Myeloid-derived suppressor cells: Immune suppressive cells that impair antitumor immunity and are sculpted by their environment[1]

By

Suzanne Ostrand-Rosenberg[*] and Catherine Fenselau[†]

[*]Department of Biological Sciences, University of Maryland Baltimore County

[†]Department of Chemistry and Biochemistry, University of Maryland, College Park

Running title: Immune suppressive MDSC

Corresponding author: S. Ostrand-Rosenberg, Department of Biological Sciences, University of Maryland Baltimore County (UMBC), srosenbe@umbc.edu; 410 455-2237 (voice); 410 455-3875 (FAX)

Abstract

Myeloid-derived suppressor cells (MDSC) are a diverse population of immature myeloid cells that have potent immune suppressive activity. Studies in both mice and humans have demonstrated that MDSC accumulate in most individuals with cancer where they promote tumor progression, inhibit antitumor immunity, and are an obstacle to many cancer immunotherapies. As a result, there has been intense interest in understanding the mechanisms and in situ conditions that regulate and sustain MDSC, and the mechanisms MDSC use to promote tumor progression. This article reviews the characterization of MDSC and how they are distinguished from neutrophils, describes the suppressive mechanisms used by MDSC to mediate their effects, and explains the role of pro-inflammatory mediators and the tumor microenvironment in driving MDSC accumulation, suppressive potency, and survival.

The term "myeloid-derived suppressor cells" (MDSC) was coined in 2007 to encompass a collection of non-macrophage cells of myeloid origin that have potent immune suppressive activity and that are phenotypically characterized by a constellation of markers, none of which are unique to MDSC (1). The name was chosen because the cells encompass a range of immature cells whose unifying characteristics are their myeloid origin and their ability to suppress T cell activation and T cell function. Cells with a similar function called "natural suppressor cells" were reported in the 1980's (2-5); reviewed by (6). Such suppressor cells were largely ignored by immunologists until the late 1990's and early 2000's when it became apparent that antitumor immunity was suppressed by cells of myeloid origin (7-12). As investigators become more aware of MDSC and tested for them in both cancer patients and mice with tumors, MDSC were increasingly recognized as being a major spoiler of antitumor immunity because they accumulate in virtually all individuals with cancer (13, 14). This review will describe the basic features of MDSC and how they are identified, and will then review some of the recent studies that have provided significant insight into how MDSC are induced and inhibit antitumor immunity, and how they are molded by the tumor microenvironment.

MDSC are immature myeloid cells

MDSC encompass a range of myeloid cells that are developmentally immature and in different stages of myelopoiesis. They are phenotypically defined by a constellation of markers. Since none of these markers are unique to MDSC, and there is overlap of some of these markers with other cell populations, phenotyping in combination with assessing immune suppressive activity is the optimal strategy for identifying MDSC. Since there has been considerable discussion about the nomenclature, phenotype, and function of this cell population, an international group of investigators in the field recently recommended nomenclature and characterization standards for MDSC (15). An international consortium of 23 laboratories has also been organized to test human MDSC with the goal of

harmonizing staining and gating procedures for analysis of human MDSC (16). The phenotypes reported in these studies are used in the following descriptions and are shown in **figure 1**.

Initial studies identified two major subtypes of MDSC in mice, monocytic (M-MDSC) and granulocytic (PMN-MDSC) (17). M-MDSC are mononuclear and PMN-MDSC are polymorphonuclear. Both types express the myeloid lineage marker CD11b and the granulocytic marker Gr1. Gr1 includes two distinct molecules, Ly6C and Ly6G. M-MDSC have a lower level of expression of Gr1 and express Ly6C, while PMN-MDSC have higher levels of Gr1 and express Ly6G. The expression of additional markers varies depending on the tumor system. Functionally, mouse M-MDSC are also characterized by their high levels of nitric oxide (NO) and inducible NO synthase (iNOS/NOS_2), while PMN-MDSC contain higher levels of reactive oxygen species (ROS).

There are also two types of human MDSC. Both types express CD11b; however, there is no equivalent to the mouse Gr1 marker. Instead, human M-MDSC are characterized by their expression of CD14 and PMN-MDSC by their expression of CD15 and CD66b. Both types also express the general myeloid maker CD33 and lack linage markers for lymphocytes and NK cells. Since these markers are also expressed by monocytes, MDSC are distinguished from monocytes by their absence of HLA-DR.

Since human peripheral blood leukocytes are frequently cryopreserved prior to testing, the effects of these treatments on MDSC have been examined. PMN-MDSC are particularly sensitive to cryopreservation (18, 19). Likewise, both arginase (Arg1) and ROS are lost with freezing (18). Given these constraints, phenotypic analysis of human MDSC is only accurate if fresh blood samples are tested. Mouse MDSC are typically assessed immediately after being harvested from mice, so freezing is usually not performed; however, mouse M-MDSC and their functions are stable when frozen at liquid nitrogen temperatures.

PMN-MDSC and neutrophils share some common features but are functionally and phenotypically distinct

Defining PMN-MDSC as a distinct population has met with controversy among some investigators since PMN-MDSC and some types of neutrophils have a similar phenotype, share a multi-lobed nuclear morphology, and share some common pro-tumor functions (20-22). Although neutrophils traditionally may have antitumor activity, investigators have ascribed immune suppressive activity to another group of neutrophils. The latter have been termed N2 neutrophils, while the former are termed N1 neutrophils (23, 24). The controversy over identification is whether N2 neutrophils are MDSC or vice-versa. Multiple clinical studies have documented that patients with a variety of solid tumors who have a high baseline level of neutrophils in the blood or in the tumor mass have a poor prognosis and do not respond to medical interventions (reviewed by (25, 26)). Because most studies use only markers shared by PMN-MDSC and neutrophils, it is not possible to retrospectively discern if neutrophils or PMN-MDSC were assessed.

