MDSC by treatment with anti-Ly6G antibody resulted in a decrease in primary tumor size, but failed to diminish cutaneous tumors (which have low infiltrates of PMN-MDSC). Furthermore, early depletion of PMN-MDSC (before primary tumor development) resulted in decreased proliferation of primary tumors, while in vitro assays demonstrated that the ability to induce cancer cell proliferation is specific to PMN-MDSC but not macrophages. These data also indicate that PMN-MDSC are able to directly induce tumor cell proliferation by secreting a soluble factor (Toh et al., 2011).

PMN-MDSC also induce epithelial-mesenchymal transition (EMT) which is the first step towards metastasis in early stage cancers (Toh et al., 2011). Depletion of PMN-MDSC reduced dissemination of tumor cells to distant metastatic sites such as the lungs and the tumor-draining lymph nodes. Primary tumor cells in control mice exhibited higher expression of mesenchymal markers S100A4 and vimentin compared with mice depleted of PMN-MDSC. In vitro, PMN-MDSC were also able to down-modulate E-Cadherin, a classical epithelial marker, in both mouse and human melanoma cells. Induction of EMT was dependent on TGF-\(\beta\), epidermal growth factor and hepatocyte growth factor, since blockade of these molecules either individually or in combination resulted in partial or complete inhibition of EMT (Toh et al., 2011).

The ability of melanomas to evade immune destruction depends on components of the host immune system. While extensive research has already been conducted on immunotherapy strategies that enhance T-cell responses against tumors, improving the efficacy of these interventions will require a better understanding of the interactions between melanoma cells and the immune system, with a particular focus on immunosuppressive MDSC populations.
Additional research will also be required to determine whether MDSC are capable of inducing cancer cell proliferation and epithelial-mesenchymal transition in other types of cancers besides melanoma.

Fig. 2. Roles of MDSC in the growth, invasion, and metastasis of melanoma.

10. Therapeutic strategies targeting MDSC

Recognition that MDSC-mediated immune suppression plays a pivotal role in tumor progression highlights these cells as an appealing target for novel cancer treatments. Agents that modulate MDSC development, differentiation and recruitment, or block the suppressive functions of these cells could represent potent new methods of limiting tumor progression, or could perhaps enhance the efficacy of existing therapies. Limiting the infiltration and activation of MDSC during chronic inflammation may even reduce the risk of de novo tumor development.

10.1 Promoting MDSC differentiation

Given the fact that MDSC are immature myeloid cells, a promising approach in cancer immunotherapy would be to drive MDSC differentiation into mature populations that no longer have suppressive activity. Vitamin A has been identified as a candidate agent that possesses this ability, since vitamin A deficiency causes systemic expansion of MDSC in mice (Kuwata et al., 2000). Vitamin A metabolites such as retinoic acid have been found to favor MDSC differentiation into mature DC, macrophages, and granulocytes. Treatment of
mouse or human MDSC with all-trans-retinoic acid (ATRA) in vitro resulted in induction of myeloid cell differentiation (Almand et al., 2001; Gabrilovich et al., 2001; Hengesbach and Hoag, 2004; Kusmartsev et al., 2008). Using adoptive transfer of MDSC into congenic mice, Kusmartsev et al. were able to demonstrate that ATRA also induced rapid differentiation of MDSC into mature myeloid cells in vivo (Kusmartsev et al., 2003). In tumor-bearing mice, ATRA administration substantially reduced the presence of MDSC and noticeably improved CD4+ and CD8+ T-cell-mediated anti-tumor immune responses. Combination of ATRA with two different types of cancer vaccines significantly prolonged the anti-tumor effect of the vaccination in two different mouse tumor models (Kusmartsev et al., 2003). Moreover, in human patients with metastatic renal cell carcinoma, effective concentrations of ATRA were shown to eliminate MDSC and improve antigen-specific T-cell responses (Kusmartsev et al., 2008; Mirza et al., 2006). Vitamin D derivatives have also been reported to drive myeloid progenitor cell differentiation both in vitro and in vivo (Duits et al., 1992; Testa et al., 1993). 25-hydroxyvitamin D3 treatment in patients with head and neck squamous cell carcinoma diminished the number of immuno-suppressive CD34+ progenitor cells and improved numerous parameters of immune responsiveness (Lathers et al., 2004).

### 10.2 Inhibiting MDSC expansion

As described above, many tumor-derived factors can induce the development and expansion of MDSC from hematopoietic precursors (see also section 2). Neutralization of these mediators is therefore another attractive strategy for novel cancer therapies. For example, stem-cell factor (SCF) has been identified as a vital mediator of MDSC expansion and accumulation, since SCF knockdown using silencing RNA decreased MDSC frequency and reversed tumor-specific T-cell tolerance in the mouse MCA26 colon cancer model. Blocking SCF interactions with its receptor, c-kit, by the use of specific antibodies dramatically reduced the MDSC population and prevented tumor-specific T-cell anergy, Treg development, and tumor angiogenesis, resulting in tumor regression and enhanced efficacy of immune-activating cancer therapy (Pan et al., 2008). Another study also reported that melanoma development is restrained in RET-transgenic mice with impaired c-kit function, or when RET mice are treated with anti-c-kit antibody. Although the authors attributed this phenomenon to the direct function of c-kit in tumor cells, we cannot exclude the possibility that the suppression of tumor development was due to attenuated MDSC expansion caused by c-kit impairment (Kato et al., 2004).

