



Published in final edited form as:

Adv Cancer Res. 2015 ; 128: 95–139. doi:10.1016/bs.acr.2015.04.002.

Myeloid-Derived Suppressor Cells: Critical Cells Driving Immune Suppression in the Tumor Microenvironment

Katherine H. Parker¹, Daniel W. Beury¹, and Suzanne Ostrand-Rosenberg²

Department of Biological Sciences, University of Maryland Baltimore County, Baltimore, Maryland, USA

Abstract

Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of immature myeloid cells that suppress innate and adaptive immunity. MDSCs are present in many disease settings; however, in cancer, they are a major obstacle for both natural antitumor immunity and immunotherapy. Tumor and host cells in the tumor microenvironment (TME) produce a myriad of pro-inflammatory mediators that activate MDSCs and drive their accumulation and suppressive activity. MDSCs utilize a variety of mechanisms to suppress T cell activation, induce other immune-suppressive cell populations, regulate inflammation in the TME, and promote the switching of the immune system to one that tolerates and enhances tumor growth. Because MDSCs are present in most cancer patients and are potent immune-suppressive cells, MDSCs have been the focus of intense research in recent years. This review describes the history and identification of MDSCs, the role of inflammation and intracellular signaling events governing MDSC accumulation and suppressive activity, immune-suppressive mechanisms utilized by MDSCs, and recent therapeutics that target MDSCs to enhance antitumor immunity.

1. MYELOID-DERIVED SUPPRESSOR CELL HISTORY

Abnormal myelopoiesis and neutrophilia were observed in cancer patients for many years; however, the role of these pathologies was not appreciated until relatively recently, when myeloid-derived suppressor cells (MDSCs) were identified and associated with immune suppression. Studies from the early and middle 1980s in tumor-free mice identified a population of so-called natural suppressor cells that inhibited T cell proliferation and the generation of cytotoxic T lymphocytes in an antigen and MHC-independent manner (Strober, 1984). In the 1990s, studies of patients with head and neck cancer described CD34⁺-suppressive myeloid cells that had the capacity to differentiate into dendritic cells (DCs) (Garrity et al., 1997). Soon after their identification in head and neck cancer patients, similar cells were discovered in patients with various other forms of cancer. These cells prevented the *in vivo* and *in vitro* activation of T cells and were chemo-attracted to the tumor microenvironment (TME) by tumor-produced vascular endothelial growth factor (VEGF) (Almand et al., 2001; Young et al., 2001). Mice with transplanted or spontaneous tumors also produced suppressive myeloid cells (Gabrilovich, Velders, Sotomayor, & Kast, 2001;

²Corresponding author: srosenbe@umbc.edu.

¹K.H.P. and D.W.B. are dual-first authors.

Melani, Chiodoni, Forni, & Colombo, 2003), which expressed the granulocyte and macrophage markers Gr1 and CD11b/Mac1, respectively. Their accumulation correlated with tumor-produced granulocyte/ monocyte-colony-stimulating factor (GM-CSF) (Bronte et al., 1999), and they inhibited antigen-specific CD8⁺ T cell activation in a contact-dependent manner (Gabrilovich et al., 2001). Early studies used a variety of terms to identify the cells, including “immature myeloid cells (IMCs),” “immature macrophages (iMacs),” or “myeloid suppressor cells (MSCs).” In 2007, the terminology “myeloid-derived suppressor cells” (MDSCs) was adopted to reflect that the cells are the product of abnormal myelopoiesis (Gabrilovich et al., 2007).

MDSCs differentiate from a common myeloid progenitor cell that also gives rise to normal DCs, monocytes, macrophages, and granulocytes (Fig. 1). Unlike other fully differentiated myeloid cells that are relatively homogeneous, MDSCs are a heterogeneous population of cells since they represent varied stages in myelopoiesis. This heterogeneity is tumor dependent and is most likely spawned from the unique inflammatory milieu released by different tumors. These tumor-released factors, in turn, modulate the recruitment and suppressive potency of tumor-infiltrating MDSCs. The phenotype and functions of MDSCs may also vary with cancer progression since tumor cells evolve and change through immunoediting (Dunn, Bruce, Ikeda, Old, & Schreiber, 2002). Within this wide array of variation, human and mouse MDSCs have been separated into two major categories: monocytic (MO-MDSC) and granulocytic (PMN-MDSC).

1.1 Mouse MDSCs

MDSCs have been identified in the bone marrow, liver, blood, spleen, and tumor of tumor-bearing mice based on their expression of surface markers and their ability to prevent T cell activation. All murine MDSCs express the plasma membrane markers Gr1 and CD11b. The granulocyte marker Gr1 includes the isoforms Ly6C and Ly6G. The differential expression of these molecules distinguishes MO-MDSCs from PMN-MDSCs. MO-MDSCs are CD11b⁺ Ly6C⁺ Ly6G^{low/-}; PMN-MDSCs are CD11b⁺ Ly6C⁻ Ly6G⁺. MO-MDSCs are mononuclear and side scatter^{low}, while PMN-MDSCs are polymorphonuclear and side scatter^{hi}. The two subsets use different modes of suppression. PMN-MDSCs utilize reactive oxygen species (ROS) and the enzyme arginase 1 (ARG1), while MO-MDSCs use nitric oxide synthase 2 (NOS2) and ROS. These phenotypes apply to tumor-infiltrating MDSCs, as well as MDSCs residing in the spleen and blood of tumor-bearing mice. Tumor-infiltrating MDSCs are more suppressive than blood or splenic MDSCs on a per cell basis. Tumor-free mice contain cells with the same phenotype (Gr1⁺ CD11b⁺) in the blood, spleen, and bone marrow; however, they are present at much lower levels compared to tumor-bearing mice (Sinha et al., 2008, 2011).

The markers Gr1 and CD11b as well as the polymorphonuclear morphology of PMN-MDSCs are also characteristics of neutrophils, raising the question of whether MDSCs are different from neutrophils. MDSCs are not neutrophils; however, MDSCs can differentiate into neutrophils. Tumor-associated neutrophils have been categorized as N1, antitumorigenic, and as N2, protumorigenic, with their induction dependent on the presence of IFN β or TGF β , respectively (Fridlender et al., 2009; Jablonska, Leschner, Westphal,

Lienenklaus, & Weiss, 2010). N1 neutrophils are characterized as TNF α ^{hi}, CCL3^{hi}, ICAM-1^{hi}, and ARG1^{low}, while N2 neutrophils are high in CCL2, 3, 4, 8, 12, and 17 as well as in CXCL1, 2, 6, and 16 (Sionov, Fridlender, & Granot, 2014). In contrast to MDSCs, neutrophils do not express CD244 (M-CSF receptor), are more phagocytic than MDSCs, produce lower levels of ROS, have enhanced chemokine secretion, express higher levels of TNF α , and most importantly cannot suppress T cell activation (Youn, Collazo, Shalova, Biswas, & Gabrilovich, 2012).

1.2 Human MDSCs

Human MDSCs have been isolated from patients with solid tumors who display elevated MDSC levels that directly correlate with clinical cancer stage and metastatic burden. MDSCs have been found in patients with breast cancer (Alizadeh et al., 2014; Diaz-Montero et al., 2009), head and neck squamous cell carcinoma (Brandau et al., 2011), nonsmall cell lung cancer (Huang et al., 2013; Srivastava et al., 2008), colon and colorectal cancer (OuYang et al., 2015), renal cell carcinoma (Rodriguez et al., 2009), bladder cancer (Eruslanov et al., 2012), gastrointestinal cancer (Wang et al., 2013), pancreatic adenocarcinoma (Porembka et al., 2012), esophageal cancer (Gabitass, Annels, Stocken, Pandha, & Middleton, 2011), prostate cancer (Vuk-Pavlovi et al., 2010), urothelial tract cancer (Brandau et al., 2011), sarcoma, carcinoid, gall bladder, adrenocortical, thyroid, and hepatocellular carcinoma (Shen, Wang, He, Wang, & Zheng, 2014). Patients with multiple myeloma and non-Hodgkin's lymphoma also exhibit elevated levels of MDSCs in their blood (Brimnes et al., 2010; Lin et al., 2011).

Since humans lack an analog to Gr1, human MDSCs are characterized by the monocyte/macrophage marker CD11b, the monocyte differentiation antigen CD14, the mature monocyte marker CD15, the myeloid lineage markers CD33, and the absence of HLA-DR, which is commonly expressed on myeloid cells (Dumitru, Moses, Trellakis, Lang, & Brandau, 2012). Similar to murine MDSCs, human MDSCs lack lineage markers characteristic of other hematopoietic-derived cells. Human PMN-MDSCs are CD11b⁺ CD14⁻ CD15⁺ HLA-DR^{low/-} CD33⁺; MO-MDSCs are CD11b⁺ CD14⁺ CD15⁻ IL4R α ⁺ HLA-DR^{low} CD33⁺ (Montero, Diaz-Montero, Kyriakopoulos, Bronte, & Mandruzzato, 2012). Since none of the individual markers are unique to MDSCs, definitive identification of MDSCs requires demonstration of immune-suppressive function.

2. MDSC DEVELOPMENT AND SUPPRESSIVE FUNCTIONS ARE INDUCED BY INFLAMMATION

Studies evaluating patients on long-term use of nonsteroidal anti-inflammatory drugs, epidemiological analyses, and trials involving blockade of inflammatory molecules have demonstrated that inflammation contributes to the onset of cancer (Balkwill & Mantovani, 2001). Four main sources of inflammation promote carcinogenesis: environmental inflammation, therapy-induced inflammation, tumor-associated inflammation, and chronic inflammation or infection.

Particulates from tobacco smoke are an example of an environmental source of inflammation. They cause chronic obstructive pulmonary disease which is associated with increased lung cancer (Punturieri, Szabo, Croxton, Shapiro, & Dubinett, 2009). Therapy-induced inflammation occurs following radiation and chemotherapy. It causes necrotic death of cancer cells and tumor stromal cells and initiates an inflammatory response similar to wound-healing (Zong & Thompson, 2006). Therapy-induced inflammation may enhance presentation of tumor antigens; however, it may also create tumor-promoting inflammation (Zitvogel, Apetoh, Ghiringhelli, & Kroemer, 2008). Many tumors are inherently inflammatory due to their production of inflammatory mediators such as IL-6 and prostaglandins. The resulting inflammation recruits immunosuppressive cells that also release cytokines and feed the inflammatory environment. As solid tumors outpace their blood supply and become deprived of nutrients and oxygen, necrosis sets in causing the chronic release of pro-inflammatory mediators such as IL-1 and high-mobility group box 1 (HMGB1), which in turn promote neoangiogenesis (Vakkila & Lotze, 2004). Long-term infection may also cause chronic inflammation and increased cancer risk. Examples include hepatocellular carcinoma in patients infected with hepatitis B or C viruses (Karin, 2006), and bladder and colon cancer in individuals infected with *Schistosoma* or *Bacteroides*, respectively (Mostafa, Sheweita, & O'Connor, 1999; Wu et al., 2009).

Chronic inflammation promotes tumor development through various mechanisms including the production of proangiogenic factors, matrix metalloproteinases (MMPs), and damage-associated molecular pattern molecules (DAMPs), all of which drive MDSC accumulation and MDSC suppressive functions. Proangiogenic factors such as VEGF stimulate tumor neovascularization, while MMPs facilitate invasion and metastasis (Shacter & Weitzman, 2002). DAMPs such as S100A8/A9 chemoattract leukocytes and promote the expansion of MDSCs leading to an influx of inflammatory molecules within the TME (Cheng et al., 2008; Sinha et al., 2008).

The TME is a complex network that includes both tumor cells and host cells. MDSCs in this environment are therefore subjected to diverse pro-inflammatory factors. Since the TME varies between tumor types and individuals with cancer, as well as with stage of tumor progression, it is not surprising that MDSCs are a heterogeneous population that may vary from individual to individual.

Almost a decade ago, the connection between MDSCs and inflammation was established with the findings that the pro-inflammatory cytokines IL-1 β , IL-6, and PGE₂ promote MDSC accumulation and suppressive function (Bunt, Sinha, Clements, Leips, & Ostrand-Rosenberg, 2006; Bunt et al., 2007; Ezernitchi et al., 2006; Sinha, Clements, Fulton, & Ostrand-Rosenberg, 2007; Song et al., 2005). Other studies demonstrated that additional cytokines, transcription factors, and DAMPs, including, but not limited to, C5a, PGE₂, COX₂, VEGF, GM-CSF, G-CSF, IL-17, IDO, HMGB1, and S100A8/A9, C/EBP β , and chop, also drive MDSCs. The effects of these factors are discussed in the following section and are illustrated in Fig. 2.

2.1 Vascular Endothelial Growth Factor

VEGF is a pro-inflammatory growth factor that stimulates angiogenesis, and tumors producing high levels of VEGF have a poor prognosis. VEGF inhibits nuclear factor kappa-light-chain-enhancer (NF- κ B) activation which blocks DC development while simultaneously driving MDSC accumulation (Gabrilovich et al., 1998). MDSCs express the VEGF receptor enabling VEGF to function as a chemoattractant for MDSCs. ROS production by MDSCs increases oxidative stress which upregulates MDSC expression of the VEGF receptor (Kusmartsev et al., 2008). Since other factors in solid tumors also contribute to oxidative stress, the TME is a critical factor in determining the responsiveness of MDSCs to VEGF.

In addition to tumor cells, MDSCs themselves produce VEGF, thereby creating an autocrine feedback loop that sustains MDSC accumulation (Kujawski et al., 2008). VEGF has been shown to be released from the extracellular matrix by MMP9, a matrix degrading enzyme (Bergers et al., 2000). Soluble MMP9 is produced by tumor cells and promotes MDSC accumulation and tumor angiogenesis (Melani, Sangaletti, Barazzetta, Werb, & Colombo, 2007). Therefore, MDSCs have multiple modes of generating VEGF.

2.2 Granulocyte-Macrophage Colony-Stimulating Factor and Granulocyte Colony-Stimulating Factor

GM-CSF is a growth factor for leukocytes. It is required for DC differentiation and is used to expand DC *ex vivo*. However, high levels of GM-CSF induce MDSC accumulation *in vivo* and *in vitro*, while *in vivo* knockdown of GM-CSF reduces MDSC expansion (Morales, Kmiecik, Knutson, Bear, & Manjili, 2010; Serafini et al., 2004). Inclusion of GM-CSF in cultures of bone marrow progenitor cells drives the differentiation of MDSCs, demonstrating that GM-CSF is a growth factor for MDSCs (Nefedova et al., 2004).

MDSC differentiation is also positively regulated by the growth factor granulocyte colony-stimulating factor (G-CSF). G-CSF plays a critical role in mobilizing bone marrow stem cells and is essential for differentiation of granulocytic lineages (Lieschke et al., 1994). Administration of G-CSF to tumor-bearing mice drives tumor growth and angiogenesis, while blockade of G-CSF reduces MDSC levels (Okazaki et al., 2006). G-CSF also preconditions metastatic sites by mobilizing MDSCs (Kowanetz et al., 2010). When G-CSF and VEGF are both inhibited, tumor growth is reduced (Okazaki et al., 2006). While the role of G-CSF in MDSC development is clear, the impact of G-CSF on MDSC function is more complicated. In mice bearing MCA203 sarcomas, G-CSF induced Gr1^{hi}CD11b⁺ cells that were less suppressive than Gr1^{int}CD11b⁺ cells, while in MMTV-PyMT transgenic mice with mammary carcinoma, G-CSF caused CD11b⁺Ly6G⁺Ly6C⁺ cells to secrete Bv8. Bv8 is an endocrine analog of VEGF and functions as a proangiogenic protein that promotes hematopoiesis (Dolcetti et al., 2010; Kowanetz et al., 2010). Therefore, G-CSF differentially affects MDSC function depending on the type of tumor.

2.3 Prostaglandin E2 and Cyclooxygenase 2

Prostaglandin E2 (PGE₂) is a potent inflammatory mediator that is generated by cyclooxygenase 2 (COX₂) conversion of arachidonic acid. PGE₂ supports tumor growth by

promoting angiogenesis, stimulating tumor-cell proliferation, and protecting tumor cells from apoptosis. Many human and mouse tumors as well as tumor-infiltrating cells produce COX₂ and PGE₂. PGE₂ promotes MDSC differentiation at the expense of DC, while inhibition of COX₂ or PGE₂ in tumor-bearing mice blocks MDSC differentiation and delays tumor progression (Eruslanov, Daurkin, Ortiz, Vieweg, & Kusmartsev, 2010; Sinha, Clements, Fulton, et al., 2007). In the TME, PGE₂ mediates its effects through four integral membrane G-protein-coupled prostanoid receptors: EP1, EP2, EP3, and EP4. Mice deficient in EP2 display delayed tumor progression and reduced MDSC levels (Sinha, Clements, Fulton, et al., 2007). Blockade of PGE₂ or EP4 in tumor-bearing mice reduces MDSC production of ARG1 (Rodriguez et al., 2005). PGE₂ promotes the differentiation of progenitor cells in human blood to MDSCs (CD11b⁺CD33⁺ cells) from human blood progenitor cells that have elevated levels of NOS2, ARG1, IL-10, and IL-4R α (Obermajer, Muthuswamy, Lesnock, Edwards, & Kalinski, 2011). Therefore, for mouse and human MDSCs, PGE₂ not only regulates the differentiation of MDSCs, but several suppressive mechanisms as well.