However, there are definitive differences between classical neutrophils and PMN-MDSC. The transcriptomes of mouse neutrophils, tumor-associated neutrophils (TANs) and PMN-MDSC differ. Although neutrophils lack immune suppressive activity, their mRNA repertoire is more similar to PMN-MDSC, while the transcriptome of TANs and PMN-MDSC are less related (27). The predominant differences are in cytokine and MHC antigen presentation transcripts. At the proteomic level mass spectrometry studies also revealed that MDSC express a distinct profile (28-31), with S100A8 and S100A9 being prominently expressed by MDSC (28, 32) .

PMN-MDSC and neutrophils also differ in their expression of at least some cell surface markers. For example, human neutrophils undergoing ER stress, as occurs within solid tumors, become immune suppressive PMN-MDSC and express the lectin-type oxidized low-density lipoprotein receptor 1 *OLR1* (LOX-1) (33). Similarly, tumor-induced MDSC from C57BL/6 mice express CD115 and CD224, while

neutrophils express neither of these plasma membrane molecules (34). The latter study also demonstrated that whereas mouse PMN-MDSC expressed more Arg1, myeloperoxidase, and ROS, neutrophils produced more TNFα and lysosomal proteins, and were more phagocytic.

Whether PMN-MDSC are a type of neutrophil or are a distinct granulocyte population remains to be resolved. The distinction is relevant for semantic and categorizing purposes. However, a goal of immunotherapy is to neutralize or eliminate immune suppression, so an in-depth understanding of the functional properties of relevant immune suppressive cells is essential, and whether the cells are called PMN-MDSC or neutrophils may be less important.

Environmental conditions within tumors drive MDSC accumulation and suppressive potency

MDSC are generated in the bone marrow from myeloid progenitor cells and then traffic through the circulatory system where they can mix with circulating malignant cells of hematopoietic origin, or migrate into solid tumors. Tumor-produced growth factors are responsible for increasing the generation of M-MDSC and PMN-MDSC and recruiting them from the bone marrow (and in mice also from the spleen) to solid tumors and for sustaining their levels in blood. However once in the tumor microenvironment most M-MDSC differentiate into immune suppressive tumor-associated macrophages (TAMs) (35, 36).

The tumor microenvironment is a complex and evolving milieu of tumor cells and host cells, and there is extensive cross-talk between and among cell populations. This cross-talk reciprocally molds the phenotype and function of both MDSC and host tumor-infiltrating cells (37). **Figure 2** shows the growth factors and tumor microenvironment elements that regulate MDSC induction and accumulation and are discussed below.

Growth factors regulating myelopoiesis are important molecules for inducing the accumulation and suppressive activity of MDSC. Cancer patients frequently have abnormal or “emergency”

myelopoiesis due to dysregulated production of hematopoietic growth factors. VEGF is predominantly thought of as a growth factor that supports tumor progression by promoting neoangiogenesis. It is frequently present and upregulated by hypoxia in the tumor microenvironment. Studies in non-small-cell lung cancer (NSCLC) patients demonstrated that VEGF is a chemoattractant for MDSC (38-40), and mouse studies have demonstrated that MDSC produce VEGF (41). GM-CSF and G-CSF, important growth factors that regulate myelopoiesis, also drive the accumulation and suppressive function of MDSC in both mice (42-45) and patients (46) with cancer.

Emergency myelopoiesis, which is controlled by the C/EBPβ transcription factor, often accompanies the chronic inflammation that exists in many solid tumors, and is also present in other inflammatory conditions, including infections, autoimmunity, obesity, and stress. These conditions have led to the understanding that chronic inflammation is a major driving force for MDSC, and the hypothesis that one of the mechanisms by which inflammation drives cancer risk and tumor progression is through the suppression of antitumor immunity (47). Studies with tumor-bearing mice have shown that a variety of pro-inflammatory mediators can drive MDSC.

IL-6 and IL-1β are potent pro-inflammatory mediators that have been linked to the induction and progression of multiple cancers. Early studies using knockout mice and gene over-expression demonstrated the role of these molecules in driving both the accumulation and suppressive potency of mouse MDSC (48-51). IL-6 is likely down-stream of IL-1β since MDSC induction is restored in IL-1R-deficient mice by provision of IL-6 (49). TNFα, another potent pro-inflammatory mediator that is commonly found in the tumor microenvironment, also increases the quantity and suppressive activity of MDSC (52). Drug inhibition of TNFα during early stages of inflammation allows immature myeloid cells to exit the MDSC state and differentiate into DC and macrophages. It also reverses the down-regulation of the TcRζ chain in T cells that is characteristic of MDSC-mediated T cell suppression (53, 54).

Prostaglandin E2 (PGE_2) and cyclooxygenase-2 (COX_2), the enzyme that generates PGE_2 from arachidonic acid, are key pro-inflammatory mediators that are produced by many mouse and human cancer cells. In vitro studies using PGE_2 receptor inhibitors, PGE_2 receptor knockout mice, and non-steroidal anti-inflammatory drugs that block PGE_2 established that in mice PGE_2 drives the differentiation of MDSC from bone marrow progenitor cells (55-57). PGE_2 also drives the differentiation of MDSC from human hematopoietic stem cells (58), and for M-MDSC the induction is via the p38MAPK/ERK pathway (59, 60). PGE_2 drives the suppressive potency of MDSC by increasing their content of Arg1 (61).

IL-17 is another cytokine that is present in the environment of many solid tumors and regulates the accumulation and suppressive activity of MDSC. Studies in IL-17-deficient tumor-bearing mice demonstrated that IL-17 increases intratumoral levels of MDSC and raises their intracellular levels of Arg1, COX_2, and the immune suppressive molecule indole-amine 2,3 dioxygenase (IDO) (62). PMN-MDSC and total MDSC levels are increased in colorectal cancer by the production of IL-17, TNFα, and GM-CSF by $\gamma\delta$ T17 cells (63).

Other mediators that are major contributors to an inflammatory tumor microenvironment have also been shown to induce MDSC. The pro-inflammatory calcium-binding proteins S100A8 and S100A9 are particularly active. These proteins function as a heterodimer (S100A8/A9) and are ubiquitously present in the microenvironment of most tumors. They are also present in the plasma membrane of tumor-induced mouse MDSC as shown by spectral counting and mass spectrometry (28). S100A8/A9 are regulated by STAT3 and NF-κB, and their overexpression results in an increase in MDSC at the expense of fewer DC and macrophages (64, 65). MDSC themselves produce S100A8/A9 which increases MDSC suppressive activity and serves as a chemoattractant for MDSC. The heterodimer acts by binding to the N-glycan motif of the receptor for advanced glycation end products (RAGE) (65). Interestingly,

TNFα also drives the suppressive activity of MDSC by increasing S100A8/A9 levels which signal through RAGE (53).