MMP9 inhibition is another logical therapeutic approach in cancer therapy due to the MDSC requirement for MMP9 in supporting their expansion and function. In a spontaneous mouse mammary tumor model, treatment with a MMP9 inhibitor (amino-biphosphonate) was shown to significantly reduce MDSC expansion and impair tumor growth, while simultaneously enhancing tumor necrosis and improving the anti-tumor responses induced by immunotherapy (Melani et al., 2007). Finally, targeting the intracellular signaling pathways that are involved in MDSC expansion is also a promising strategy. Using selective STAT3 inhibitors, such as JSI-124 (cucurbitacin I), or tyrosine kinase inhibitors, such as sunitinib, can augment anti-tumor immune responses by reducing the presence of MDSC. Sunitinib combination therapy with IL-12 and 4-1BB activation significantly improved the long-term survival rate of mice bearing MCA26 colon tumors (Ko et al., 2009; Nefedova et al., 2005; Ozao-Choy et al., 2009). In a more recent study, treating mice with docetaxel anti-
mitotic, chemotherapeutic reagent was found to polarize MDSC towards a M1-like phenotype by inhibiting STAT3 activation, and consequently restored CD4+ and CD8+ T-cell function to reduce 4T1-Neu tumor burden (Kodumudi et al., 2010).

10.3 Eliminating MDSC

Direct elimination of MDSC can be achieved with chemotherapeutic drugs such as gemcitabine, which dramatically and specifically reduces MDSC numbers in tumor-bearing mice, but spares CD4+ T cells, CD8+ T cells, NK cells, macrophages, and B cells. This beneficial loss of MDSC is also accompanied by an increase in the anti-tumor activity of the preserved CD8+ T-cell and NK-cell pool (Suzuki et al., 2005). Treatment with 5-fluorouracil (5FU) has also been shown to induce selective apoptosis of MDSC, thereby decreasing the burden of these cells in murine spleen and tumor beds, but without depleting host T cells, NK cells, dendritic cells, or B cells. The elimination of MDSC by 5FU treatment also increased IFN-γ production by tumor-specific CD8+ T cells and promoted T-cell—dependent anti-tumor responses (Apetoh et al., 2011; Vincent et al., 2010).

10.4 Blocking MDSC suppressive function

Another approach to restricting MDSC support for tumor progression is to block the immunosuppressive function of these cells. Since ARG1 and NOS2 are the primary mediators of MDSC immunosuppression, these enzymes are the most likely targets for novel therapeutic interventions. Various different drugs including nitro-aspirin, COX-2 inhibitors, and phosphodiesterase-5 (PDE5) inhibitors have been shown to profoundly inhibit both ARG1 and NOS2 activity in MDSC. By removing MDSC suppressive mediators, these drugs exhibited a potent ability to restore anti-tumor immune responses and delayed tumor progression in several mouse models (De Santo et al., 2005; Serafini et al., 2006; Talmadge et al., 2007; Zea et al., 2005). Interestingly, in addition to inhibiting MDSC function, COX2 inhibitors also blocked the systemic development of MDSC as well as CCL2-mediated accumulation of these cells in the tumor microenvironment in a mouse model of glioma (Fujita et al., 2011).

11. Conclusion

In recent years, it is becoming increasingly apparent that the immuno-suppressive mechanisms operating in cancer patients significantly contribute to tumor progression and attenuate the efficacy of immunotherapies. The tumor microenvironment incorporates several distinct immunosuppressive cell populations that play dominant roles in this process. MDSC are a heterogeneous population of immature myeloid cells that possess potent ability to inhibit immune responses. These MDSC also have the capacity to promote angiogenesis, cancer cell proliferation, and epithelial-mesenchymal transition, and thus enhance cancer growth, invasion, and metastasis. Controlling the expansion and accumulation of MDSC or blocking their suppressive functions represents promising novel approaches in cancer therapy. However, vital questions remain to be answered if this potential is to be fully realized. What is the predominant mechanism driving the differentiation and activation of MDSC? Which mechanisms primarily contribute to the suppressive activities of MDSC? What are the dynamics of MDSC migration into tumor...
tissues and peripheral lymphatic organs, and which factors affect their trafficking? Do different subsets of MDSC differ in their function, and does this difference depend on the cancer sub-type? Are there better specific markers that would allow investigators to identify functional MDSC and distinguish various subpopulations of MDSC (particularly in humans)? Solving these questions will advance our understanding of the critical role of MDSC in cancer and could aid the development of novel interventions for cancer treatment.

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13. References


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Myeloid Derived Suppressor Cells: Subsets, Expansion, and Role in Cancer Progression


Tumor microenvironment represents an extremely dynamic niche shaped by the interplay of different cell types (e.g. tumor cells, stromal cells), their soluble products (e.g. cytokines, chemokines and growth factors) and varied physico-chemical conditions (e.g. low oxygen concentration or hypoxia). Recent studies have identified myelomonocytic cells as key players in regulating the tumor microenvironment and hence, tumor progression in a variety of cancers. In view of these findings, the present book attempts to provide a comprehensive account of the diversity of tumor microenvironment across different cancers and how myelomonocytic cells have taken the center-stage in regulating this niche to direct cancer progression. A better understanding of the myelomonocytic cells and the mechanisms by which they regulate cancer progression will open new vistas in cancer therapeutics.

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