2.4 CCAAT/Enhancer Binding Protein β and C/EBP Homologous Protein

C/EBP proteins are a family of leucine zipper transcription factors that regulate inflammation and myeloid cell differentiation. While there are various isoforms of C/EBP proteins, CCAAT/enhancer binding protein β (C/EBP β) acts during stress/inflammation-induced myelopoiesis. C/EBP β has three isoforms: LAP* and LAP (liver-enriched activator proteins), and LIP (liver-enriched inhibitory protein). LAP* and LIP are transcriptional activators that drive inflammatory myelopoiesis by inducing IL-6 and ARG1. In contrast, LIP inhibits LAP signaling promoting an anti-inflammatory response. In inflammatory settings such as the TME, LAP* and LAP are active and drive inflammation-induced myelopoiesis. C/EBP β is also required for the *ex vivo* generation of immunosuppressive MDSCs from bone marrow progenitor cells, via IL-6 and GM-CSF (Marigo et al., 2010).

2.5 Complement Component C5a

C5a (also known as anaphylatoxin) is a pro-inflammatory member of the complement and lectin pathway. When the complement pathway is activated, C5a in the blood becomes fixed in tissues. C5a triggers degranulation of mast cells (MCs), aids in vascular permeability, and stimulates smooth muscle contraction. In a tumor setting, C5a increases MDSC-mediated immune suppression by chemoattracting C5a receptor⁺ MDSCs to tumor vasculature and by increasing MDSC production of ROS and ARG1 (Markiewski et al., 2008).

2.6 S100A8/A9

S100A8/A9 proteins are pro-inflammatory danger signals. They are calcium binding proteins that are localized in the cytoplasm or nucleus of myeloid cells, and are released in response to cell damage, infection, or inflammation. Mice deficient in S100A9 reject transplanted tumors, while elevated expression of S100A8/A9 in solid tumors perpetuates inflammation by chemoattracting leukocytes that produce additional inflammatory molecules (Cheng et al., 2008; Sinha et al., 2008). MDSCs are one of the leukocyte populations that are chemoattracted by S100A8/A9, and chemoattraction is dependent on

signaling through receptor for advanced glycation end-products (RAGE) (Sinha et al., 2008). Heterodimeric S100A8/A9 mediates its pro-inflammatory effects by binding to the plasma membrane receptors TLR4, carboxylated N-glycans, RAGE, or heparin sulfate (Bresnick, Weber, & Zimmer, 2015). MDSCs amplify their own accumulation by secreting S100A8/A9, thus creating a self-sustained feedback loop (Sinha et al., 2008).

2.7 High-Mobility Group Box 1

HMGB1 is the second most abundant protein within a cell and is released from myeloid cells as a danger response to sepsis, infection, or arthritis. HMGB1 can signal through a number of receptors including thrombospondin, CD24, TLR2, 4, 7, and 9, as well as RAGE. HMGB1 consists of two functional domains, the A and B boxes, and an acidic tail. The A box is a RAGE antagonist and prevents HMGB1-mediated release of IL-1 β and TNF α . The B box and part of the linker before the acidic tail is a RAGE agonist with pro-inflammatory properties (Bianchi & Manfredi, 2007). The B box signals via TLR4 on macrophages which initiate the release of IL-1 β , IL-6, TNF α , MIP-2, and IL-10. The A box is anti-inflammatory as it prevents HMGB1-mediated release of IL-1 β and TNF α . Whether HMGB1 functions in a pro-inflammatory or anti-inflammatory manner is determined by its redox state. In the normal extracellular environment, the disulfide bridge between residues Cys₂₃ and Cys₄₅ maintains the A box in a dysfunctional conformation, so the B box is exclusively active. With inflammation, the microenvironment becomes oxidatively stressed and ROS is produced. ROS terminally oxidizes Cys₂₃ and Cys₄₅, thereby breaking the disulfide bridge and allowing A box to resolve the inflammation (Venereau et al., 2012).

Elevated levels of HMGB1 are associated with numerous cancers and are known to directly promote tumor growth. However, HMGB1 also drives tumor progression by modulating MDSCs. Inhibition of HMGB1 prevents the expansion of MDSCs from bone marrow progenitor cells *in vitro*, demonstrating that HMGB1 is required for the differentiation of MDSCs. *In vivo* inhibition of HMGB1 in tumor-bearing mice reduces MDSC levels in the tumor, spleen, and blood, confirming HMGB1 as a driver of MDSCs. MDSC-mediated downregulation of T cell L-selectin (CD62L) is also HMGB1 dependent, since HMGB1 increases MDSC extracellular expression of A disintegrin and metalloproteinase 17 (ADAM17), a protease that cleaves L-selectin. Secretion of the protumor cytokines IL-10 and IL-1 β by MDSCs is also increased by HMGB1 (Parker, Sinha, Horn, Clements, & Ostrand-Rosenberg, 2014), and HMGB1-driven MDSC accumulation facilitates metastasis (Li et al., 2013). Preliminary studies indicate that HMGB1 mediates its effects on MDSCs through RAGE and/or TLR4 (K.H. Parker & S. Ostrand-Rosenberg, unpublished). HMGB1 also binds to other receptors, but it is unknown if MDSCs are activated through additional receptors.

2.8 IL-1 β , IL-6, and Indoleamine 2,3-Dioxygenase

The causative relationship between inflammation, cancer, and immune suppression was first proposed following the finding that IL-1 β was a potent inducer of MDSC accumulation and suppressive activity (Ostrand-Rosenberg & Sinha, 2009). Mice bearing 4T1 tumor cells that were trans-fected to constitutively express high levels of IL-1 β exhibit increased MDSC accumulation and more suppressive MDSCs compared to mice bearing parental 4T1 tumors.

4T1 tumor-bearing mice that lack the IL-1 receptor antagonist, an inhibitor for IL-1 β , also develop elevated levels of MDSCs that are more suppressive. Similarly, mice deficient for the IL-1R display slower tumor growth and their MDSCs are less suppressive (Bunt et al., 2006, 2007; Elkabets et al., 2010; Song et al., 2005). Since IL-1 β induces the production of other mediators, including VEGF, IL-6, PGE₂, and GM-CSF, some of the effects of IL-1 β on MDSCs may be indirect. 4T1 tumor cells transfected to constitutively express IL-6 induce elevated levels of MDSCs and restore MDSC levels in tumor-bearing IL-1 receptor knockout mice, indicating that IL-6 effects on MDSCs are either downstream of IL-1 β , or have an overlapping mechanism of action with IL-1 β (Bunt et al., 2007). Since MDSCs produce IL-6 and IL-1 β , these studies also raise the question of whether MDSC production of IL-6 is regulated by IL-1 β , and if MDSC production of IL-1 β enhances MDSC production of IL-6. Indole amine 2,3 dioxygenase (IDO), which is utilized by MDSCs as an immune-suppressive mechanism, also regulates IL-6, and tumor-bearing IDO1-deficient mice have less suppressive MDSCs, reduced levels of IL-6, and delayed primary tumor growth and metastatic disease (Smith et al., 2012). Provision of IL-6 to tumor-bearing indoleamine 2,3-dioxygenase (IDO) knockout mice restores MDSC levels and suppressive potency (Smith et al., 2012).

2.9 IL-17

IL-17 is a pro-inflammatory cytokine secreted by CD4 Th17 and CD8 Tc17 cells. Tumor growth is suppressed and MDSC levels are decreased in IL-17-deficient mice, while administration of IL-17 raises MDSC levels (He et al., 2010; Wang et al., 2009). Patients with gastrointestinal cancers show a strong positive correlation between serum IL-17 and MDSC levels, further supporting a role for IL-17 as an inducer of MDSCs (Yazawa et al., 2013). The effects of IL-17 may be either direct or indirect. Most cells have IL-17 receptors so MDSCs may be directly impacted. However, IL-17 triggers the production of IL-6 which in turn activates STAT3, so many effects on MDSCs may be directly mediated by IL-6 and indirectly by IL-17 (Chatterjee et al., 2013; Wang et al., 2009).

3. MDSC ARE REGULATED BY MULTIPLE MOLECULAR MECHANISMS

Multiple signal transduction pathways, transcription factors, and micro-RNAs (miRNAs) regulate MDSC accumulation and function (Fig. 3).

3.1 Signal Transducer and Activator of Transcription 1

MDSC function is positively regulated by STAT1. STAT1 is activated by IFN γ or IL-1 β and regulates the induction of NOS2 and ARG1 (Kusmartsev & Gabrilovich, 2005). MDSC accumulation is also dependent on STAT1 as tumor-bearing mice deficient in STAT1 exhibit reduced MDSC levels (Hix et al., 2013). Whether IFN γ is the ligand that activates MO-MDSCs is unclear. Early studies indicated that IFN γ was essential for the development of MO-MDSCs (Movahedi et al., 2008); however, subsequent experiments demonstrated that MDSC function, accumulation, and phenotype are independent of IFN γ as tumor-bearing IFN $\gamma^{+/+}$, IFN $\gamma^{-/-}$, IFN $\gamma R^{+/+}$, and IFN $\gamma R^{-/-}$ mice with equal-sized tumors contained equal numbers of equivalently suppressive MDSCs (Sinha, Parker, Horn, & Ostrand-Rosenberg, 2012).

3.2 Signal Transducer and Activator of Transcription 3 and 6

MDSC accumulation and function are enhanced by activation of both STAT3 and STAT6. Activation of STAT6 occurs from the binding of IL-4 or IL-13 to IL-4R α resulting in the upregulation of ARG1 and TGF β (Bronte et al., 2003; Sinha, Clements, & Ostrand-Rosenberg, 2005a; Terabe et al., 2003). In STAT6-deficient mice, signaling through IL-4R α does not occur, MDSCs are less suppressive and accumulate more slowly, and spontaneous metastatic disease is delayed (Sinha, Clements, & Ostrand-Rosenberg, 2005b). STAT3 activation increases the half-life and proliferation of both human and mouse MDSCs by driving the expression of the antiapoptotic genes Bcl-xL, c-myc, and the proliferation gene cyclin D1 (Nefedova et al., 2005; Xin et al., 2009). STAT3 also increases the differentiation of MDSCs by inducing the pro-inflammatory mediators S100A8/A9 (Cheng et al., 2008) and by downregulating the transcription factor PKC β II in hematopoietic progenitor cells (Farren, Carlson, & Lee, 2010). Since MDSCs and DCs are derived from a common progenitor cell, the increase in MDSC differentiation is accompanied by a decrease in DC expansion. In addition to regulating MDSC expansion, STAT3 also enhances MDSC suppressive activity (Kujawski et al., 2008). Tumor-derived exosomes containing heat-shock protein 72 on their membranes induce MDSC production of IL-6 which subsequently activates STAT3 and increases MDSC-mediated T cell suppression (Chalmin et al., 2010).

C/EBP β is another transcription factor activated by STAT3. Activated C/EBP β binds to the c-myc promoter and induces c-myc expression which stimulates cell proliferation. C/EBP β is a key molecule for induction of MDSCs since multiple factors (GM-CSF, G-CSF, and IL-6) activate MDSCs via C/EBP β , (Marigo et al., 2010). C/EBP β regulation of MDSCs is associated with chop. ROS produced by tumors upregulates MDSC expression of chop (Thevenot et al., 2014). Chop expression in MDSCs activates C/EBP β and induces STAT3 signaling. MDSCs from chop-deficient mice have decreased ability to inhibit T cell proliferation and accumulate to lower levels. This reduced accumulation and decreased potency of MDSCs is attributed to lower levels of IL-6 and reduced phosphorylation of STAT3. Overexpression of IL-6 in chop-deficient mice rescues MDSC suppressive activity (Thevenot et al., 2014).

MDSC production of ROS is also regulated by STAT3. ROS are generated intracellularly by the NAD(P)H oxidase enzyme complex (NOX), which consists of membrane-bound gp91 and p22, and cytosolic p40, p47, and p67. This complex catalyzes the production of superoxide through the reduction of oxygen, with NAD(P)H serving as the one electron donor. Activation of STAT3 increases ROS levels through upregulation of p47 and gp91 (Corzo et al., 2009); however, it is not known which of the several activators of STAT3 upregulate p47 and gp91. Solid tumors contain oxidatively stressed hypoxic regions, and cells within these regions contain activated hypoxia-inducible factor-1 alpha (HIF-1 α). Activated HIF-1 α induces STAT3 signaling. Therefore, STAT3 induction of ROS may be regulated by HIF-1 α .

3.3 Nuclear Factor Kappa-Light-Chain-Enhancer

Activation of NF- κ B also promotes MDSC accumulation and function and occurs following ligation of MyD88-dependent TLRs. Exposure to a variety of pro-inflammatory mediators

including S100A8/A9, HMGB1, and IL-1 β activates the NF- κ B pathway in MDSCs (Parker et al., 2014; Sinha et al., 2008; Tu et al., 2008).

3.4 Interferon Regulatory Factor-8

Interferon regulatory factor 8 (IRF-8) is a transcription factor that is essential for the normal development of granulocyte/monocyte lineage cells. IRF-8-deficient mice have myeloproliferative disorders and accumulate high levels of MDSCs. Expression of IRF-8 is downregulated by G-CSF and GM-CSF, so treating mice with these cytokines blocks IRF-8 activation and drives the accumulation of MDSCs (Stewart, Liewehr, Steinberg, Greenelch, & Abrams, 2009; Waight et al., 2013). IRF-8 may also negatively regulate MDSC survival as IRF-8 downregulates antiapoptotic genes Bcl-2 and Bcl-xL and upregulates the proapoptotic gene caspase-3 (Burchert et al., 2004; Gabriele et al., 1999). Inhibition of Bcl-2 and Bcl-xL enhances MDSC susceptibility to Fas-mediated apoptosis (Hu et al., 2013).

3.5 Notch

Another transcription factor implicated in the development of MDSCs from hematopoietic progenitor cells is Notch. Notch signaling permits the differentiation of MDSCs into DCs. Inhibition of notch signaling by casein kinase 2 (CK2) drives abnormal myeloid cell differentiation (Cheng et al., 2014).

3.6 Hypoxia-Inducible Factor-1 Alpha

The HIF complex consists of the subunits HIF-1 α and HIF-1 β , both of which are constitutively expressed. Hypoxia stabilizes HIF-1 α and allows it to translocate from the cytoplasm into the nucleus where it dimerizes with HIF-1 β . The HIF complex upregulates multiple target genes (e.g., VEGF, NOS2, and MMPs) by associating with their hypoxia response elements. HIF-1 α is overexpressed in various cancers, where it increases MDSC expression of ARG1 and NOS2, rendering MDSCs more immune suppressive and facilitating their conversion to tumor-associated macrophages (TAMs) (Corzo et al., 2010). The capacity of HIF-1 α to modulate the function of MDSCs highlights the plasticity of MDSCs and further demonstrates that MDSC function is governed by their environment.

3.7 MicroRNAs

miRNAs are noncoding single-stranded RNAs approximately 22 nucleotides long that regulate gene expression. miRNAs in the RNA-induced silencing complex bind to complementary target mRNAs causing target mRNA degradation. The generation of miRNAs is regulated by cell- and tissue-specific transcription factors as well as proteins involved in the processing of miRNA, both of which can be influenced by chronic inflammation (El Gazzar & McCall, 2012).

miRNAs enhance and inhibit MDSC accumulation and suppressive potency. For example, miRNAs 146a and 223 prevent MDSC accumulation (Boldin et al., 2011; Liu et al., 2011). miRNA-146a blocks inflammation, while miRNA-223 is needed for the development of granulocytes. In contrast, miRNAs 494, 155, and 21 facilitate the accumulation of MDSCs. miRNA-494 induces MMPs 2, 13, and 14 which drive MDSC growth and survival signals, and by inhibiting phosphatase and tensin homolog (PTEN) which promotes STAT3

activation (Liu et al., 2012). miRNAs 155 and 21 promote MDSC accumulation by activating STAT3, which, as previously discussed, drives both MDSC accumulation and suppressive potency (Li et al., 2014). miRNAs also negatively regulate MDSC suppressive function. These include miRNAs 17-5 and 20a which silence STAT3, thereby reducing MDSC production of ROS and hydrogen peroxide (H₂O₂) (Zhang et al., 2011).

MDSCs also use miRNAs to modulate cancer cell growth. An example is the MDSC-mediated activation of miRNA-101 in ovarian cancer cells (Cui et al., 2013). miRNA-101 increases cancer cell stemness as well as metastatic and tumorigenic potential (Cui et al., 2013).

3.8 MDSC Turnover

MDSC turnover *in vitro* and *in vivo* varies with tumor type, with half-life ranging for only a few days. T cells may contribute to this rapid turnover since when activated, T cells express FasL and cause apoptosis of Fas⁺ MDSCs. Inflammation counteracts the T cell effect by increasing MDSC resistance to Fas-mediated lysis (Chornoguz et al., 2011; Sinha et al., 2011).