Recent studies have identified even more factors that regulate MDSC levels and activity. High mobility group box protein 1 (HMGB1) is present in the nucleus where it forms a scaffolding for DNA, but when released from cells, functions as a damage associated molecular pattern molecule (DAMP) or alarmin. As for S100A8/A9, HMGB1 is ubiquitously present in the tumor microenvironment and RAGE is one of its plasma membrane receptors. HMGB1 drives the differentiation of MDSC from bone marrow progenitor cells, increases MDSC production of IL-10, enhances MDSC cross-talk with macrophages, and promotes MDSC-mediated down-regulation of L-selectin expression on naïve T cells.

Using head and neck squamous cell carcinoma cells (HNSCC), the induction of human MDSC has also been ascribed to semaphorin 4D (Sema4D), a proangiogenic cytokine that is produced by several types of cancers (66). Inhibition of Sema4D in the supernatants of cultured HNSCC cells resulted in decreased levels of MDSC with reduced content of Arg1, TGFβ, and IL-10, and concurrent restoration of T cell activation.

Estrogen also induces both mouse and human MDSC. In mouse tumor models in which the tumor cells are non-responsive to estrogen, estrogen has been shown to dysregulate myelopoiesis and drive the accumulation and suppressive activity of MDSC (67). Estrogen mediates its effects by binding to its receptor on myeloid progenitor cells in bone marrow and subsequently activating the STAT3 pathway and enhancing JAK2 and SRC. These findings suggest that estrogen antagonists may decrease tumor progression and enhance antitumor immunity even in individuals whose tumor cells do not express estrogen receptors.

Complement has also been shown to modulate the anti-tumor immune response. Tumor progression has been demonstrated in mice carrying TC-1 cervical carcinoma to be reduced after

knockout of complement proteins C3 or C4, and complement component C5a was found to drive accumulation of MDSC (68).

Because of the extensive redundancy in MDSC inducers, depletion of one mediator may be compensated by the presence of other mediators. As a result, elimination of a single inducer may reduce the levels and suppressive potency of some MDSC, but is unlikely to eliminate all MDSC.

MDSC survival

Circulating and tumor-infiltrating M-MDSC and PMN-MDSC have a short in vivo lifespan of approximately one to two days. PMN-MDSC also do not survive longer in vitro, while M-MDSC are viable in vitro for several days. Higher levels of inflammation result in more circulating MDSC, suggesting that inflammation may prolong the half-life of MDSC (49). Mass spectrometry studies identified Fas pathway and caspase network proteins in MDSC induced under a heightened inflammatory milieu, and cellular studies with Fas agonists demonstrated that inflammation increases MDSC resistance to Fas-mediated apoptosis (29, 69, 70). As described above, HMGB1 is a pro-inflammatory mediator that is ubiquitously present in the tumor microenvironment. In addition to driving the development of MDSC, HMGB1 also regulates MDSC survival by rendering MDSC more autophagic. MDSC in the blood have a default autophagic phenotype, and tumor-infiltrating MDSC are more autophagic due to the inflammatory tumor microenvironment (71).

Although ROS are toxic to most cells, MDSC are largely resistant to both their internal content of ROS and the ROS they release extracellularly. Nuclear factor erythroid 2-related factor 2 (Nrf2), a transcription factor that regulates a battery of genes that ameliorate oxidative stress, enhances MDSC resistance to ROS. Studies with Nrf2-deficient mice demonstrated that Nrf2 reduces oxidative stress and apoptosis of tumor-infiltrating MDSC, thereby increasing the half-life of MDSC within solid tumors. Interestingly, these studies also demonstrated that there is a strong homeostatic regulation of MDSC

and that when the half-life of tumor-infiltrating MDSC is decreased, there is a compensatory increase in the rate of MDSC production in the bone marrow so that steady-state levels of MDSC in the blood are maintained (72).

MDSC use a variety of mechanisms to promote tumor progression

MDSC mediate both immune and non-immune suppressive mechanisms (**Figure 3**). They promote tumor growth by facilitating neovascularization through their production of VEGF, and by driving invasion and metastasis through their production of matrix metalloproteases (41). Arg1 and ROS are the classic molecules used by MDSC to prevent T cell activation and function. Arg1 depletes arginine, an essential amino acid for T cell activation and function, while ROS kills target cells by causing oxidative stress. Since these mechanisms have been recently comprehensively reviewed in other publications (73-77), they will not be further described here.

MDSC also use other mechanisms to inhibit innate and adaptive immunity. They sequester cysteine, another essential amino acid for T cell activation and function (78), polarize macrophages towards a tumor-promoting phenotype by down-regulating macrophage production of the type 1 cytokine IL-12 (79, 80), inhibit NK-mediated tumor cell lysis (81), and induce and recruit T regulatory cells (82-84). MDSC mediate these suppressive mechanisms by cell-to-cell contact with either T cells, macrophages, or NK cells, and these events predominantly occur in the tumor microenvironment. However, MDSC also inhibit T cell activation and function via a systemic mechanism involving MDSC-mediated down-regulation of L-selectin (CD62L) on circulating naïve T cells (85). L-selectin is essential for naïve T cell extravasation from blood vessels and entry into lymph nodes and subsequent T cell activation in lymph nodes. Recent in vivo imaging studies have confirmed that while circulating in the blood MDSC down-regulate T cell expression of L-selectin, and that this down-regulation prevents naïve T cells from entering lymph nodes and becoming activated (86).