4. MDSCs UTILIZE A NETWORK OF EFFECTOR AND SIGNALING MOLECULES TO MODULATE THE INFLAMMATORY MILIEU AND DECREASE IMMUNE SURVEILLANCE

MDSCs utilize multiple suppressive mechanisms to induce a tolerogenic, tumor-promoting environment. MDSCs directly suppress T cells by starving them of amino acids, inducing apoptosis, reducing homing to lymph nodes, or inhibiting their intracellular signaling pathways required for activation. MDSCs also indirectly suppress T cells by altering the ability of antigen-presenting cells (APCs) to activate T cells and by inducing immunosuppressive T regulatory cells (Tregs). In addition, MDSCs impact other cells involved in an antitumor response because they alter the inflammatory milieu in the TME by cross talk with macrophages, tumor cells, and MCs. These mechanisms are described below and are illustrated in Fig. 4.

4.1 MDSC Depletion of Amino Acids

Following initial contact with antigen, T cells undergo metabolic changes that are essential for their activation and clonal expansion. MDSCs limit several amino acids necessary for these processes and thereby inhibit T cell activation. An early event of amino-acid starvation is the accumulation of empty aminoacyl tRNAs, which activate serine–threonine kinase GCN2. GCN2 phosphorylates eIF2 α , which binds eIF2B and suppresses the translation initiation complex from binding charged aminoacyl tRNA, thereby causing a global decrease in protein translation. Simultaneously, GCN2 enhances the translation of GCN4, which results in the transcription of genes required for the synthesis of amino acids (Wang & Green, 2012). MDSCs deplete the local environment of L-arginine (L-Arg), L-tryptophan (L-Trp), and L-cysteine (L-Cys) through different mechanisms.

One of the first suppressive mechanisms attributed to MDSCs was the inhibition of T cell activation and proliferation by the depletion of L-Arg. L-Arg is a nonessential amino acid and is a substrate for several enzymes: (i) NOS 1, 2, and 3 which metabolize L-Arg into L-citrulline and nitric oxide (NO); (ii) ARG 1 and 2 which convert L-Arg to L-ornithine and urea; (iii) arginine:glycine amidinotransferase which transfers the amidino group from L-Arg to L-glycine, yielding L-ornithine and glycoylamine; and (iv) arginine decarboxylase, which catalyzes the reaction of L-Arg to agmatine and CO₂ (Bronte & Zanovello, 2005).

In the absence of L-Arg, T cells decrease their expression of CD3 ζ , which is required for signal transduction through the antigen-specific T cell receptor (TCR) (Rodriguez et al., 2002; Zea et al., 2004). L-Arg-depleted T cells are arrested in G₀–G₁ due to the failure to upregulate cyclin D3 and cyclin-dependent kinase 4 (cdk4). Cyclin D3 and cdk4 are not upregulated due to decreased mRNA stability and lower translation rates (Rodriguez et al., 2010). Despite their inability to proliferate, L-Arg-starved T cells express early activation markers and secrete IL-2, indicating that the early events of T cell activation are not L-Arg dependent (Fletcher et al., 2015). *In vivo* studies confirmed the critical role of MDSCs in L-Arg depletion since renal cell carcinoma patients and mice with chronic inflammation have elevated levels of MDSCs and low levels of serum L-Arg, which is correlated with decreased T cell activation (Ezernitchi et al., 2006; Zea et al., 2005). Depletion of L-Arg is mediated by ARG1, and MDSC synthesis of ARG1 is regulated by PGE₂ (Rodriguez et al., 2005). Tumor-derived MDSCs deplete their local environment of L-Arg by internalizing L-Arg through the cationic amino-acid transporter 2B (Rodriguez et al., 2004) and by secreting ARG1 (Rodriguez et al., 2009).

L-Trp metabolism by MDSCs also facilitates T cell suppression. MDSCs express IDO, which degrades the essential amino-acid L-Trp into *N*-formylkynurenine. IDO causes T cell suppression by enhancing GCN2 kinase in a similar manner as L-Arg starvation (Munn et al., 2005). Expression of IDO in MDSCs is regulated by STAT3 (Yu et al., 2013). However, not all MDSCs express IDO (Smith et al., 2012), indicating that IDO is not a universal mechanism utilized by MDSCs to suppress T cell activation.

MDSCs also prevent T cell activation by sequestering L-Cys. In the extracellular oxidizing environment, L-Cys exists as the dipeptide cystine (L-Cys₂). Naïve T cells must acquire L-Cys from APCs because they lack the cystine transporter x_c⁻ and therefore cannot import L-Cys₂, and cannot *de novo* synthesize L-Cys because they lack cystathionase, the enzyme that converts methionine to L-Cys. MDSCs also lack cystathionase and therefore must scavenge L-Cys₂. Since MDSCs do not export L-Cys due to their lack of the neutral amino-acid alanine-serine-cysteine transporter 1 (ASC), high levels of MDSCs quickly deplete their local environment of L-Cys₂, thereby limiting the ability of APCs to provide T cells with L-Cys. The role of MDSCs and their biological relevance in L-Cys depletion is supported by the correlation between high levels of MDSCs and reduced serum L-Cys₂ in tumor-bearing mice (Srivastava, Sinha, Clements, Rodriguez, & Ostrand-Rosenberg, 2010). Since activated T cells express x_c⁻, theoretically they should be resistant to this suppressive mechanism (Levring et al., 2012). However, since ARG1 production by MDSCs suppresses T cell activation, it is unclear if T cell upregulation of x_c⁻ is functionally relevant.

4.2 MDSC Production of NO

NOS also catabolizes L-Arg and contributes to MDSC-mediated immune suppression (Bronte & Zanovello, 2005; Raber et al., 2014). MDSCs produce NO by the action of NOS2 and NOS3. PMN-MDSCs are NOS2^{low} NOS3^{hi}, while MO-MDSCs are NOS2^{hi} NOS3^{low} (Raber et al., 2014). NOS2 generates more NO than NOS3 and is induced by pro-inflammatory cytokines, endotoxin, hypoxia, and oxidative stress, while NOS3 is constitutively expressed (Fukumura, Kashiwagi, & Jain, 2006).

NO is labile and reacts with multiple compounds to produce many toxic and regulatory factors. For example, NO reacts with (i) cysteine thiol groups on proteins and peptides, which form S-nitrosothiols, thereby altering a protein's tertiary structure; (ii) superoxide anions (O₂⁻), which form peroxynitrite (PNT, ONOO⁻), a molecule that alters protein structure; (iii) divalent cations (e.g., Fe²⁺ and Zn²⁺), which regulate the function of various transcription factors and enzymes; (iv) nucleic acids, which cause mutagenesis; and (v) unsaturated lipids, which lead to the formation of nitrolipids that can have pro- or anti-inflammatory activity (Bogdan, 2015). Since NO influences many biological processes, it is not surprising that NO is capable of pro- and antitumor activity. NO can induce tumor-cell apoptosis and inhibit metastasis, or enhance tumor-cell invasion, proliferation, and angiogenesis (Fukumura et al., 2006). However, MDSC-produced NO negatively impacts T cells. NO inhibits JAK3, STAT5, ERK, and AKT, which prevents IL-2 signaling, thereby impairing the generation of effector and memory T cells (Mazzoni et al., 2002). NO directly inhibits these signaling proteins by S-nitrosothiolation, or indirectly by activating guanylate cyclase and cyclic-GMP-dependent kinases (Serafini, 2013). S-nitrosothiolation of ARG1 enhances ARG1 affinity for L-Arg which subsequently increases ARG1 activity, thereby establishing a synergistic relationship between ARG1- and NO-mediated immune suppression (Santhanam et al., 2007).

4.3 MDSC Production of ROS

NOX is a membrane-bound enzyme complex that is utilized by MDSCs to suppress T cell activation. MDSCs from tumor-bearing mice have enhanced expression of the NOX subunits gp91, p22, and p47 and produce more ROS than MDSCs from tumor-free mice (Corzo et al., 2009). NOX generates superoxide which spontaneously reacts with many molecules to produce a variety of ROS including H₂O₂, hydroxyl radical, and hypochlorous acid. These ROS damage proteins, lipids, and nucleic acids, thereby enhancing inflammation and promoting apoptosis. For example, H₂O₂ production in cancer patients reduces T cell production of cytokines and expression of CD3ζ (Schmielau & Finn, 2001). Superoxide also reacts with NO to form PNT, which is produced by PMN-MDSCs through the action of gp91 and NOS3 (Raber et al., 2014). PNT nitrates/nitrosylates the TCR and MHC (Lu & Gabrilovich, 2012), thereby disrupting TCR-MHC I/peptide binding and rendering tumor cells resistant to CTL-mediated apoptosis (Lu et al., 2011). Due to the short half-life of PNT, these reactions are limited to short distances and require close cell-to-cell contact. PNT also reacts with the chemoattractant CCL2, thereby inhibiting T cell infiltration into tumors (Molon et al., 2011).

4.4 MDSCs Inhibit T Cell Migration by Downregulating L- and E-Selectins

Activation of tumor-reactive T cells requires entry of naïve T cells into tumor-draining lymph nodes or migration to the TME. L-selectin mediates the first step in extravasation by facilitating T cell adhesion to high endothelial venules (HEVs). Naïve T cells with low expression of L-selectin do not adhere efficiently to HEVs and fail to enter lymph nodes (J. Mihich, S. Evans, S. Abrams, & S. Ostrand-Rosenberg, unpublished data). In tumor-bearing mice, MDSCs prevent T cell entry into lymph nodes by downregulating L-selection through their extracellular expression of ADAM17, the enzyme that cleaves L-selectin on naïve T cells (Hanson, Clements, Sinha, Ilkovitch, & Ostrand-Rosenberg, 2009; Parker et al., 2014).

In squamous cell carcinoma patients, MDSCs also prevent the homing of T cells to tumor sites by downregulating E-selectin on tumor vessels. In order for T cells to adhere to tumor vessels and subsequently enter the tumor mass, they must first bind to E-selectin. However, NO produced by MDSCs decreases E-selectin levels, thereby limiting T cell access to tumor (Gehad et al., 2012).

4.5 MDSCs Express Programmed Death-Ligand 1

Tumor cells escape antitumor immunity through their expression of programmed death-ligand 1 (PD-L1). When PD-L1 binds to its receptor PD-1 on T cells, it induces T cell exhaustion/apoptosis. MDSCs from some tumor-bearing mice and patients express PD-L1 (Youn, Nagaraj, Collazo, & Gabrilovich, 2008; Zhang, Wang, et al., 2013). Some tumor-infiltrating MDSCs have elevated expression of PD-L1 due to hypoxia-induced upregulation of HIF-1 α (Noman et al., 2014). However, MDSCs do not universally express PD-L1, and PD-L1 blockade does not always decrease MDSC suppressive activity (Youn et al., 2008).

4.6 MDSCs Induce Tregs and Th17 Cells

Tregs play an important role in the control of immune reactivity against self- and non-self-antigens, and in some animal models, they protect tumors from antitumor immunity. Tregs are characterized as CD4⁺ FoxP3⁺ cells. MDSCs induce/expand Tregs *in vitro* and *in vivo* in multiple tumor models (Adeegbe et al., 2011; Huang et al., 2006; MacDonald et al., 2005; Zoso et al., 2014). MDSCs induce Tregs by secreting IL-10 and TGF β (Hoechst et al., 2008; Huang et al., 2006) and activate Tregs by presenting tumor-specific antigens in an ARG-dependent and TGF β -independent manner (Serafini, Mgebroff, Noonan, & Borrello, 2008). MDSC expression of CD40 is required for MDSC-mediated Treg induction, since CD40-deficient MDSCs do not drive Treg expansion (Pan et al., 2010). Given the link between MDSCs and Tregs, therapies targeting MDSCs may also reduce Treg populations.

Th17 cells are a pro-inflammatory CD4⁺ T cell subset (CD4⁺ ROR γ t⁺ IL-17⁺). Since they have both pro- and antitumor effects, their role in antitumor immunity is controversial (Ye, Livergood, & Peng, 2013). MDSCs induce Th17 cells by producing IL-6 and TGF β (Chatterjee et al., 2013). IFN γ - or TNF α -activated MDSCs also recruit Th17 cells through their production of CCL4, which is a Th17 chemoattractant (Ortiz et al., 2015). As previously mentioned, IL-17 drives the accumulation of MDSCs. Therefore, MDSCs and Th17 cells may induce each other.

4.7 MDSCs Impair NK Cell-Mediated Cytotoxicity

MDSCs impair NK function via contact-dependent mechanisms. MDSCs produce TGF β and H₂O₂ which decrease NK cell expression of the activating receptors NKG2D, NKp46, and NKp44, thereby making NK cells more difficult to activate (Elkabets et al., 2010; Mao et al., 2014). MDSCs also decrease the ability of NK cells to induce apoptosis by downregulating NK cell production of perforin which is essential for NK-mediated target cell lysis. In addition, MDSCs suppress NK cells by limiting their response to IL-2, a growth factor that enhances NK cell proliferation and cytolytic activity (Liu et al., 2007).

4.8 Cross Talk Between MDSCs, Macrophages, Tumor Cells, and MCs Enhances Inflammation and Promotes MDSC Suppressive Activity

Solid tumors are a complex and frequently inflamed microenvironment. Both tumor and host (macrophages, DCs, MCs, MDSCs, and fibroblasts) cells within solid tumors participate in cross talk that regulates the release of pro- and anti-inflammatory cytokines and drive the accumulation and suppressive function of immune-suppressive cells such as Tregs, TAMs, and MDSCs.

Macrophages can be either tumoricidal (M1-like) or tumor-promoting (M2-like) (Sica & Mantovani, 2012). MDSCs subvert macrophages toward an M2 phenotype through their production of IL-10 which downregulates macrophage production of IL-12 and TNF α , while simultaneously enhancing macrophage production of NO (Beury et al., 2014; Sinha, Clements, Bunt, Albelda, & Ostrand-Rosenberg, 2007). IL-12 downregulation is mediated by both intact MDSCs and MDSC-derived exosomes (Burke, Choksawangkar, Edwards, Ostrand-Rosenberg, & Fenselau, 2014). MDSC production of IL-10 involves TLR4 signaling and is increased by inflammation and direct cell-to-cell contact with macrophages (Bunt, Clements, Hanson, Sinha, & Ostrand-Rosenberg, 2009; Sinha, Clements, Bunt, et al., 2007), and via the adenosine A2A receptor (Cekic, Day, Sag, & Linden, 2014). MDSCs also decrease macrophage expression of MHC II through both IL-10-dependent and -independent mechanisms (P. Sinha, D. Beury, V. Clements, & S. Ostrand-Rosenberg, unpublished) and upregulate PD-L1 on macrophages in the liver (Ilkovitch & Lopez, 2009).

MDSCs and tumor cells also participate in cross talk. Tumor cells increase MDSC production of IL-6, and in turn, MDSCs enhance tumor-cell production of IL-6. IL-6 also increases MDSC suppressive activity, but inhibits MDSC production of IL-10 (Beury et al., 2014). In addition, tumor cells enhance MDSC production of IL-28, which facilitates tumor-cell invasion, migration, and angiogenesis (Mucha, Majchrzak, Taciak, Hellmen, & Krol, 2014).

MCs and MDSCs also interact. MDSC and MC cross talk drives inflammation by increasing production of TNF α , CCL3, IL-4, IL-13, IL-6, and CCL2 (Danelli et al., 2015; Martin et al., 2014; Saleem et al., 2012). The latter two molecules are regulated by ligation of MC CD40L to CD40 on MDSCs. Activated MCs release histamine which signals through histamine receptors 1, 2, and 3 on MDSCs and enhances MDSC expression of IL-4 and IL-13. Histamine upregulates ARG1 and NOS2 in MO-MDSCs and decreases ARG1 and NOS2 in PMN-MDSCs (Martin et al., 2014). Since histamine increases MO-MDSC production of

NO and down-regulates immune-suppressive mediators of PMN-MDSC, the net effect of histamine is to increase MO-MDSC suppressive activity (Danelli et al., 2015).

5. MDSCs IN NONCANCER SETTINGS

MDSCs are also elevated in noncancer settings, where they can be either detrimental or beneficial. For example, elevated levels of MDSC decrease immune responsiveness in patients with toxoplasmosis (Voisin, Buzoni-Gatel, Bout, & Velge-Roussel, 2004) and trypanosomiasis (Goñi, Alcaide, & Fresno, 2002). MDSCs are also elevated in mice with antigen-induced autoimmune enterocolitis, where adoptive transfer of additional MDSCs reduces disease symptoms, suggesting a protective role for MDSCs (Haile et al., 2008). Likewise, mice with experimental autoimmune encephalomyelitis have elevated levels of immune-suppressive MDSCs in their spleens and blood, which are likely to be beneficial in limiting autoreactivity (Zhu et al., 2007). Elevated levels of MDSCs are also found in the serum of patients with sepsis where they polarize immunity toward an antibody-promoting Type 2 response (Delano et al., 2007). Whether the MDSCs are beneficial or detrimental in sepsis is unclear.

Both stress and aging are also associated with increased MDSC levels. For example, postsurgery traumatic stress in mice is accompanied by increased levels of splenic MDSCs that suppress T cell proliferation by an ARG1-dependent mechanism (Makarenkova, Bansal, Matta, Perez, & Ochoa, 2006). Psychological stress in breast cancer patients further elevates circulating MDSCs (Mundy-Bosse, Thornton, Yang, Andersen, & Carson, 2011). MDSCs also increase with aging as shown in a study of adults ages 19–59, 61–76 (seniors), and 67–99 (elderly). The elderly cohort had the highest levels of MDSCs as well as increased serum levels of IL-6 and IL-1 β (Verschoor et al., 2013). Studies in aging mice similarly show increases in MDSCs (Grizzle et al., 2007; Hanson et al., 2009).

MDSCs have also been implicated in driving asthma, an allergy caused by a hyper Th2 response that disrupts the normal Th1/Th2 balance. Children with asthma have elevated serum levels of MDSCs and IL-10, and reduced levels of IL-12 (Zhang, Luan, et al., 2013). Since MDSCs produce IL-10 which decreases macrophage production of IL-12 (Sinha, Clements, Bunt, et al., 2007), MDSCs are likely increasing the severity of disease by exacerbating polarization toward a type 2 response. In contrast, in a mouse asthma model, MDSCs appear to reduce disease because injection of tumor-derived MDSCs restored the Th1/Th2 balance by reducing the type 2 cytokine IL-4 and increasing the type 1 cytokine IFN- γ (Song et al., 2014).

MDSCs may play a beneficial role in obesity, which is considered a chronic low-grade inflammatory disease. Obese individuals have elevated levels of MDSCs in their peripheral tissues. These MDSCs counterbalance some of the detrimental effects of obesity by promoting insulin sensitivity and reducing inflammation. This latter effect occurs because MDSCs in adipose tissue skew macrophages toward an anti-inflammatory M2 phenotype (Xia et al., 2011), possibly by their production of IL-10. However, MDSCs may be detrimental in obese individuals undergoing vaccination, since mice with diet-induced

obesity and elevated levels of MDSC displayed decreased antigen-specific T cell responses following vaccination (Chen et al., 2015).

Because of their immunosuppressive potency, MDSCs have been tested as therapeutic agents for autoimmune diseases or when tolerance is required. For example, adoptive transfer of bone marrow-generated MDSCs has been used to combat graft-versus-host disease (Highfill et al., 2010), ameliorate experimental autoimmune encephalomyelitis (Ioannou et al., 2012), aid in the retention of allogeneic islet grafts (Chou et al., 2012), and induce Tregs to protect against type1 diabetes (Yin et al., 2010). MDSCs may also be needed in mice to maintain maternal–fetal tolerance during the development of allogeneic fetuses (P. Sinha & S. Ostrand-Rosenberg, unpublished). The presence of elevated levels of immune-suppressive MDSCs in both mice and women pregnant with allogeneic embryos further supports the concept that MDSCs contribute to maternal–fetal tolerance and suggests that reduced levels of MDSCs in pregnant women could lead to miscarriage (Köstlin et al., 2014).

6. THERAPEUTIC TARGETING OF MDSCs

Because of their central role in immune suppression, many investigators have focused on neutralizing MDSCs in individuals with cancer. Strategies include targeting MDSC suppressive mechanisms, inducing MDSCs to differentiate into nonsuppressive mature APCs, blocking development of MDSCs, and killing of MDSCs. Table 1 lists recent approaches. Older therapies are reviewed in Wesolowski, Markowitz, and Carson (2013). It should be noted that none of these approaches universally neutralize MDSCs.

7. CONCLUSIONS

MDSCs encompass a range of immature immune-suppressive myeloid cells. Their suppressive activity and accumulation are induced by many inflammatory mediators with unique and redundant signaling pathways. MDSCs inhibit antitumor immunity through several mechanisms including (i) depletion of the local environment of the amino acids L -Arg, L -Trp, and L -Cys, which inhibits T cell activation and/or proliferation; (ii) secretion of NO, PNT, and ROS, which causes T cell apoptosis, inhibits peptide recognition by T cells, and inhibits T cell activation; (iii) induction of immunosuppressive Tregs; and (iv) impairment of T cell trafficking to lymph nodes. MDSCs also alter the inflammatory milieu by inducing inflammatory Th17 cells, participating in cross talk with macrophages, tumor cells, and MCs which promotes a protumor environment that enhances tumor-cell growth, invasion, and metastasis. Therefore, neutralizing MDSCs is an obvious strategy to enhance natural antitumor immunity and boost the efficacy of immunotherapies.

The concept of activating a patient's immune system to destroy their endogenous cancer cells has been a goal of immunotherapy for many years. Unfortunately, many cancer immunotherapy clinical trials have failed to show therapeutic efficacy. MDSCs may be responsible for at least some of these failures since they are present in many cancer patients, and have the ability to prevent T cell activation. Analysis of blood samples from non-responder patients indicated a correlation between lack of response and MDSC levels

(Kimura et al., 2013). Regardless of the outcome of such studies, it is likely that cancer immunotherapies involving *in vivo* activation or proliferation of tumor-reactive T cells will require adjunctive treatment that neutralizes MDSCs.

Accumulation and suppressive potency of MDSCs are regulated by a complex milieu of inflammatory mediators. Environmental conditions such as hypoxia and inflammation act through similar signaling networks. These networks converge on common transcription factors such as STAT3 and NF- κ B and regulate additional transcription factors, miRNAs, and proteins that mediate MDSC accumulation and suppression. Since multiple ligands initiate signaling through these pathways, MDSC regulation is highly redundant. This redundancy allows for the development of MDSCs under a broad range of conditions and may explain why MDSCs are so widespread in cancer patients. The redundancy also complicates therapeutic approaches for neutralizing MDSCs, since different inducers compensate for each other.

The past decade has seen remarkable progress in recognizing MDSCs as key players that inhibit antitumor immunity and facilitate tumor progression. Advances in understanding the mechanisms that drive MDSC accumulation and function have also been extensive. Hopefully, these studies will lead to the development of therapeutic strategies that are universally effective in neutralizing or eliminating MDSCs in cancer patients.

REFERENCES

- Adeegbe D, Serafini P, Bronte V, Zoso A, Ricordi C, Inverardi L. In vivo induction of myeloid suppressor cells and CD4(+)Foxp3(+) T regulatory cells prolongs skin allograft survival in mice. *Cell Transplantation*. 2011; 20:941–954. [PubMed: 21054938]
- Alizadeh D, Trad M, Hanke NT, Larmonier CB, Janikashvili N, Bonnotte B, et al. Doxorubicin eliminates myeloid-derived suppressor cells and enhances the efficacy of adoptive T-cell transfer in breast cancer. *Cancer Research*. 2014; 74:104–118. [PubMed: 24197130]
- Almand B, Clark JI, 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. *Journal of Immunology*. 2001; 166:678–689.
- Annels NE, Shaw VE, Gabitass RF, Billingham L, Corrie P, Eatock M, et al. The effects of gemcitabine and capecitabine combination chemotherapy and of low-dose adjuvant GM-CSF on the levels of myeloid-derived suppressor cells in patients with advanced pancreatic cancer. *Cancer Immunology, Immunotherapy*. 2014; 63:175–183. [PubMed: 24292263]
- Balkwill F, Mantovani A. Inflammation and cancer: Back to Virchow? *Lancet*. 2001; 357:539–545. [PubMed: 11229684]
- Bergers G, Brekken R, McMahon G, Vu TH, Itoh T, Tamaki K, et al. Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. *Nature Cell Biology*. 2000; 2:737–744. [PubMed: 11025665]
- Beury DW, Parker KH, Nyandjo M, Sinha P, Carter KA, Ostrand-Rosenberg S. Cross-talk among myeloid-derived suppressor cells, macrophages, and tumor cells impacts the inflammatory milieu of solid tumors. *Journal of Leukocyte Biology*. 2014; 96:1109–1118. [PubMed: 25170116]
- Bianchi ME, Manfredi AA. High-mobility group box 1 (HMGB1) protein at the crossroads between innate and adaptive immunity. *Immunological Reviews*. 2007; 220:35–46. [PubMed: 17979838]
- Bogdan C. Nitric oxide synthase in innate and adaptive immunity: An update. *Trends in Immunology*. 2015; 36:161–178. [PubMed: 25687683]
- Boldin MP, Taganov KD, Rao DS, Yang L, Zhao JL, Kalwani M, et al. miR-146a is a significant brake on autoimmunity, myeloproliferation, and cancer in mice. *The Journal of Experimental Medicine*. 2011; 208:1189–1201. [PubMed: 21555486]

- Brandau S, Trellakis S, Bruderek K, Schmaltz D, Steller G, Elian M, et al. Myeloid-derived suppressor cells in the peripheral blood of cancer patients contain a subset of immature neutrophils with impaired migratory properties. *Journal of Leukocyte Biology*. 2011; 89:311–317. [PubMed: 21106641]
- Bresnick AR, Weber DJ, Zimmer DB. S100 proteins in cancer. *Nature Reviews. Cancer*. 2015; 15:96–109. [PubMed: 25614008]
- Brimnes MK, Vangstedt AJ, Knudsen LM, Gimsing P, Gang AO, Johnsen HE, et al. Increased level of both CD4+FOXP3+ regulatory T cells and CD14+HLA-DR⁻/low myeloid-derived suppressor cells and decreased level of dendritic cells in patients with multiple myeloma. *Scandinavian Journal of Immunology*. 2010; 72:540–547. [PubMed: 21044128]
- 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. *Journal of Immunology*. 1999; 162:5728–5737.
- Bronte V, Serafini P, De Santo C, Marigo I, Tosello V, Mazzoni A, et al. IL-4-induced arginase 1 suppresses alloreactive T cells in tumor-bearing mice. *Journal of Immunology*. 2003; 170:270–278.
- Bronte V, Zanovello P. Regulation of immune responses by l-arginine metabolism. *Nature Reviews. Immunology*. 2005; 5:641–654.
- Bunt SK, Clements VK, Hanson EM, Sinha P, Ostrand-Rosenberg S. Inflammation enhances myeloid-derived suppressor cell cross-talk by signaling through Toll-like receptor 4. *Journal of Leukocyte Biology*. 2009; 85:996–1004. [PubMed: 19261929]
- Bunt SK, Mohr AM, Bailey JM, Grandgenett PM, Hollingsworth MA. Rosiglitazone and Gemcitabine in combination reduces immune suppression and modulates T cell populations in pancreatic cancer. *Cancer Immunology, Immunotherapy*. 2013; 62:225–236. [PubMed: 22864396]
- Bunt SK, Sinha P, Clements VK, Leips J, Ostrand-Rosenberg S. Inflammation induces myeloid-derived suppressor cells that facilitate tumor progression. *Journal of Immunology*. 2006; 176:284–290.
- Bunt SK, Yang L, Sinha P, Clements VK, Leips J, Ostrand-Rosenberg S. Reduced inflammation in the tumor microenvironment delays the accumulation of myeloid-derived suppressor cells and limits tumor progression. *Cancer Research*. 2007; 67:10019–10026. [PubMed: 17942936]
- Burchert A, Cai D, Hofbauer LC, Samuelsson MK, Slater EP, Duyster J, et al. Interferon consensus sequence binding protein (ICSBP; IRF-8) antagonizes BCR/ABL and down-regulates bcl-2. *Blood*. 2004; 103:3480–3489. [PubMed: 14656881]
- Burke M, Choksawangkar W, Edwards N, Ostrand-Rosenberg S, Fenselau C. Exosomes from myeloid-derived suppressor cells carry biologically active proteins. *Journal of Proteome Research*. 2014; 13:836–843. [PubMed: 24295599]
- Cekic C, Day YJ, Sag D, Linden J. Myeloid expression of adenosine A2A receptor suppresses T and NK cell responses in the solid tumor microenvironment. *Cancer Research*. 2014; 74:7250–7259. [PubMed: 25377469]
- Chalmin F, Ladoire S, Mignot G, Vincent J, Bruchard M, Remy-Martin JP, et al. Membrane-associated Hsp72 from tumor-derived exosomes mediates STAT3-dependent immunosuppressive function of mouse and human myeloid-derived suppressor cells. *The Journal of Clinical Investigation*. 2010; 120:457–471. [PubMed: 20093776]
- Chatterjee S, Das S, Chakraborty P, Manna A, Chatterjee M, Choudhuri SK. Myeloid derived suppressor cells (MDSCs) can induce the generation of Th17 response from naive CD4+ T cells. *Immunobiology*. 2013; 218:718–724. [PubMed: 22995935]
- Chen S, Akbar SM, Miyake T, Abe M, Al-Mahtab M, Furukawa S, et al. Diminished immune response to vaccinations in obesity: Role of myeloid-derived suppressor and other myeloid cells. *Obesity Research & Clinical Practice*. 2015; 9:35–44. [PubMed: 25660173]
- Cheng P, Corzo CA, Luetette 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. *The Journal of Experimental Medicine*. 2008; 205:2235–2249. [PubMed: 18809714]

- Cheng P, Kumar V, Liu H, Youn JI, Fishman M, Sherman S, et al. Effects of notch signaling on regulation of myeloid cell differentiation in cancer. *Cancer Research*. 2014; 74:141–152. [PubMed: 24220241]
- Chornoguz O, Grmai L, Sinha P, Artemenko KA, Zubarev RA, Ostrand-Rosenberg S. Proteomic pathway analysis reveals inflammation increases myeloid-derived suppressor cell resistance to apoptosis. *Molecular & Cellular Proteomics*. 2011; 10 M110.002980.
- Chou HS, Hsieh CC, Charles R, Wang L, Wagner T, Fung JJ, et al. Myeloid-derived suppressor cells protect islet transplants by B7-H1 mediated enhancement of T regulatory cells. *Transplantation*. 2012; 93:272–282. [PubMed: 22179405]
- Corzo CA, Condamine T, Lu L, Cotter MJ, Youn JI, Cheng P, et al. HIF-1 α regulates function and differentiation of myeloid-derived suppressor cells in the tumor microenvironment. *The Journal of Experimental Medicine*. 2010; 207:2439–2453. [PubMed: 20876310]
- 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. *Journal of Immunology*. 2009; 182:5693–5701.
- Cui TX, Kryczek I, Zhao L, Zhao E, Quick R, Roh MH, et al. Myeloid-derived suppressor cells enhance stemness of cancer cells by inducing microRNA101 and suppressing the corepressor CtBP2. *Immunity*. 2013; 39:611–621. [PubMed: 24012420]
- Danelli L, Frossi B, Gri G, Mion F, Guarnotta C, Bongiovanni L, et al. Mast cells boost myeloid-derived suppressor cell activity and contribute to the development of tumor-favoring microenvironment. *Cancer Immunology Research*. 2015; 3:85–95. [PubMed: 25351848]
- Delano MJ, Scumpia PO, Weinstein JS, Coco D, Nagaraj S, Kelly-Scumpia KM, et al. MyD88-dependent expansion of an immature GR-1(+)CD11b(+) population induces T cell suppression and Th2 polarization in sepsis. *The Journal of Experimental Medicine*. 2007; 204:1463–1474. [PubMed: 17548519]
- Diaz-Montero CM, Salem ML, Nishimura MI, Garrett-Mayer E, Cole DJ, Montero AJ. Increased circulating myeloid-derived suppressor cells correlate with clinical cancer stage, metastatic tumor burden, and doxorubicin-cyclophosphamide chemotherapy. *Cancer Immunology, Immunotherapy*. 2009; 58:49–59. [PubMed: 18446337]
- Dolcetti L, Peranzoni E, Ugel S, Marigo I, Fernandez Gomez A, Mesa C, et al. Hierarchy of immunosuppressive strength among myeloid-derived suppressor cell subsets is determined by GM-CSF. *European Journal of Immunology*. 2010; 40:22–35. [PubMed: 19941314]
- Dumitru CA, Moses K, Trellakis S, Lang S, Brandau S. Neutrophils and granulocytic myeloid-derived suppressor cells: Immunophenotyping, cell biology and clinical relevance in human oncology. *Cancer Immunology, Immunotherapy*. 2012; 61:1155–1167. [PubMed: 22692756]
- Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: From immunosurveillance to tumor escape. *Nature Immunology*. 2002; 3:991–998. [PubMed: 12407406]
- El Gazzar M, McCall CE. MicroRNAs regulatory networks in myeloid lineage development and differentiation: Regulators of the regulators. *Immunology and Cell Biology*. 2012; 90:587–593. [PubMed: 21912420]
- Elkabets M, Ribeiro VS, Dinarello CA, Ostrand-Rosenberg S, Di Santo JP, Apte RN, et al. IL-1 β regulates a novel myeloid-derived suppressor cell subset that impairs NK cell development and function. *European Journal of Immunology*. 2010; 40:3347–3357. [PubMed: 21110318]
- Eruslanov E, Daurkin I, Ortiz J, Vieweg J, Kusmartsev S. Pivotal advance: Tumor-mediated induction of myeloid-derived suppressor cells and M2-polarized macrophages by altering intracellular PGE₂ catabolism in myeloid cells. *Journal of Leukocyte Biology*. 2010; 88:839–848. [PubMed: 20587738]
- Eruslanov E, Neuberger M, Daurkin I, Perrin GQ, Algood C, Dahm P, et al. Circulating and tumor-infiltrating myeloid cell subsets in patients with bladder cancer. *International Journal of Cancer*. 2012; 130:1109–1119.
- Espagnolle N, Barron P, Mandron M, Blanc I, Bonnin J, Agnel M, et al. Specific inhibition of the VEGFR-3 tyrosine kinase by SAR131675 reduces peripheral and tumor associated immunosuppressive myeloid cells. *Cancers*. 2014; 6:472–490. [PubMed: 24589997]