MDSC also contribute to the process of malignant transformation and tumor progression through non-immune suppressive mechanisms. In a mouse model of epidermal carcinogenesis, immature myeloid cells with the phenotype of MDSC accumulated in the skin prior to the onset of malignancy. The MDSC secreted CCL4 which chemoattracted Th17-producing $CD4^+$ T cells and resulted in increased papilloma formation. Depletion of either CCL4 or the $CD4^+$ T cells prevented the effect, demonstrating that MDSC indirectly drive epidermal carcinogenesis (87). MDSC also promote tumor progression by endowing breast cancer cells with stem-like characteristics. They mediate this effect through their production of IL-6 which activates STAT3 in cancer cells and by their production of NO which activates the Notch pathway and sustains STAT3 activation (88). MDSC of ovarian cancer patients increased cancer cell stemness by driving miRNA101 expression in the cancer cells. miRNA101, in turn, down-regulated the corepressor gene C-terminal binding protein-2 (CtBP2), resulting in increased expression of cancer stem cell genes (89).

Cancer stem cells reciprocally effect MDSC. In a mouse model of glioblastoma, cancer stem cells were found to secrete macrophage migration inhibitory factor (MIF). MIF increased the suppressive potency of MDSC by increasing Arg1 levels via a CXCR2-dependent pathway (90).

MDSC accumulation and function are regulated by multiple transcription and epigenetic factors

MDSC are regulated through multiple and overlapping signal transduction pathways, demonstrating their ability to be induced and function by varied environment conditions. This section will briefly review the more prominent pathways (see **figure 2**). A more in-depth description can be found in a recent review (91).

During normal myelopoiesis in healthy individuals, the common myeloid progenitor cell (CMP) gives rise to dendritic cells, macrophages, and granulocytes (neutrophils, basophils, and eosinophils). However, under the influence of tumor-produced factors including pro-inflammatory cytokines, myeloid

cells deviate from their normal differentiation pathway and become immune suppressive MDSC. Early studies identified signal transducer activator of transcription 3 (STAT3) as a key player in the accumulation of mouse MDSC (92). Multiple subsequent studies confirmed the role of STAT3 (93). Established inducers of MDSC such as G-CSF, GM-CSF, and IL-6 act by turning-on STAT3 (94). Given the critical role of STAT3, drugs such as sunitinib that prevent STAT3 activation have been used to limit the accumulation of MDSC in both mice and humans carrying tumor (95-97).

STAT3 promotes MDSC accumulation and function through multiple mechanisms. It facilitates the survival of both mouse and human MDSC by up-regulating the proliferation gene cyclin D1 and the antiapoptotic genes Bcl-xl and c-myc (98, 99). It also induces S100A8/A9 expression, which drives the accumulation/differentiation of MDSC (64). Phosphorylation of STAT3 drives MDSC suppressive potency by increasing the expression of gp47 and gp91, two subunits of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX2). NOX2 generates superoxide by reducing oxygen (100). When superoxide reacts with nitric oxide (NO), peroxynitrite is produced. Peroxynitrite released by MDSC nitrates the TcR and MHC class I, thereby perturbing T cell recognition and preventing both T cell activation and T cell function (101, 102). MDSC that are deficient for $gp91^{phox}$ lack suppressive activity and differentiate to DC and macrophages (100). Phosphorylation of STAT3 is also likely to be responsible for the increased suppressive potency of MDSC within hypoxic regions of solid tumors since these regions contain activated hypoxia-inducible factor-1 alpha (HIF1α) which activates STAT3 (35, 36).

Interferon-inducible regulatory factor 8 (IRF8) is another transcription factor that is important in MDSC accumulation. In contrast to STAT3 which promotes MDSC accumulation, IRF8 deters PMN-MDSC accumulation. IRF8 is activated in the GMP stage of myelopoiesis. It diverts differentiation away from granulocytic lineages and blocks the accumulation of MDSC because it prevents STAT3 activation and thereby limits the level of ROS. As a result, IRF8-deficient mice have high levels of PMN-MDSC (103, 104), and over-expression of IRF8 in the myeloid lineage reduces MDSC accumulation (105, 106). Similar

to IRF8, IRF4 expression also decreases MDSC levels in tumor-bearing mice, and myeloid-specific deletion of IRF4 produces an increase in MDSC (107).

Additional transcription factors and their receptors are also involved in regulating MDSC levels. C/EBPβ preferentially regulates M-MDSC since mice deficient for this transcription factor have reduced levels of M-MDSC (108). Likewise, elements of the ER stress pathway also contribute to MDSC accumulation. MDSC of both tumor-bearing mice and cancer patients contain elevated levels of C/EBP homologous protein (CHOP), an indicator of ER stress (109), and induction of ER stress in tumor-bearing mice increased the levels of tumor-infiltrating MDSC and increased their suppressive potency by up-regulating Arg1 and iNOS (110). MDSC levels are reduced and the remaining MDSC lose their immune suppressive function and become antigen-presenting cells in CHOP-deficient mice, indicating that CHOP is a positive regulator of MDSC (111). CHOP mediates this effect by impairing C/EBPβ signaling in MDSC which, in turn, decreases MDSC production of IL-6. Translocation of NF-κB from the cytosol to the nucleus also enhances MDSC suppressive function. Studies have shown that such activation is MyD88-dependent and occurs in conjunction with TLR2 or TLR4-mediated signaling (112-114). The retinoic-acid-related orphan receptor (RORC1/RORγ) directs emergency myelopoiesis in individuals with cancer, and ablation of RORC1 in hematopoietic cells reduces the accumulation of MDSC, and prevents tumor development. RORC1 is active in both mouse and human MDSC (and in macrophages) and acts by promoting C/EBPβ and suppressing negative regulators Socs3 and Bcl3 (115).

Accumulation and function of mouse MDSC are also regulated by an epigenetic mechanism. Production of the type 2 cytokine IL-10 is a key distinguishing characteristic of MDSC and contributes to the ability of MDSC to polarize immunity towards a tumor-promoting phenotype. Histone deacetylase 11 (HDAC11) regulates IL-10 production, and HDAC11-knockout mice have higher levels and more suppressive MDSC, suggesting that HDAC11 is a negative regulator for the development of MDSC (116).

Collectively, these studies demonstrate that MDSC are regulated by a variety of mechanisms and through multiple signal transduction pathways.