- Ezernitchi AV, Vaknin I, Cohen-Daniel L, Levy O, Manaster E, Halabi A, et al. TCR zeta down-regulation under chronic inflammation is mediated by myeloid suppressor cells differentially distributed between various lymphatic organs. *Journal of Immunology*. 2006; 177:4763–4772.
- Farren MR, Carlson LM, Lee KP. Tumor-mediated inhibition of dendritic cell differentiation is mediated by down regulation of protein kinase C beta II expression. *Immunologic Research*. 2010; 46:165–176. [PubMed: 19756409]
- Fletcher M, Ramirez ME, Sierra RA, Raber P, Thevenot P, Al-Khami AA, et al. l-Arginine depletion blunts antitumor T-cell responses by inducing myeloid-derived suppressor cells. *Cancer Research*. 2015; 75:275–283. [PubMed: 25406192]
- Forghani P, Khorramzadeh MR, Waller EK. Silibinin inhibits accumulation of myeloid-derived suppressor cells and tumor growth of murine breast cancer. *Cancer Medicine*. 2014; 3:215–224. [PubMed: 24574320]
- Fridlender ZG, Sun J, Kim S, Kapoor V, Cheng G, Ling L, et al. Polarization of tumor-associated neutrophil phenotype by TGF-beta: “N1” versus “N2” TAN. *Cancer Cell*. 2009; 16:183–194. [PubMed: 19732719]
- Fukumura D, Kashiwagi S, Jain RK. The role of nitric oxide in tumour progression. *Nature Reviews. Cancer*. 2006; 6:521–534. [PubMed: 16794635]
- Gabitass RF, Anells NE, Stocken DD, Pandha HA, Middleton GW. Elevated myeloid-derived suppressor cells in pancreatic, esophageal and gastric cancer are an independent prognostic factor and are associated with significant elevation of the Th2 cytokine interleukin-13. *Cancer Immunology, Immunotherapy*. 2011; 60:1419–1430. [PubMed: 21644036]
- Gabriele L, Phung J, Fukumoto J, Segal D, Wang IM, Giannakakou P, et al. Regulation of apoptosis in myeloid cells by interferon consensus sequence-binding protein. *The Journal of Experimental Medicine*. 1999; 190:411–421. [PubMed: 10430629]
- Gabrilovich DI, Bronte V, Chen SH, Colombo MP, Ochoa A, Ostrand-Rosenberg S, et al. The terminology issue for myeloid-derived suppressor cells. *Cancer Research*. 2007; 67:425. author reply 426. [PubMed: 17210725]
- Gabrilovich D, Ishida T, Oyama T, Ran S, Kravtsov V, Nadaf S, et al. Vascular endothelial growth factor inhibits the development of dendritic cells and dramatically affects the differentiation of multiple hematopoietic lineages in vivo. *Blood*. 1998; 92:4150–4166. [PubMed: 9834220]
- Gabrilovich DI, Velders MP, Sotomayor EM, Kast WM. Mechanism of immune dysfunction in cancer mediated by immature Gr-1+ myeloid cells. *Journal of Immunology*. 2001; 166:5398–5406.
- Garrity T, Pandit R, Wright MA, Benefield J, Keni S, Young MR. Increased presence of CD34+ cells in the peripheral blood of head and neck cancer patients and their differentiation into dendritic cells. *International Journal of Cancer*. 1997; 73:663–669.
- Gehad AE, Lichtman MK, Schmults CD, Teague JE, Calarese AW, Jiang Y, et al. Nitric oxide-producing myeloid-derived suppressor cells inhibit vascular E-selectin expression in human squamous cell carcinomas. *The Journal of Investigative Dermatology*. 2012; 132:2642–2651. [PubMed: 22718118]
- Gleason MK, Ross JA, Warlick ED, Lund TC, Verneris MR, Wiernik A, et al. CD16xCD33 bispecific killer cell engager (BiKE) activates NK cells against primary MDS and MDSC CD33+ targets. *Blood*. 2014; 123:3016–3026. [PubMed: 24652987]
- Goñi O, Alcaide P, Fresno M. Immunosuppression during acute *Trypanosoma cruzi* infection: Involvement of Ly6G (Gr1(+))CD11b(+)immature myeloid suppressor cells. *International Immunology*. 2002; 14:1125–1134. [PubMed: 12356678]
- Grizzle WE, Xu X, Zhang S, Stockard CR, Liu C, Yu S, et al. Age-related increase of tumor susceptibility is associated with myeloid-derived suppressor cell mediated suppression of T cell cytotoxicity in recombinant inbred BXD12 mice. *Mechanisms of Ageing and Development*. 2007; 128:672–680. [PubMed: 18036633]
- Haile LA, von Wasielewski R, Gamrekelashvili J, Krüger C, Bachmann O, Westendorf AM, et al. Myeloid-derived suppressor cells in inflammatory bowel disease: A new immunoregulatory pathway. *Gastroenterology*. 2008; 135:871–881. 881.e1–5. [PubMed: 18674538]

- Hanson EM, Clements VK, Sinha P, Ilkovitch D, Ostrand-Rosenberg S. Myeloid-derived suppressor cells down-regulate L-selectin expression on CD4+ and CD8+ T cells. *Journal of Immunology*. 2009; 183:937–944.
- He D, Li H, Yusuf N, Elmets CA, Li J, Mountz JD, et al. IL-17 promotes tumor development through the induction of tumor promoting microenvironments at tumor sites and myeloid-derived suppressor cells. *Journal of Immunology*. 2010; 184:2281–2288.
- Highfill SL, Rodriguez PC, Zhou Q, Goetz CA, Koehn BH, Veenstra R, et al. Bone marrow myeloid-derived suppressor cells (MDSCs) inhibit graft-versus-host disease (GVHD) via an arginase-1-dependent mechanism that is up-regulated by interleukin-13. *Blood*. 2010; 116:5738–5747. [PubMed: 20807889]
- Hix LM, Karavitis J, Khan MW, Shi YH, Khazaie K, Zhang M. Tumor STAT1 transcription factor activity enhances breast tumor growth and immune suppression mediated by myeloid-derived suppressor cells. *The Journal of Biological Chemistry*. 2013; 288:11676–11688. [PubMed: 23486482]
- Hoehst B, Ormandy LA, Ballmaier M, Lehner F, Kruger C, Manns MP, et al. A new population of myeloid-derived suppressor cells in hepatocellular carcinoma patients induces CD4(+)CD25(+)Foxp3(+) T cells. *Gastroenterology*. 2008; 135:234–243. [PubMed: 18485901]
- Horlad H, Fujiwara Y, Takemura K, Ohnishi K, Ikeda T, Tsukamoto H, et al. Corosolic acid impairs tumor development and lung metastasis by inhibiting the immunosuppressive activity of myeloid-derived suppressor cells. *Molecular Nutrition & Food Research*. 2013; 57:1046–1054. [PubMed: 23417831]
- Hu X, Bardhan K, Paschall AV, Yang D, Waller JL, Park MA, et al. Deregulation of apoptotic factors Bcl-xL and Bax confers apoptotic resistance to myeloid-derived suppressor cells and contributes to their persistence in cancer. *The Journal of Biological Chemistry*. 2013; 288:19103–19115. [PubMed: 23677993]
- Huang B, Pan PY, Li Q, Sato AI, Levy DE, Bromberg J, et al. Gr-1+CD115+ immature myeloid suppressor cells mediate the development of tumor-induced T regulatory cells and T-cell anergy in tumor-bearing host. *Cancer Research*. 2006; 66:1123–1131. [PubMed: 16424049]
- Huang A, Zhang B, Wang B, Zhang F, Fan KX, Guo YJ. Increased CD14(+)HLA-DR (–/low) myeloid-derived suppressor cells correlate with extrathoracic metastasis and poor response to chemotherapy in non-small cell lung cancer patients. *Cancer Immunology, Immunotherapy*. 2013; 62:1439–1451. [PubMed: 23760662]
- Iclozan C, Antonia S, Chiappori A, Chen DT, Gabrilovich D. Therapeutic regulation of myeloid-derived suppressor cells and immune response to cancer vaccine in patients with extensive stage small cell lung cancer. *Cancer Immunology, Immunotherapy*. 2013; 62:909–918. [PubMed: 23589106]
- Ilkovitch D, Lopez DM. The liver is a site for tumor-induced myeloid-derived suppressor cell accumulation and immunosuppression. *Cancer Research*. 2009; 69:5514–5521. [PubMed: 19549903]
- Ioannou M, Alissafi T, Lazaridis I, Deraos G, Matsoukas J, Gravanis A, et al. Crucial role of granulocytic myeloid-derived suppressor cells in the regulation of central nervous system autoimmune disease. *Journal of Immunology*. 2012; 188:1136–1146.
- Jablonska J, Leschner S, Westphal K, Lienenklaus S, Weiss S. Neutrophils responsive to endogenous IFN-beta regulate tumor angiogenesis and growth in a mouse tumor model. *The Journal of Clinical Investigation*. 2010; 120:1151–1164. [PubMed: 20237412]
- Karin M. Nuclear factor-kappaB in cancer development and progression. *Nature*. 2006; 441:431–436. [PubMed: 16724054]
- Kim K, Skora AD, Li Z, Liu Q, Tam AJ, Blosser RL, et al. Eradication of metastatic mouse cancers resistant to immune checkpoint blockade by suppression of myeloid-derived cells. *Proceedings of the National Academy of Sciences of the United States of America*. 2014; 111:11774–11779. [PubMed: 25071169]
- Kimura T, McKolanis JR, Dzubinski LA, Islam K, Potter DM, Salazar AM, et al. MUC1 vaccine for individuals with advanced adenoma of the colon: A cancer immunoprevention feasibility study. *Cancer Prevention Research (Philadelphia, PA)*. 2013; 6:18–26.

- Köstlin N, Kugel H, Spring B, Leiber A, Marmé A, Henes M, et al. Granulocytic myeloid derived suppressor cells expand in human pregnancy and modulate T-cell responses. *European Journal of Immunology*. 2014; 44:2582–2591. [PubMed: 24894988]
- Kowanetz M, Wu X, Lee J, Tan M, Hagenbeek T, Qu X, et al. Granulocyte-colony stimulating factor promotes lung metastasis through mobilization of Ly6G+Ly6C+ granulocytes. *Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107:21248–21255. [PubMed: 21081700]
- Kujawski M, Kortylewski M, Lee H, Herrmann A, Kay H, Yu H. Stat3 mediates myeloid cell-dependent tumor angiogenesis in mice. *The Journal of Clinical Investigation*. 2008; 118:3367–3377. [PubMed: 18776941]
- Kusmartsev S, Eruslanov E, Kubler H, Tseng T, Sakai Y, Su Z, et al. Oxidative stress regulates expression of VEGFR1 in myeloid cells: Link to tumor-induced immune suppression in renal cell carcinoma. *Journal of Immunology*. 2008; 181:346–353.
- Kusmartsev S, Gabrilovich DI. STAT1 signaling regulates tumor-associated macrophage-mediated T cell deletion. *Journal of Immunology*. 2005; 174:4880–4891.
- Levring TB, Hansen AK, Nielsen BL, Kongsbak M, von Essen MR, Woetmann A, et al. Activated human CD4+ T cells express transporters for both cysteine and cystine. *Scientific Reports*. 2012; 2:266. [PubMed: 22355778]
- Li W, Wu K, Zhao E, Shi L, Li R, Zhang P, et al. HMGB1 recruits myeloid derived suppressor cells to promote peritoneal dissemination of colon cancer after resection. *Biochemical and Biophysical Research Communications*. 2013; 436:156–161. [PubMed: 23707808]
- Li L, Zhang J, Diao W, Wang D, Wei Y, Zhang CY, et al. MicroRNA-155 and MicroRNA-21 promote the expansion of functional myeloid-derived suppressor cells. *Journal of Immunology*. 2014; 192:1034–1043.
- Lieschke GJ, Grahl D, Hodgson G, Metcalf D, Stanley E, Cheers C, et al. Mice lacking granulocyte colony-stimulating factor have chronic neutropenia, granulocyte and macrophage progenitor cell deficiency, and impaired neutrophil mobilization. *Blood*. 1994; 84:1737–1746. [PubMed: 7521686]
- Lin Y, Gustafson MP, Bulur PA, Gastineau DA, Witzig TE, Dietz AB. Immunosuppressive CD14+HLA-DR(low)/- monocytes in B-cell non-Hodgkin lymphoma. *Blood*. 2011; 117:872–881. [PubMed: 21063024]
- Liu Y, Lai L, Chen Q, Song Y, Xu S, Ma F, et al. MicroRNA-494 is required for the accumulation and functions of tumor-expanded myeloid-derived suppressor cells via targeting of PTEN. *Journal of Immunology*. 2012; 188:5500–5510.
- Liu C, Yu S, Kappes J, Wang J, Grizzle WE, Zinn KR, et al. Expansion of spleen myeloid suppressor cells represses NK cell cytotoxicity in tumor-bearing host. *Blood*. 2007; 109:4336–4342. [PubMed: 17244679]
- Liu Q, Zhang M, Jiang X, Zhang Z, Dai L, Min S, et al. miR-223 suppresses differentiation of tumor-induced CD11b+ Gr1+ myeloid-derived suppressor cells from bone marrow cells. *International Journal of Cancer*. 2011; 129:2662–2673.
- Lu T, Gabrilovich DI. Molecular pathways: Tumor-infiltrating myeloid cells and reactive oxygen species in regulation of tumor microenvironment. *Clinical Cancer Research*. 2012; 18:4877–4882. [PubMed: 22718858]
- Lu T, Ramakrishnan R, Altiock S, Youn JI, Cheng P, Celis E, et al. Tumor-infiltrating myeloid cells induce tumor cell resistance to cytotoxic T cells in mice. *The Journal of Clinical Investigation*. 2011; 121:4015–4029. [PubMed: 21911941]
- MacDonald KP, Rowe V, Clouston AD, Welply JK, Kuns RD, Ferrara JL, et al. Cytokine expanded myeloid precursors function as regulatory antigen-presenting cells and promote tolerance through IL-10-producing regulatory T cells. *Journal of Immunology*. 2005; 174:1841–1850.
- Makarenkova VP, Bansal V, Matta BM, Perez LA, Ochoa JB. CD11b+/ Gr-1+ myeloid suppressor cells cause T cell dysfunction after traumatic stress. *Journal of Immunology*. 2006; 176:2085–2094.

- Mao Y, Sarhan D, Steven A, Seliger B, Kiessling R, Lundqvist A. Inhibition of tumor-derived prostaglandin-e2 blocks the induction of myeloid-derived suppressor cells and recovers natural killer cell activity. *Clinical Cancer Research*. 2014; 20:4096–4106. [PubMed: 24907113]
- Marigo I, Bosio E, Solito S, Mesa C, Fernandez A, Dolcetti L, et al. Tumor-induced tolerance and immune suppression depend on the C/EBPbeta transcription factor. *Immunity*. 2010; 32:790–802. [PubMed: 20605485]
- Markiewski MM, DeAngelis RA, Benencia F, Ricklin-Lichtsteiner SK, Koutoulaki A, Gerard C, et al. Modulation of the antitumor immune response by complement. *Nature Immunology*. 2008; 9:1225–1235. [PubMed: 18820683]
- Martin RK, Saleem SJ, Folgosa L, Zellner HB, Damle SR, Nguyen GK, et al. Mast cell histamine promotes the immunoregulatory activity of myeloid-derived suppressor cells. *Journal of Leukocyte Biology*. 2014; 96:151–159. [PubMed: 24610880]
- 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. *Journal of Immunology*. 2002; 168:689–695.
- Melani C, Chiodoni C, Forni G, Colombo MP. Myeloid cell expansion elicited by the progression of spontaneous mammary carcinomas in c-erbB-2 transgenic BALB/c mice suppresses immune reactivity. *Blood*. 2003; 102:2138–2145. [PubMed: 12750171]
- Melani C, Sangaletti S, Barazzetta FM, Werb Z, Colombo MP. Amino-biphosphonate-mediated MMP-9 inhibition breaks the tumor-bone marrow axis responsible for myeloid-derived suppressor cell expansion and macrophage infiltration in tumor stroma. *Cancer Research*. 2007; 67:11438–11446. [PubMed: 18056472]
- Mikyskova R, Indrova M, Vlkova V, Bieblova J, Simova J, Parackova Z, et al. DNA demethylating agent 5-azacytidine inhibits myeloid-derived suppressor cells induced by tumor growth and cyclophosphamide treatment. *Journal of Leukocyte Biology*. 2014; 95:743–753.
- Molon B, Ugel S, Del Pozzo F, Soldani C, Zilio S, Avella D, et al. Chemo-kine nitration prevents intratumoral infiltration of antigen-specific T cells. *The Journal of Experimental Medicine*. 2011; 208:1949–1962. [PubMed: 21930770]
- Montero AJ, Diaz-Montero CM, Kyriakopoulos CE, Bronte V, Mandruzzato S. Myeloid-derived suppressor cells in cancer patients: a clinical perspective. *Journal of Immunotherapy*. 2012; 35:107–115. [PubMed: 22306898]
- Morales JK, Kmiecik M, Knutson KL, Bear HD, Manjili MH. GM-CSF is one of the main breast tumor-derived soluble factors involved in the differentiation of CD11b–Gr1- bone marrow progenitor cells into myeloid-derived suppressor cells. *Breast Cancer Research and Treatment*. 2010; 123:39–49. [PubMed: 19898981]
- Mostafa MH, Sheweita SA, O'Connor PJ. Relationship between schisto-somiasis and bladder cancer. *Clinical Microbiology Reviews*. 1999; 12:97–111. [PubMed: 9880476]
- Movahedi K, Williams M, Van den Bossche J, Van den Bergh R, Gysemans C, Beschin A, et al. Identification of discrete tumor-induced myeloid-derived suppressor cell subpopulations with distinct T cell-suppressive activity. *Blood*. 2008; 111:4233–4244. [PubMed: 18272812]
- Mucha J, Majchrzak K, Taciak B, Hellmen E, Krol M. MDSCs mediate angiogenesis and predispose canine mammary tumor cells for metastasis via IL-28/IL-28RA (IFN-lambda) signaling. *PLoS One*. 2014; 9:e103249. [PubMed: 25075523]
- Mundy-Bosse BL, Thornton LM, Yang HC, Andersen BL, Carson WE. Psychological stress is associated with altered levels of myeloid-derived suppressor cells in breast cancer patients. *Cellular Immunology*. 2011; 270:80–87. [PubMed: 21600570]
- Munn DH, Sharma MD, Baban B, Harding HP, Zhang Y, Ron D, et al. GCN2 kinase in T cells mediates proliferative arrest and anergy induction in response to indoleamine 2,3-dioxygenase. *Immunity*. 2005; 22:633–642. [PubMed: 15894280]
- Nefedova Y, Cheng P, Gilkes D, Blaskovich M, Beg AA, Sebti SM, et al. Activation of dendritic cells via inhibition of Jak2/STAT3 signaling. *Journal of Immunology*. 2005; 175:4338–4346.
- Nefedova Y, Huang M, Kusmartsev S, Bhattacharya R, Cheng P, Salup R, et al. Hyperactivation of STAT3 is involved in abnormal differentiation of dendritic cells in cancer. *Journal of Immunology*. 2004; 172:464–474.

- Noman MZ, Desantis G, Janji B, Hasmim M, Karray S, Dessen P, et al. PD-L1 is a novel direct target of HIF-1 α , and its blockade under hypoxia enhanced MDSC-mediated T cell activation. *The Journal of Experimental Medicine*. 2014; 211:781–790. [PubMed: 24778419]
- Obermajer N, Muthuswamy R, Lesnock J, Edwards RP, Kalinski P. Positive feedback between PGE2 and COX2 redirects the differentiation of human dendritic cells toward stable myeloid-derived suppressor cells. *Blood*. 2011; 118:5498–5505. [PubMed: 21972293]
- Okazaki T, Ebihara S, Asada M, Kanda A, Sasaki H, Yamaya M. Granulocyte colony-stimulating factor promotes tumor angiogenesis via increasing circulating endothelial progenitor cells and Gr1+CD11b+ cells in cancer animal models. *International Immunology*. 2006; 18:1–9. [PubMed: 16352631]
- Ortiz ML, Kumar V, Martner A, Mony S, Donthireddy L, Condamine T, et al. Immature myeloid cells directly contribute to skin tumor development by recruiting IL-17-producing CD4+ T cells. *The Journal of Experimental Medicine*. 2015; 212:351–367. [PubMed: 25667306]
- Ostrand-Rosenberg S, Sinha P. Myeloid-derived suppressor cells: Linking inflammation and cancer. *Journal of Immunology*. 2009; 182:4499–4506.
- OuYang LY, Wu XJ, Ye SB, Zhang RX, Li ZL, Liao W, et al. Tumor-induced myeloid-derived suppressor cells promote tumor progression through oxidative metabolism in human colorectal cancer. *Journal of Translational Medicine*. 2015; 13:47. [PubMed: 25638150]
- Pan PY, Ma G, Weber KJ, Ozao-Choy J, Wang G, Yin B, et al. Immune stimulatory receptor CD40 is required for T-cell suppression and T regulatory cell activation mediated by myeloid-derived suppressor cells in cancer. *Cancer Research*. 2010; 70:99–108. [PubMed: 19996287]
- Panka DJ, Liu Q, Geissler AK, Mier JW. Effects of HDM2 antagonism on sunitinib resistance, p53 activation, SDF-1 induction, and tumor infiltration by CD11b+/Gr-1+ myeloid derived suppressor cells. *Molecular Cancer*. 2013; 12:17. [PubMed: 23497256]
- Parker K, Sinha P, Horn L, Clements V, Ostrand-Rosenberg S. HMGB1 enhances immune suppression by facilitating the differentiation and suppressive activity of myeloid-derived suppressor cells. *Cancer Research*. 2014; 74:5723–5733. [PubMed: 25164013]
- Porembka MR, Mitchem JB, Belt BA, Hsieh CS, Lee HM, Herndon J, et al. Pancreatic adenocarcinoma induces bone marrow mobilization of myeloid-derived suppressor cells which promote primary tumor growth. *Cancer Immunology, Immunotherapy*. 2012; 61:1373–1385. [PubMed: 22215137]
- Punturieri A, Szabo E, Croxton TL, Shapiro SD, Dubinett SM. Lung cancer and chronic obstructive pulmonary disease: Needs and opportunities for integrated research. *Journal of the National Cancer Institute*. 2009; 101:554–559. [PubMed: 19351920]
- Raber PL, Thevenot P, Sierra R, Wyczechowska D, Halle D, Ramirez ME, et al. Subpopulations of myeloid-derived suppressor cells impair T cell responses through independent nitric oxide-related pathways. *International Journal of Cancer*. 2014; 134:2853–2864.
- Rodriguez PC, Ernstoff MS, Hernandez C, Atkins M, Zabaleta J, Sierra R, et al. Arginase I-producing myeloid-derived suppressor cells in renal cell carcinoma are a subpopulation of activated granulocytes. *Cancer Research*. 2009; 69:1553–1560. [PubMed: 19201693]
- Rodriguez PC, Hernandez CP, Morrow K, Sierra R, Zabaleta J, Wyczechowska DD, et al. l-Arginine deprivation regulates cyclin D3 mRNA stability in human T cells by controlling HuR expression. *Journal of Immunology*. 2010; 185:5198–5204.
- Rodriguez PC, Hernandez CP, Quiceno D, Dubinett SM, Zabaleta J, Ochoa JB, et al. Arginase I in myeloid suppressor cells is induced by COX-2 in lung carcinoma. *The Journal of Experimental Medicine*. 2005; 202:931–939. [PubMed: 16186186]
- Rodriguez PC, Quiceno DG, Zabaleta J, Ortiz B, Zea AH, Piazeulo MB, et al. Arginase I production in the tumor microenvironment by mature myeloid cells inhibits T-cell receptor expression and antigen-specific T-cell responses. *Cancer Research*. 2004; 64:5839–5849. [PubMed: 15313928]
- Rodriguez PC, Zea AH, Culotta KS, Zabaleta J, Ochoa JB, Ochoa AC. Regulation of T cell receptor CD3zeta chain expression by l-arginine. *The Journal of Biological Chemistry*. 2002; 277:21123–21129. [PubMed: 11950832]

- Sakamaki I, Kwak LW, Cha SC, Yi Q, Lerman B, Chen J, et al. Lenalidomide enhances the protective effect of a therapeutic vaccine and reverses immune suppression in mice bearing established lymphomas. *Leukemia*. 2014; 28:329–337. [PubMed: 23765229]
- Saleem SJ, Martin RK, Morales JK, Sturgill JL, Gibb DR, Graham L, et al. Cutting edge: Mast cells critically augment myeloid-derived suppressor cell activity. *Journal of Immunology*. 2012; 189:511–515.
- Santhanam L, Lim HK, Miriel V, Brown T, Patel M, Balanson S, et al. Inducible NO synthase dependent S-nitrosylation and activation of arginase1 contribute to age-related endothelial dysfunction. *Circulation Research*. 2007; 101:692–702. [PubMed: 17704205]
- Santilli G, Piotrowska I, Cantilena S, Chayka O, D'Alicarnasso M, Morgenstern DA, et al. Polyphenon [corrected] E enhances the antitumor immune response in neuroblastoma by inactivating myeloid suppressor cells. *Clinical Cancer Research*. 2013; 19:1116–1125. [PubMed: 23322899]
- Schilling B, Sucker A, Griewank K, Zhao F, Weide B, Gorgens A, et al. Vemurafenib reverses immunosuppression by myeloid derived suppressor cells. *International Journal of Cancer*. 2013; 133:1653–1663.
- Schmielau J, Finn OJ. Activated granulocytes and granulocyte-derived hydrogen peroxide are the underlying mechanism of suppression of t-cell function in advanced cancer patients. *Cancer Research*. 2001; 61:4756–4760. [PubMed: 11406548]
- Serafini P. Myeloid derived suppressor cells in physiological and pathological conditions: The good, the bad, and the ugly. *Immunologic Research*. 2013; 57:172–184. [PubMed: 24203443]
- Serafini P, Carbley R, Noonan KA, Tan G, Bronte V, Borrello I. High-dose granulocyte-macrophage colony-stimulating factor-producing vaccines impair the immune response through the recruitment of myeloid suppressor cells. *Cancer Research*. 2004; 64:6337–6343. [PubMed: 15342423]
- Serafini P, Mgebroff S, Noonan K, Borrello I. Myeloid-derived suppressor cells promote cross-tolerance in B-cell lymphoma by expanding regulatory T cells. *Cancer Research*. 2008; 68:5439–5449. [PubMed: 18593947]
- Shacter E, Weitzman SA. Chronic inflammation and cancer. *Oncology (Williston Park)*. 2002; 16:217–226. 229. discussion 230–232. [PubMed: 11866137]
- Shen P, Wang A, He M, Wang Q, Zheng S. Increased circulating Lin(–/low) CD33(+) HLA-DR(–) myeloid-derived suppressor cells in hepatocellular carcinoma patients. *Hepatology Research*. 2014; 44:639–650. [PubMed: 23701406]
- Sica A, Mantovani A. Macrophage plasticity and polarization: In vivo veritas. *The Journal of Clinical Investigation*. 2012; 122:787–795. [PubMed: 22378047]
- Sinha P, Chornoguz O, Clements VK, Artemenko KA, Zubarev RA, Ostrand-Rosenberg S. Myeloid-derived suppressor cells express the death receptor Fas and apoptose in response to T cell-expressed FasL. *Blood*. 2011; 117:5381–5390. [PubMed: 21450901]
- Sinha P, Clements VK, Bunt SK, Albelda SM, Ostrand-Rosenberg S. Cross-talk between myeloid-derived suppressor cells and macrophages subverts tumor immunity toward a type 2 response. *Journal of Immunology*. 2007; 179:977–983.
- Sinha P, Clements VK, Fulton AM, Ostrand-Rosenberg S. Prostaglandin E2 promotes tumor progression by inducing myeloid-derived suppressor cells. *Cancer Research*. 2007; 67:4507–4513. [PubMed: 17483367]
- Sinha P, Clements VK, Ostrand-Rosenberg S. Interleukin-13-regulated M2 macrophages in combination with myeloid suppressor cells block immune surveillance against metastasis. *Cancer Research*. 2005a; 65:11743–11751. [PubMed: 16357187]
- Sinha P, Clements VK, Ostrand-Rosenberg S. Reduction of myeloid-derived suppressor cells and induction of M1 macrophages facilitate the rejection of established metastatic disease. *Journal of Immunology*. 2005b; 174:636–645.
- Sinha P, Okoro C, Foell D, Freeze HH, Ostrand-Rosenberg S, Srikrishna G. Proinflammatory S100 proteins regulate the accumulation of myeloid-derived suppressor cells. *Journal of Immunology*. 2008; 181:4666–4675.

- Sinha P, Ostrand-Rosenberg S. Myeloid-derived suppressor cell function is reduced by Withaferin A, a potent and abundant component of *Withania somnifera* root extract. *Cancer Immunology, Immunotherapy*. 2013; 62:1663–1673. [PubMed: 23982485]
- Sinha P, Parker KH, Horn L, Ostrand-Rosenberg S. Tumor-induced myeloid-derived suppressor cell function is independent of IFN- γ and IL-4R α . *European Journal of Immunology*. 2012; 42:2052–2059. [PubMed: 22673957]
- Sionov RV, Fridlender ZG, Granot Z. The multifaceted roles neutrophils play in the tumor microenvironment. *Cancer Microenvironment*. 2014
- Smith C, Chang MY, Parker KH, Beury DW, DuHadaway JB, Flick HE, et al. IDO is a nodal pathogenic driver of lung cancer and metastasis development. *Cancer Discovery*. 2012; 2:722–735. [PubMed: 22822050]
- Song X, Krelin Y, Dvorkin T, Bjorkdahl O, Segal S, Dinarello CA, et al. CD11b+/Gr-1+ immature myeloid cells mediate suppression of T cells in mice bearing tumors of IL-1 β -secreting cells. *Journal of Immunology*. 2005; 175:8200–8208.
- Song C, Yuan Y, Wang XM, Li D, Zhang GM, Huang B, et al. Passive transfer of tumour-derived MDSCs inhibits asthma-related airway inflammation. *Scandinavian Journal of Immunology*. 2014; 79:98–104. [PubMed: 24313384]
- Srivastava MK, Bosch JJ, Thompson JA, Ksander BR, Edelman MJ, Ostrand-Rosenberg S. Lung cancer patients' CD4(+) T cells are activated in vitro by MHC II cell-based vaccines despite the presence of myeloid-derived suppressor cells. *Cancer Immunology, Immunotherapy*. 2008; 57:1493–1504. [PubMed: 18322683]
- Srivastava MK, Sinha P, Clements VK, Rodriguez P, Ostrand-Rosenberg S. Myeloid-derived suppressor cells inhibit T-cell activation by depleting cystine and cysteine. *Cancer Research*. 2010; 70:68–77. [PubMed: 20028852]
- Stewart TJ, Liewehr DJ, Steinberg SM, Greenelch KM, Abrams SI. Modulating the expression of IFN regulatory factor 8 alters the protumorigenic behavior of CD11b+Gr-1+ myeloid cells. *Journal of Immunology*. 2009; 183:117–128.
- Strober S. Natural suppressor (NS) cells, neonatal tolerance, and total lymphoid irradiation: Exploring obscure relationships. *Annual Review of Immunology*. 1984; 2:219–237.
- Terabe M, Matsui S, Park JM, Mamura M, Noben-Trauth N, Donaldson DD, et al. Transforming growth factor- β production and myeloid cells are an effector mechanism through which CD1d-restricted T cells block cytotoxic T lymphocyte-mediated tumor immunosurveillance: Abrogation prevents tumor recurrence. *The Journal of Experimental Medicine*. 2003; 198:1741–1752. [PubMed: 14657224]
- Thevenot PT, Sierra RA, Raber PL, Al-Khami AA, Trillo-Tinoco J, Zarrei P, et al. The stress-response sensor chop regulates the function and accumulation of myeloid-derived suppressor cells in tumors. *Immunity*. 2014; 41:389–401. [PubMed: 25238096]
- Tu S, Bhagat G, Cui G, Takaishi S, Kurt-Jones EA, Rickman B, et al. Over-expression of interleukin-1 β induces gastric inflammation and cancer and mobilizes myeloid-derived suppressor cells in mice. *Cancer Cell*. 2008; 14:408–419. [PubMed: 18977329]
- Vakkila J, Lotze MT. Inflammation and necrosis promote tumour growth. *Nature Reviews Immunology*. 2004; 4:641–648. [PubMed: 15286730]
- Venereau E, Casalgrandi M, Schiraldi M, Antoine DJ, Cattaneo A, De Marchis F, et al. Mutually exclusive redox forms of HMGB1 promote cell recruitment or proinflammatory cytokine release. *The Journal of Experimental Medicine*. 2012; 209:1519–1528. [PubMed: 22869893]
- Verschoor CP, Johnstone J, Millar J, Dorrington MG, Habibagahi M, Lelic A, et al. Blood CD33(+)HLA-DR(-) myeloid-derived suppressor cells are increased with age and a history of cancer. *Journal of Leukocyte Biology*. 2013; 93:633–637. [PubMed: 23341539]
- Voisin MB, Buzoni-Gatel D, Bout D, Velge-Roussel F. Both expansion of regulatory GR1+ CD11b+ myeloid cells and anergy of T lymphocytes participate in hyporesponsiveness of the lung-associated immune system during acute toxoplasmosis. *Infection and Immunity*. 2004; 72:5487–5492. [PubMed: 15322051]