MDSC-derived exosomes are immune suppressive

In individuals with cancer, body fluids and the local tumor microenvironment contain an abundance of exosomal vesicles derived from tumor cells. These vesicles contain proteins, RNAs, and miRNAs and act as intercellular messengers. Many of these vesicles contribute to the immune suppression common in cancer patients (117, 118), and in mouse models, tumor-derived exosomes have been shown to induce MDSC (119).

In addition to exosomes shed by tumor cells, host cells in the tumor microenvironment also generate exosomes, and recent studies demonstrated that MDSC-derived exosomes mediate some of the immune suppressive effects attributed to their parental cells. Using mass spectrometry and bottom-up proteomic analysis, >400 proteins were identified in exosomes derived from mouse tumor-induced MDSC (120). Spectral counting demonstrated a greater abundance of 63 of these proteins if the MDSC developed in a more inflammatory tumor microenvironment, indicating that the level of inflammation impacts MDSC content. Chemotactic assays indicated that >90 % of the MDSC associated pro-inflammatory S100A8/A9 proteins were carried in exosomes. Functional studies demonstrated that the MDSC-derived exosomes efficiently polarized macrophages towards a type 2 tumor-promoting phenotype and were chemotactic for intact MDSC. Both activities were mediated by S100A8/A9 (120). A more recent proteomic analysis of 1188 proteins indicates that the neutrophil degranulation pathway is enhanced by inflammation in MDSC exosomes (121).

Mass spectrometry studies have also revealed that MDSC-derived exosomes contain ubiquitinated proteins using the conservative requirement that tryptic peptides contain glycinylglycine-modified lysine residues. S100A8/A9 and HMGB1 were among the ubiquitinated species detected (122).

In vitro studies using antibody blocking demonstrated that ubiquitinated proteins in exosomes mediate MDSC chemotaxis (121). This latter observation is surprising since although ubiquitinated species are implicated in endosomal trafficking, their function in chemotaxis has not previously been reported.

In addition to delivering their soluble contents to target cells, MDSC-derived exosomes also display plasma membrane glycoproteins that impact target cell communication and function (28). Using cell surface chemistry, 93 N-linked glycoproteins were identified on the surface of parental tumor-induced MDSC (123). Twenty-one of these glycoproteins were also present in the membranes of MDSC-derived exosomes, and included CD47, the "don't eat me" molecule, and its two ligands SIRPα and thrombospondin-1 (TSP). In vitro studies demonstrated that exosomal CD47 served as a powerful chemotactic signal for parental MDSC with TSP-1 being the predominant ligand and SIRPα playing a lesser role (123). Because CD47 protects cells from macrophage phagocytosis (124), MDSC expression of CD47 may be another mechanism sustaining MDSC survival in the tumor microenvironment.

Next-generation sequencing and bottom-up proteomics were used in an integrated study of proteins, mRNA and miRNA carried in exosomes shed by MDSC (125). Over 40,000 mRNAs were present in the exosomes and approximately 34% of these mRNAs were more abundant in the exosomes than in parental MDSC. A majority of the mRNA transcripts found were capped and translationally competent. Over 1400 miRNAs were also present and approximately half of these were more abundant in MDSC that developed in a heightened inflammatory environment. Ninety-one percent of the proteins carried by these immunosuppressive exosomes were also encoded by the mRNA transcripts present, suggesting mechanistic redundancy. The mRNA and miRNA results also suggest that MDSC may amplify and sustain their immune suppressive activity by transferring nucleic acids that have the potential to be incorporated into the genetic makeup of target cells.

Collectively, the studies with MDSC-derived exosomes demonstrate that MDSC mediate their suppressive effects not only directly but also indirectly via exosomes, thereby increasing the range over which they function.

Conclusions

During the last approximately 10 years MDSC have come to be recognized as a major obstacle to both natural antitumor immunity and to many immunotherapies. This recognition has occurred as clinical studies have demonstrated the presence of MDSC in most cancer patients. The relevance of MDSC as roadblocks of antitumor immunity has been further recognized in the context of checkpoint inhibitor immunotherapy because MDSC are likely a limiting factor for the efficacy of checkpoint inhibition therapy (126-128)

Extensive work in mouse systems and to a lesser extent in human systems, has demonstrated that MDSC are a heterogeneous population of immature myeloid cells that are induced by multiple myeloid growth factors and that inflammation is a key driving mechanism for enhancing MDSC levels and suppressive potency. Studies have also revealed that MDSC have significant plasticity and that they undergo cross-talk with their neighboring cells. Accordingly, accumulation and function of MDSC are dependent on and sculpted by their microenvironment. Furthermore, these multi-talented cells use a variety of apparently independent mechanisms to impair antitumor immunity and promote tumor growth. Given the critical role that MDSC play as a spoiler of antitumor immunity, many on-going studies are aimed at discovering therapeutic strategies for neutralizing or eliminating MDSC.

Given the detrimental effects of MDSC, one wonders why such a population of cells would be evolutionarily maintained. Recent work in two conditions provides potential insight into this question (see **Figure 3**). During pregnancy, maternal tolerance is essential for maintenance of the allogeneic fetus, and there is evidence that maternal-fetal tolerance is at least partially due to MDSC. PMN-MDSC

accumulate in the peripheral blood of pregnant women and in the cord blood of healthy newborns. Within days after birth the level of MDSC in the mother's blood reverts to the levels of non-pregnant women. These MDSC share characteristics with tumor-induced PMN-MDSC in that they suppress T cell activation via Arg1 and ROS, and they polarize immunity towards a type 2 cytokine response (129, 130). Confirmation that these MDSC contribute to maternal-fetal tolerance and are not merely bystanders or passenger cells has been obtained in mice (131). Similar to pregnant women, levels of MDSC increase significantly in the blood of female mice carrying allogeneic fetuses, and MDSC are present in uteri containing viable fetuses. The cells are predominantly PMN-MDSC and they suppress T cell activation and impair naïve T cell trafficking into lymph nodes. Knockout and replacement studies demonstrated that the MDSC are essential for successful implantation and maintenance of pregnancy. Low grade inflammation occurs in the uterus following conception, providing a mechanism for the induction of MDSC during pregnancy.