- Vuk-Pavlovi S, Bulur PA, Lin Y, Qin R, Szumlanski CL, Zhao X, et al. Immunosuppressive CD14+HLA-DRlow/- monocytes in prostate cancer. *Prostate*. 2010; 70:443–455. [PubMed: 19902470]
- Waight JD, Netherby C, Hensen ML, Miller A, Hu Q, Liu S, et al. Myeloid-derived suppressor cell development is regulated by a STAT/IRF-8 axis. *The Journal of Clinical Investigation*. 2013; 123:4464–4478. [PubMed: 24091328]
- Wang L, Chang EW, Wong SC, Ong SM, Chong DQ, Ling KL. Increased myeloid-derived suppressor cells in gastric cancer correlate with cancer stage and plasma S100A8/A9 proinflammatory proteins. *Journal of Immunology*. 2013; 190:794–804.
- Wang R, Green DR. Metabolic checkpoints in activated T cells. *Nature Immunology*. 2012; 13:907–915. [PubMed: 22990888]
- Wang L, Yi T, Kortylewski M, Pardoll DM, Zeng D, Yu H. IL-17 can promote tumor growth through an IL-6-Stat3 signaling pathway. *The Journal of Experimental Medicine*. 2009; 206:1457–1464. [PubMed: 19564351]
- Weed DT, Vella JL, Reis IM, De la Fuente AC, Gomez C, Sargi Z, et al. Tadalafil reduces myeloid-derived suppressor cells and regulatory T cells and promotes tumor immunity in patients with head and neck squamous cell carcinoma. *Clinical Cancer Research*. 2015; 21:39–48. [PubMed: 25320361]
- Wesolowski R, Markowitz J, Carson WE 3rd. Myeloid derived suppressor cells—A new therapeutic target in the treatment of cancer. *Journal for Immunotherapy of Cancer*. 2013; 1:10. [PubMed: 24829747]
- Wu S, Rhee KJ, Albesiano E, Rabizadeh S, Wu X, Yen HR, et al. A human colonic commensal promotes colon tumorigenesis via activation of T helper type 17 T cell responses. *Nature Medicine*. 2009; 15:1016–1022.
- Wu J, Zhang R, Tang N, Gong Z, Zhou J, Chen Y, et al. Dopamine inhibits the function of Gr-1+CD115+ myeloid-derived suppressor cells through D1-like receptors and enhances anti-tumor immunity. *Journal of Leukocyte Biology*. 2015; 97:191–200. [PubMed: 25341727]
- Xia S, Sha H, Yang L, Ji Y, Ostrand-Rosenberg S, Qi L. Gr-1+ CD11b+ myeloid-derived suppressor cells suppress inflammation and promote insulin sensitivity in obesity. *The Journal of Biological Chemistry*. 2011; 286:23591–23599. [PubMed: 21592961]
- Xin H, Zhang C, Herrmann A, Du Y, Figlin R, Yu H. Sunitinib inhibition of Stat3 induces renal cell carcinoma tumor cell apoptosis and reduces immunosuppressive cells. *Cancer Research*. 2009; 69:2506–2513. [PubMed: 19244102]
- Yazawa T, Shibata M, Gonda K, Machida T, Suzuki S, Kenjo A, et al. Increased IL-17 production correlates with immunosuppression involving myeloid-derived suppressor cells and nutritional impairment in patients with various gastrointestinal cancers. *Molecular and Clinical Oncology*. 2013; 1:675–679. [PubMed: 24649227]
- Ye J, Livergood RS, Peng G. The role and regulation of human Th17 cells in tumor immunity. *The American Journal of Pathology*. 2013; 182:10–20. [PubMed: 23159950]
- Yin Y, Huang X, Lynn KD, Thorpe PE. Phosphatidylserine-targeting antibody induces M1 macrophage polarization and promotes myeloid-derived suppressor cell differentiation. *Cancer Immunology Research*. 2013; 1:256–268. [PubMed: 24777853]
- Yin B, Ma G, Yen CY, Zhou Z, Wang GX, Divino CM, et al. Myeloid-derived suppressor cells prevent type 1 diabetes in murine models. *Journal of Immunology*. 2010; 185:5828–5834.
- Youn JI, Collazo M, Shalova IN, Biswas SK, Gabrilovich DI. Characterization of the nature of granulocytic myeloid-derived suppressor cells in tumor-bearing mice. *Journal of Leukocyte Biology*. 2012; 91:167–181. [PubMed: 21954284]
- Youn JI, Nagaraj S, Collazo M, Gabrilovich DI. Subsets of myeloid-derived suppressor cells in tumor-bearing mice. *Journal of Immunology*. 2008; 181:5791–5802.
- Young MR, Petruzzelli GJ, Kolesiak K, Achille N, Lathers DM, Gabrilovich DI. Human squamous cell carcinomas of the head and neck chemoattract immune suppressive CD34(+) progenitor cells. *Human Immunology*. 2001; 62:332–341. [PubMed: 11295465]

- Yu J, Du W, Yan F, Wang Y, Li H, Cao S, et al. Myeloid-derived suppressor cells suppress antitumor immune responses through IDO expression and correlate with lymph node metastasis in patients with breast cancer. *Journal of Immunology*. 2013; 190:3783–3797.
- Yuan H, Cai P, Li Q, Wang W, Sun Y, Xu Q, et al. Axitinib augments anti-tumor activity in renal cell carcinoma via STAT3-dependent reversal of myeloid-derived suppressor cell accumulation. *Biomedicine & Pharmacotherapy*. 2014; 68:751–756. [PubMed: 25081318]
- Zea AH, Rodriguez PC, Atkins MB, Hernandez C, Signoretti S, Zabaleta J, et al. Arginase-producing myeloid suppressor cells in renal cell carcinoma patients: A mechanism of tumor evasion. *Cancer Research*. 2005; 65:3044–3048. [PubMed: 15833831]
- Zea AH, Rodriguez PC, Culotta KS, Hernandez CP, DeSalvo J, Ochoa JB, et al. l-Arginine modulates CD3zeta expression and T cell function in activated human T lymphocytes. *Cellular Immunology*. 2004; 232:21–31. [PubMed: 15922712]
- Zhang M, Liu Q, Mi S, Liang X, Zhang Z, Su X, et al. Both miR-17-5p and miR-20a alleviate suppressive potential of myeloid-derived suppressor cells by modulating STAT3 expression. *Journal of Immunology*. 2011; 186:4716–4724.
- Zhang YL, Luan B, Wang XF, Qiao JY, Song L, Lei RR, et al. Peripheral blood MDSCs, IL-10 and IL-12 in children with asthma and their importance in asthma development. *PLoS One*. 2013; 8:e63775. [PubMed: 23717481]
- Zhang B, Wang Z, Wu L, Zhang M, Li W, Ding J, et al. Circulating and tumor-infiltrating myeloid-derived suppressor cells in patients with colorectal carcinoma. *PLoS One*. 2013; 8:e57114. [PubMed: 23437326]
- Zhu B, Bando Y, Xiao S, Yang K, Anderson AC, Kuchroo VK, et al. CD11b+Ly-6C(hi) suppressive monocytes in experimental autoimmune encephalomyelitis. *Journal of Immunology*. 2007; 179:5228–5237.
- Zitvogel L, Apetoh L, Ghiringhelli F, Kroemer G. Immunological aspects of cancer chemotherapy. *Nature Reviews. Immunology*. 2008; 8:59–73.
- Zong WX, Thompson CB. Necrotic death as a cell fate. *Genes & Development*. 2006; 20:1–15. [PubMed: 16391229]
- Zoso A, Mazza EM, Bicciato S, Mandruzzato S, Bronte V, Serafini P, et al. Human fibrocytic myeloid-derived suppressor cells express IDO and promote tolerance via Treg-cell expansion. *European Journal of Immunology*. 2014; 44:3307–3319. [PubMed: 25113564]

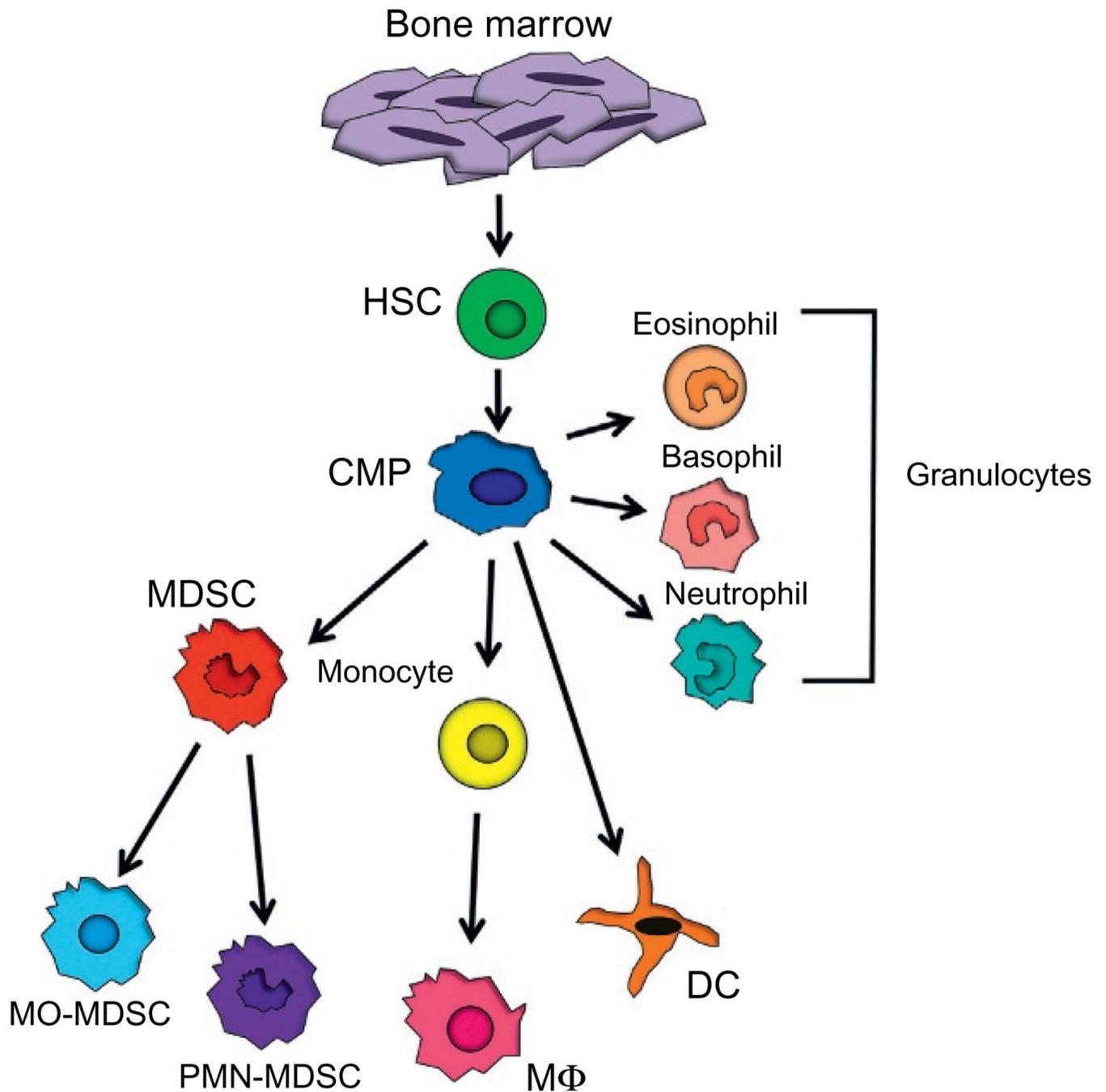


Figure 1.

Myeloid cell differentiation under normal and tumor-induced conditions. Myeloid cells originate from bone marrow-derived hematopoietic stem cells (HSCs) that differentiate into common myeloid progenitors (CMPs). During normal myelopoiesis, CMPs differentiate into granulocytes including eosinophils, basophils, and neutrophils, as well as monocytes, macrophages, and dendritic cells. MDSCs also differentiate from CMPs and are categorized as MO-MDSCs or PMN-MDSCs. HSC, hematopoietic stem cell; CMP, common myeloid

progenitor; DC, dendritic cell; M Φ , macrophage; MO-MDSCs, monocytic myeloid-derived suppressor cells; PMN-MDSCs, polymorphonuclear myeloid-derived suppressor cells.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

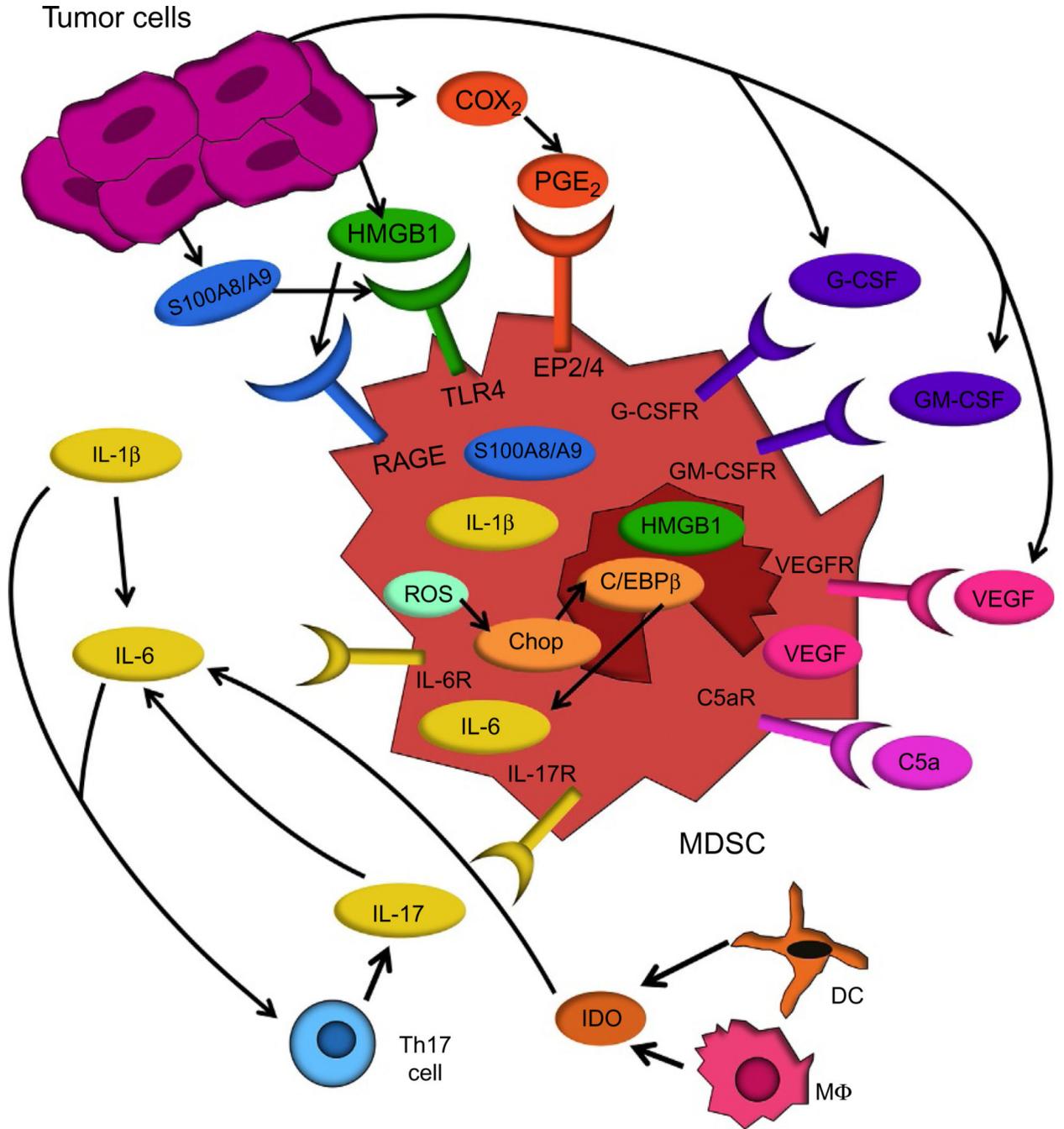


Figure 2. Inflammation drives MDSC development and function. Chronic inflammation induces the production of HMGB1, S100A8/A9, IL-1 β , IL-6, C5a, and IL-17, all of which induce the accumulation of MDSC. Induction of MDSCs by IL-1 β is mediated through IL-17 and IL-6. IL-1 β induces Th17 cells to produce IL-17 which induces the production of IL-6. IL-6 production is also upregulated by IDO produced by DCs and macrophages (M Φ). C/EBP β , which is activated by chop following MDSC production of ROS, also induces IL-6. MDSCs also produce VEGF, IL-6, IL-1 β , HMGB1, and S100A8/A9, thereby perpetuating their