MDSC also appear to play a beneficial role in the metabolic dysfunction associated with obesity and long term high fat diet, situations in which low grade inflammation is chronic (132, 133). Obese *Ob/Ob* mice or mice on a high fat diet for extended periods develop elevated blood glucose levels and insulin tolerance, and their levels of circulating MDSC and MDSC in adipose tissue increase significantly. Tumor progression is more rapid in these mice due to the elevated levels of MDSC. However, MDSC depletion further increases glucose levels and insulin tolerance, indicating that MDSC protect against metabolic dysfunction.

Therefore, although MDSC are detrimental in individuals with cancer because they suppress antitumor immunity and thereby promote tumor growth, they may have evolved because of their protective effects with respect to diet and pregnancy. Additionally, there may be no negative selective pressure on MDSC since cancer is predominantly a disease of individuals beyond reproductive age. We do not at present understand the evolutionary origin of MDSC. However, if the current obesity epidemic

continues, and if MDSC continue to limit the metabolic dysfunction associated with obesity, then evolution may favor increasing MDSC levels, and the rates of tumor onset and progression may also increase.

References

1. Gabrilovich, D. I., V. Bronte, S. H. Chen, M. P. Colombo, A. Ochoa, S. Ostrand-Rosenberg, and H. Schreiber. 2007. The terminology issue for myeloid-derived suppressor cells. *Cancer Res.* 67: 425; author reply 426.
2. Strober, S. 1984. Natural suppressor (NS) cells, neonatal tolerance, and total lymphoid irradiation: exploring obscure relationships. *Annu. Rev. Immunol.* 2: 219-237.
3. Choi, K. L., T. Maier, J. H. Holda, and H. N. Claman. 1988. Suppression of cytotoxic T-cell generation by natural suppressor cells from mice with GVHD is partially reversed by indomethacin. *Cellular immunology* 112: 271-278.
4. Subiza, J. L., J. E. Vinuela, R. Rodriguez, J. Gil, M. A. Figueredo, and E. G. De La Concha. 1989. Development of splenic natural suppressor (NS) cells in Ehrlich tumor-bearing mice. *International journal of cancer* 44: 307-314.
5. Young, M. R., S. Aquino, and M. E. Young. 1989. Differential induction of hematopoiesis and immune suppressor cells in the bone marrow versus in the spleen by Lewis lung carcinoma variants. *J Leukoc Biol* 45: 262-273.
6. Talmadge, J. E., and D. I. Gabrilovich. 2013. History of myeloid-derived suppressor cells. *Nat Rev Cancer* 13: 739-752.
7. Almand, B., J. I. Clark, E. Nikitina, J. van Beynen, N. R. English, S. C. Knight, D. P. Carbone, and D. I. Gabrilovich. 2001. Increased production of immature myeloid cells in cancer patients: a mechanism of immunosuppression in cancer. *J. Immunol.* 166: 678-689.
8. Bronte, V., E. Apolloni, A. Cabrelle, R. Ronca, P. Serafini, P. Zamboni, N. P. Restifo, and P. Zanovello. 2000. Identification of a CD11b(+)/Gr-1(+)/CD31(+) myeloid progenitor capable of activating or suppressing CD8(+) T cells. *Blood* 96: 3838-3846.

9. Bronte, V., D. B. Chappell, E. Apolloni, A. Cabrelle, M. Wang, P. Hwu, and N. P. Restifo. 1999. Unopposed production of granulocyte-macrophage colony-stimulating factor by tumors inhibits CD8+ T cell responses by dysregulating antigen-presenting cell maturation. *J. Immunol.* 162: 5728-5737.

10. Bronte, V., M. Wang, W. W. Overwijk, D. R. Surman, F. Pericle, S. A. Rosenberg, and N. P. Restifo. 1998. Apoptotic death of CD8+ T lymphocytes after immunization: induction of a suppressive population of Mac-1+/Gr-1+ cells. *J. Immunol.* 161: 5313-5320.

11. Gabrilovich, D., T. Ishida, T. Oyama, S. Ran, V. Kravtsov, S. Nadaf, and D. P. Carbone. 1998. Vascular endothelial growth factor inhibits the development of dendritic cells and dramatically affects the differentiation of multiple hematopoietic lineages in vivo. *Blood* 92: 4150-4166.

12. Sinha, P., V. K. Clements, and S. Ostrand-Rosenberg. 2005. Interleukin-13-regulated M2 macrophages in combination with myeloid suppressor cells block immune surveillance against metastasis. *Cancer Res.* 65: 11743-11751.

13. Messmer, M. N., C. S. Netherby, D. Banik, and S. I. Abrams. 2015. Tumor-induced myeloid dysfunction and its implications for cancer immunotherapy. *Cancer Immunol. Immunother.* 64: 1-13.

14. Solito, S., I. Marigo, L. Pinton, V. Damuzzo, S. Mandruzzato, and V. Bronte. 2014. Myeloid-derived suppressor cell heterogeneity in human cancers. *Ann. N Y Acad. Sci.* 1319: 47-65.

15. Bronte, V., S. Brandau, S. H. Chen, M. P. Colombo, A. B. Frey, T. F. Greten, S. Mandruzzato, P. J. Murray, A. Ochoa, S. Ostrand-Rosenberg, P. C. Rodriguez, A. Sica, V. Umansky, R. H. Vonderheide, and D. I. Gabrilovich. 2016. Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. *Nat. Commun.* 7: 12150.

16. Mandruzzato, S., S. Brandau, C. M. Britten, V. Bronte, V. Damuzzo, C. Gouttefangeas, D. Maurer, C. Ottensmeier, S. H. van der Burg, M. J. Welters, and S. Walter. 2016. Toward harmonized

phenotyping of human myeloid-derived suppressor cells by flow cytometry: results from an interim study. *Cancer Immunol. Immunother.* 65: 161-169.

17. Movahedi, K., M. Guilliams, J. Van den Bossche, R. Van den Bergh, C. Gysemans, A. Beschin, P. De Baetselier, and J. A. Van Ginderachter. 2008. Identification of discrete tumor-induced myeloid-derived suppressor cell subpopulations with distinct T cell-suppressive activity. *Blood* 111: 4233-4244.