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

accumulation. Tumor cells may produce COX₂, PGE₂, VEGF, IL-6, G-CSF, GM-CSF, S100A8/A9, and HMGB1 all of which induce the accumulation of MDSCs and may increase the suppressive potency of MDSCs.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

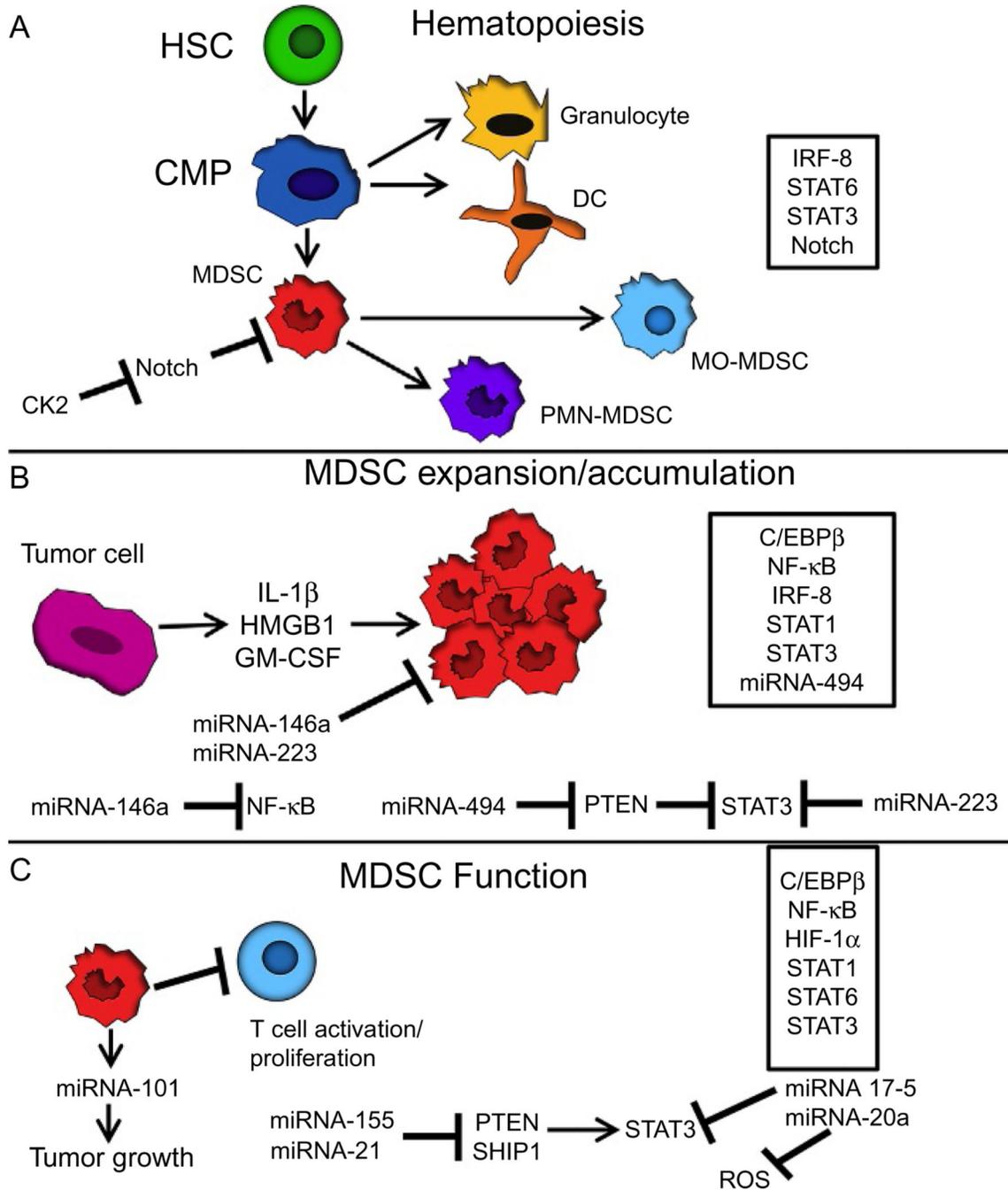


Figure 3. Multiple signal transduction pathways, transcription factors, and microRNAs regulate MDSC accumulation and function. (A) The differentiation of MDSCs from HSC and CMP is regulated by the transcription factors IRF-8, Notch, STAT6, and STAT3. IRF-8 and STAT6 regulate normal myelopoiesis and the differentiation of CMP to mature granulocytes and DCs. During abnormal myelopoiesis, which occurs in individuals with cancer, immature myeloid cells fail to terminally differentiate giving rise to elevated levels of MDSCs. Notch inhibits the differentiation of MDSCs, while CK2 blocks Notch and thereby increases

MDSCs. STAT3 promotes MDSC development and suppressive potency. (B) Tumor and host cells produce multiple inflammatory molecules that perturb myelopoiesis and induce the expansion of MDSCs by activating or inactivating transcription factors. Pro-inflammatory mediators in the tumor microenvironment, such as IL-1 β , HMGB1, and GM-CSF, drive the expansion of MDSCs by activating C/EBP β , NF- κ B, STAT1, STAT3, and miRNA-494 and downregulating IRF-8. Induction of miRNAs 146a and 223 prevents the expansion of MDSC. miRNA-494 promotes the expression of MMPs and inhibits PTEN function resulting in STAT3 induction. miRNA-146a inhibits NF- κ B signaling, while miRNA-223 blocks C/EBP β from binding to the c-myc promoter which downregulates STAT3 expression. (C) MDSC function is positively regulated by C/EBP β , NF- κ B, HIF-1 α , STAT1, STAT6, and STAT3. The miRNAs 155 and 21 inhibit PTEN and SHIP1, negative regulators of STAT3, resulting in the activation of STAT3 and increased MDSC function. miRNAs 17-5 and 20a have the opposite effect by blocking STAT3 and ROS which negatively regulates MDSC function. MDSCs themselves also promote tumor growth by activating miRNA-101 in cancer cells.

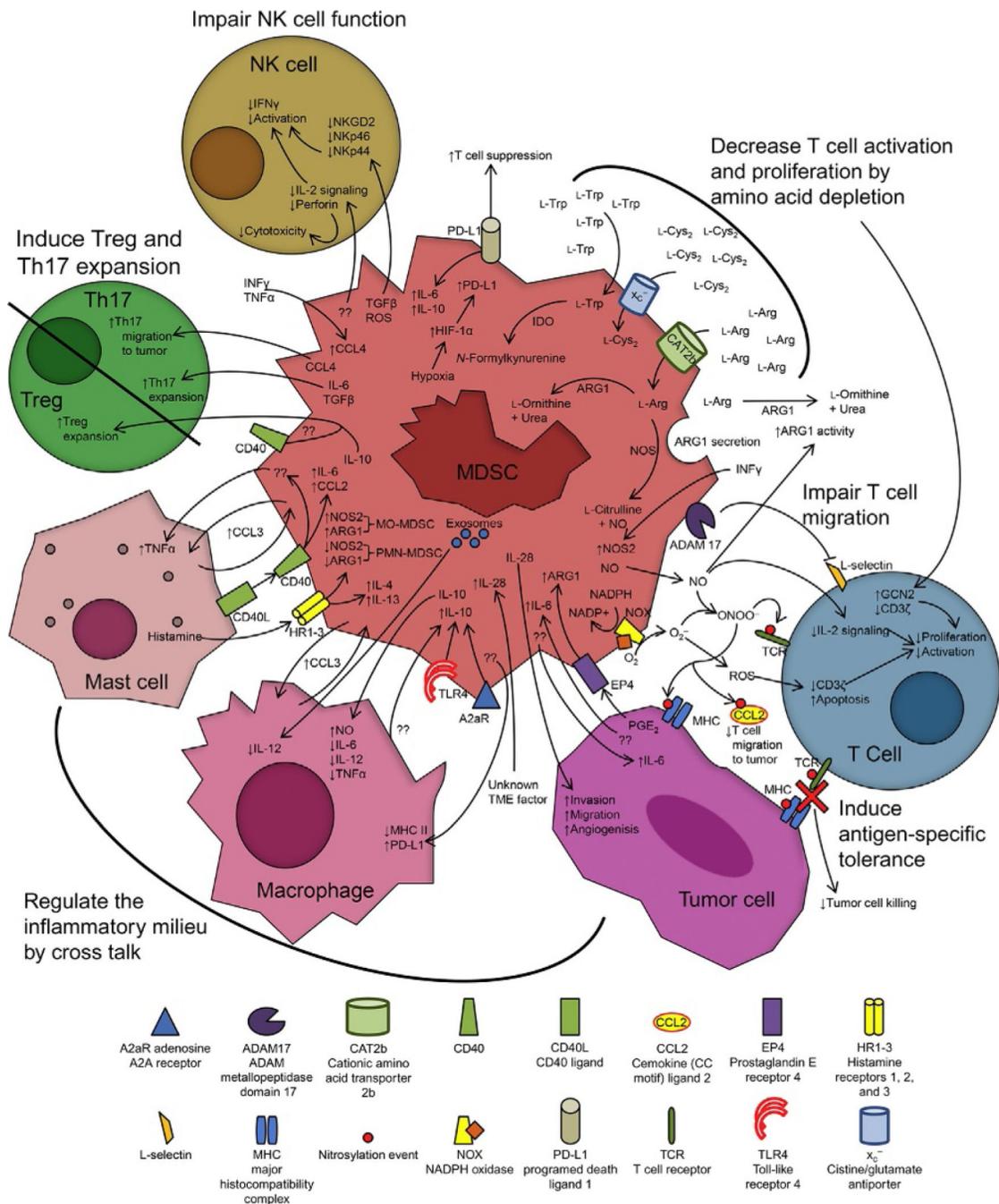


Figure 4. MDSCs suppress T cells and regulate the inflammatory milieu by multiple mechanisms. MDSCs regulate antitumor immunity by (i) secretion of IL-10, which induces Tregs; (ii) secretion of IL-6 and TGF β , which induces Th17 cells; (iii) production of ROS and TGF β , which inhibits NK cell function; (iv) degradation of amino acids essential for T cell activation and proliferation; (v) production of NO and O $_2^-$, which induces apoptosis and inhibits the activation and proliferation of T cells, and generates PNT that nitrates/nitrosylates MHC and TCR; and (vi) participation in cross talk with macrophages, tumor

cells, and mast cells to generate a protumor environment. Question marks denote an unknown mechanism or signaling molecule.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 1

Recently Reported Experimental Therapies Targeting MDSC Development, Viability, or Function

Therapy	Mechanism	Model	Effect
^a 2aG4 (phosphatidyl-serine antibody)	Reverses immunosuppressive effects by phosphatidyl-serine	LNCaP and PC3 prostate cancers (SCID mice)	Induces MDSC differentiation into M1 macrophages and DC; reduces MDSC numbers in tumor
^b 5-AZA (5-azacytidine)	Inhibits DNA methyltransferase	TRAMP-C2 prostate adenoma and TC-1/A9 pancreatic adenoma (C57BL/6 mice)	Reduces MDSC ARG1 expression, VEGF production, and suppressive activity; reduces MDSC accumulation.
^c 5-AZA+ENT (entinostat)	ENT is a class I HDAC inhibitor	CT26 colon carcinoma and 4T1 mammary carcinoma (BALB/c mice)	ENT causes apoptosis of PMN-MDSC <i>in vitro</i> , while 5-AZA has no effect on MDSCs; causes rejection of tumor when 5-AZA+ENT is used in combination with αPD-1+αCTLA-4 immunotherapy
^d ABT-737	Inhibitor of Bcl-2, Bcl-xL, and Bcl-w	CT26 colon carcinoma and 4T1 mammary carcinoma (BALB/c mice)	Increases MDSC susceptibility to FASL-mediated apoptosis; increases apoptosis of MDSC <i>in vivo</i> , not <i>in vitro</i> ; decreases MDSC accumulation
^e Antaxinib	VEGFR antagonist	RENCA renal cell carcinoma (BALB/c mice)	Inhibits STAT3 in MDSC; decreases MDSC ROS and ARG1; increases MDSC apoptosis
^f ATRA (all- <i>trans</i> retinoic acid)	Agonist for retinoic acid receptor	SCLC patients	Causes apoptosis of PMN-MDSCs; differentiates MO-MDSCs to macrophages and DCs; reduces MDSCs in SCLC patients; enhanced the number of responders to p53 vaccine
^g CD16XCD33 BiKE	Targets NK cells to CD33 ⁺ cells	Myelodysplastic syndrome (MDS) patients	Induces NK cell-mediated killing of MDSCs
^h Corosolic acid (Triterpenoid from apple pomace)	Blocks activation of STAT3 and NF-κB; inhibits polarization of macrophages to M2 phenotype	LM85 osteosarcoma (C3H mice)	Reduces MDSC suppressive activity
ⁱ Dopamine	Signals through D1-like DA receptors which inhibited MO-MDSC decreasing NO	LLC and B16 melanoma (C57BL/6 mice)	Reduces MDSC suppressive activity
^j Gemcitabine+Capecitabine	Gemcitabine is a nucleoside analog. Capecitabine is a prodrug that is enzymatically converted to fluorouracil	Pancreatic cancer patients	No direct effect on MDSCs alone, but reduces MDSCs in patients receiving GM-CSF as an adjuvant for GV1001 (GV1001 is a telomerase vaccine)
^k Gemcitabine+Rosiglitazone	Rosiglitazone activates PPARγ, thereby acting as an anti-inflammatory agent	Panc02 pancreatic carcinoma (C57BL/6 mice)	Rosiglitazone reduces early MDSC accumulation; combination therapy reduces late-stage MDSC accumulation
^c J32	PI3K inhibitor	4T1 mammary carcinoma (BALB/c mice)	Causes PMN-MDSC apoptosis; no effect on tumor growth alone; causes tumor rejection in mice when used in combination with αPD-1+αCTLA-4 immunotherapy
^l Lenalidomide (thalidomide derivative)	Inhibits NF-κB, COX-2 activity, and angiogenesis; has immunomodulatory effects	A20 lymphoma (BALB/c mice)	Reduces MDSCs <i>in vivo</i> , but does not alter MDSC levels in naive mice
^m MI-319	HDM2 inhibitor	Human renal cell carcinoma (nude mice)	Reverses sunitinib-induced MDSC infiltration into tumor (sunitinib is a RTK inhibitor and reduces angiogenesis)
ⁿ Polyphenon	Unknown mechanism	Neuroblastoma mouse models: TH-MYC transgenic mice,	Differentiates MO-MDSCs into PMN-MDSCs; reduces suppressive

Therapy	Mechanism	Model	Effect
E (green tea extract)		human SHSY5Y (NOD/SCID mice), Neuro 2A (A/J mice)	activity of MO-MDSCs; decreases ARG1 in MDSCs
^o SAR131675	Inhibits VEGFR-3 tyrosine kinase	4T1 mammary carcinoma (BALB/c mice)	Reduces the number of MDSCs in tumor; promotes M1 macrophages
^p Silibinin	Anti-inflammatory flavonoid	4T1 mammary carcinoma (BALB/c mice)	Reduces tumor volume, increases survival of tumor-bearing mice; decreases total number of MDSCs
^q Tadalafil	PDE5 inhibitor	Human HNSCC patients	Lowers MDSCs and Treg numbers; increases tumor-specific CD8 ⁺ T cells in a dose-dependent manner
^r TCBA (tetrabromocinnamic acid)	Restores Notch signaling	EL4 lymphoma (C57BL/6 mice), CT26 colon carcinoma and MethA sarcoma (BALB/c mice)	Induces differentiation of MDSCs to DCs
^s Vemurafenib	Inhibitor of B-RAF ^{V600E} , a mutation leading to constitutive activation of MAP kinase pathway	Cutaneous melanoma patients	Inhibits the release of soluble factors from melanoma cells involved in the generation of MO-MDSC <i>in vitro</i> ; decreases MO-MDSC <i>in vivo</i>
^t Withaferin A (extract from ashwagandha plant)	Antioxidant with antitumor effects; inhibits Notch signaling and NF-κB activation	4T1 mammary carcinoma (BALB/c mice)	Decreases MDSC production of ROS and IL-10; decreases MDSC suppressive activity; reduces MDSC accumulation

^aYin, Huang, Lynn, and Thorpe (2013).

^bMikyskova et al. (2014).

^cKim et al. (2014).

^dHu et al. (2013).

^eYuan et al. (2014).

^fIclozan, Antonia, Chiappori, Chen, and Gabrilovich (2013).

^gGleason et al. (2014).

^hHorlad et al. (2013).

ⁱWu et al. (2015).

^jAnnels et al. (2014).

^kBunt, Mohr, Bailey, Grandgenett, and Hollingsworth (2013).

^lSakamaki et al. (2014).

^mPanka, Liu, Geissler, and Mier (2013).

ⁿSantilli et al. (2013).

^oEspagnolle et al. (2014).

^pForghani, Khorramizadeh, and Waller (2014).

^qWeed et al. (2015).

^rCheng et al. (2014).

^sSchilling et al. (2013).

^tSinha and Ostrand-Rosenberg (2013).