18. Kotsakis, A., M. Harasymczuk, B. Schilling, V. Georgoulias, A. Argiris, and T. L. Whiteside. 2012. Myeloid-derived suppressor cell measurements in fresh and cryopreserved blood samples. *J. Immunol. Methods* 381: 14-22.

19. Trellakis, S., K. Bruderek, J. Hutte, M. Elian, T. K. Hoffmann, S. Lang, and S. Brandau. 2013. Granulocytic myeloid-derived suppressor cells are cryosensitive and their frequency does not correlate with serum concentrations of colony-stimulating factors in head and neck cancer. *Innate Immun.* 19: 328-336.

20. Pillay, J., T. Tak, V. M. Kamp, and L. Koenderman. 2013. Immune suppression by neutrophils and granulocytic myeloid-derived suppressor cells: similarities and differences. *Cell. Mol. Life Sci.* 70: 3813-3827.

21. Moses, K., and S. Brandau. 2016. Human neutrophils: Their role in cancer and relation to myeloid-derived suppressor cells. *Semin. Immunol.* 28: 187-196.

22. Dumitru, C. A., K. Moses, S. Trellakis, S. Lang, and S. Brandau. 2012. Neutrophils and granulocytic myeloid-derived suppressor cells: immunophenotyping, cell biology and clinical relevance in human oncology. *Cancer Immunol. Immunother.* 61: 1155-1167.

23. Fridlender, Z. G., J. Sun, S. Kim, V. Kapoor, G. Cheng, L. Ling, G. S. Worthen, and S. M. Albelda. 2009. Polarization of tumor-associated neutrophil phenotype by TGF-beta: "N1" versus "N2" TAN. *Cancer Cell* 16: 183-194.

24. Mishalian, I., R. Bayuh, L. Levy, L. Zolotarov, J. Michaeli, and Z. G. Fridlender. 2013. Tumor-associated neutrophils (TAN) develop pro-tumorigenic properties during tumor progression. *Cancer Immunol. Immunother.* 62: 1745-1756.

25. Brandau, S., C. A. Dumitru, and S. Lang. 2013. Protumor and antitumor functions of neutrophil granulocytes. *Semin Immunopathol* 35: 163-176.

26. Donskov, F. 2013. Immunomonitoring and prognostic relevance of neutrophils in clinical trials. *Semin. Cancer Biol.* 23: 200-207.

27. Fridlender, Z. G., J. Sun, I. Mishalian, S. Singhal, G. Cheng, V. Kapoor, W. Horng, G. Fridlender, R. Bayuh, G. S. Worthen, and S. M. Albelda. 2012. Transcriptomic analysis comparing tumor-associated neutrophils with granulocytic myeloid-derived suppressor cells and normal neutrophils. *PLoS One* 7: e31524.

28. Choksawangkarn, W., L. M. Graham, M. Burke, S. B. Lee, S. Ostrand-Rosenberg, C. Fenselau, and N. J. Edwards. 2016. Peptide-based systems analysis of inflammation induced myeloid-derived suppressor cells reveals diverse signaling pathways. *Proteomics* 16: 1881-1888.

29. Chornoguz, O., L. Grmai, P. Sinha, K. A. Artemenko, R. A. Zubarev, and S. Ostrand-Rosenberg. 2011. Proteomic pathway analysis reveals inflammation increases myeloid-derived suppressor cell resistance to apoptosis. *Mol. Cell. Proteomics* 10: M110 002980.

30. Gato, M., I. Blanco-Luquin, M. Zudaire, X. M. de Morentin, E. Perez-Valderrama, A. Zabaleta, G. Kochan, D. Escors, J. Fernandez-Irigoyen, and E. Santamaria. 2016. Drafting the proteome landscape of myeloid-derived suppressor cells. *Proteomics* 16: 367-378.

31. Gato-Canas, M., X. Martinez de Morentin, I. Blanco-Luquin, J. Fernandez-Irigoyen, I. Zudaire, T. Liechtenstein, H. Arasanz, T. Lozano, N. Casares, A. Chaikuad, S. Knapp, D. Guerrero-Setas, D. Escors, G. Kochan, and E. Santamaria. 2015. A core of kinase-regulated interactomes defines the neoplastic MDSC lineage. *Oncotarget* 6: 27160-27175.

32. Geis-Asteggiante, L., S. Ostrand-Rosenberg, C. Fenselau, and N. J. Edwards. 2016. Evaluation of Spectral Counting for Relative Quantitation of Proteoforms in Top-Down Proteomics. *Anal. Chem.* 88: 10900-10907.

33. Condamine, T., G. A. Dominguez, J. I. Youn, A. V. Kossenkov, S. Mony, K. Alicea-Torres, E. Tcyganov, A. Hashimoto, Y. Nefedova, C. Lin, S. Partlova, A. Garfall, D. T. Vogl, X. Xu, S. C. Knight, G. Malietzis, G. H. Lee, E. Eruslanov, S. M. Albelda, X. Wang, J. L. Mehta, M. Bewtra, A. Rustgi, N. Hockstein, R. Witt, G. Masters, B. Nam, D. Smirnov, M. A. Sepulveda, and D. I. Gabrilovich. 2016. Lectin-type oxidized LDL receptor-1 distinguishes population of human polymorphonuclear myeloid-derived suppressor cells in cancer patients. *Sci. Immunol.* 1.

34. Youn, J. I., M. Collazo, I. N. Shalova, S. K. Biswas, and D. I. Gabrilovich. 2012. Characterization of the nature of granulocytic myeloid-derived suppressor cells in tumor-bearing mice. *J. Leukoc. Biol.* 91: 167-181.

35. Corzo, C. A., T. Condamine, L. Lu, M. J. Cotter, J. I. Youn, P. Cheng, H. I. Cho, E. Celis, D. G. Quiceno, T. Padhya, T. V. McCaffrey, J. C. McCaffrey, and D. I. Gabrilovich. 2010. HIF-1alpha regulates function and differentiation of myeloid-derived suppressor cells in the tumor microenvironment. *J. Exp. Med.* 207: 2439-2453.

36. Doedens, A. L., C. Stockmann, M. P. Rubinstein, D. Liao, N. Zhang, D. G. DeNardo, L. M. Coussens, M. Karin, A. W. Goldrath, and R. S. Johnson. 2010. Macrophage expression of hypoxia-inducible factor-1 alpha suppresses T-cell function and promotes tumor progression. *Cancer Res.* 70: 7465-7475.

37. Beury, D. W., K. H. Parker, M. Nyandjo, P. Sinha, K. A. Carter, and S. Ostrand-Rosenberg. 2014. Cross-talk among myeloid-derived suppressor cells, macrophages, and tumor cells impacts the inflammatory milieu of solid tumors. *J. Leukoc. Biol.* 96: 1109-1118.

38. Gabrilovich, D. I., H. L. Chen, K. R. Girgis, H. T. Cunningham, G. M. Meny, S. Nadaf, D. Kavanaugh, and D. P. Carbone. 1996. Production of vascular endothelial growth factor by human tumors inhibits the functional maturation of dendritic cells. *Nat. Med.* 2: 1096-1103.

39. Young, M. R., K. Kolesiak, M. A. Wright, and D. I. Gabrilovich. 1999. Chemoattraction of femoral CD34+ progenitor cells by tumor-derived vascular endothelial cell growth factor. *Clin. Exp. Metastasis* 17: 881-888.

40. Kusmartsev, S., E. Eruslanov, H. Kubler, T. Tseng, Y. Sakai, Z. Su, S. Kaliberov, A. Heiser, C. Rosser, P. Dahm, D. Siemann, and J. Vieweg. 2008. Oxidative stress regulates expression of VEGFR1 in myeloid cells: link to tumor-induced immune suppression in renal cell carcinoma. *J. Immunol.* 181: 346-353.

41. Yang, L., L. M. DeBusk, K. Fukuda, B. Fingleton, B. Green-Jarvis, Y. Shyr, L. M. Matrisian, D. P. Carbone, and P. C. Lin. 2004. Expansion of myeloid immune suppressor Gr+CD11b+ cells in tumor-bearing host directly promotes tumor angiogenesis. *Cancer Cell* 6: 409-421.

42. Dolcetti, L., E. Peranzoni, S. Ugel, I. Marigo, A. Fernandez Gomez, C. Mesa, M. Geilich, G. Winkels, E. Traggiai, A. Casati, F. Grassi, and V. Bronte. 2010. Hierarchy of immunosuppressive strength among myeloid-derived suppressor cell subsets is determined by GM-CSF. *Eur. J. Immunol.* 40: 22-35.

43. Morales, J. K., M. Kmieciak, K. L. Knutson, H. D. Bear, and M. H. Manjili. 2010. 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 Res. Treat.* 123: 39-49.

44. Serafini, P., R. Carbley, K. A. Noonan, G. Tan, V. Bronte, and I. Borrello. 2004. High-dose granulocyte-macrophage colony-stimulating factor-producing vaccines impair the immune response through the recruitment of myeloid suppressor cells. *Cancer Res.* 64: 6337-6343.

45. Shojaei, F., X. Wu, X. Qu, M. Kowanetz, L. Yu, M. Tan, Y. G. Meng, and N. Ferrara. 2009. G-CSF-initiated myeloid cell mobilization and angiogenesis mediate tumor refractoriness to anti-VEGF therapy in mouse models. *Proc. Natl. Acad. Sci. U S A* 106: 6742-6747.

46. Filipazzi, P., R. Valenti, V. Huber, L. Pilla, P. Canese, M. Iero, C. Castelli, L. Mariani, G. Parmiani, and L. Rivoltini. 2007. Identification of a new subset of myeloid suppressor cells in peripheral blood of melanoma patients with modulation by a granulocyte-macrophage colony-stimulation factor-based antitumor vaccine. *J. Clin. Oncol.* 25: 2546-2553.

47. Ostrand-Rosenberg, S., and P. Sinha. 2009. Myeloid-derived suppressor cells: linking inflammation and cancer. *J. Immunol.* 182: 4499-4506.

48. Bunt, S. K., P. Sinha, V. K. Clements, J. Leips, and S. Ostrand-Rosenberg. 2006. Inflammation Induces Myeloid-Derived Suppressor Cells that Facilitate Tumor Progression. *J. Immunol.* 176: 284-290.

49. Bunt, S. K., L. Yang, P. Sinha, V. K. Clements, J. Leips, and S. Ostrand-Rosenberg. 2007. Reduced inflammation in the tumor microenvironment delays the accumulation of myeloid-derived suppressor cells and limits tumor progression. *Cancer Res.* 67: 10019-10026.

50. Song, X., Y. Krelin, T. Dvorkin, O. Bjorkdahl, S. Segal, C. A. Dinarello, E. Voronov, and R. N. Apte. 2005. CD11b+/Gr-1+ immature myeloid cells mediate suppression of T cells in mice bearing tumors of IL-1beta-secreting cells. *J. Immunol.* 175: 8200-8208.

51. Tu, S., G. Bhagat, G. Cui, S. Takaishi, E. A. Kurt-Jones, B. Rickman, K. S. Betz, M. Penz-Oesterreicher, O. Bjorkdahl, J. G. Fox, and T. C. Wang. 2008. Overexpression of interleukin-1beta induces gastric inflammation and cancer and mobilizes myeloid-derived suppressor cells in mice. *Cancer Cell* 14: 408-419.

52. Zhao, X., L. Rong, X. Li, X. Liu, J. Deng, H. Wu, X. Xu, U. Erben, P. Wu, U. Syrbe, J. Sieper, and Z. Qin. 2012. TNF signaling drives myeloid-derived suppressor cell accumulation. *J. Clin. Invest.* 122: 4094-4104.

53. Sade-Feldman, M., J. Kanterman, E. Ish-Shalom, M. Elnekave, E. Horwitz, and M. Baniyash. 2013. Tumor necrosis factor-alpha blocks differentiation and enhances suppressive activity of immature myeloid cells during chronic inflammation. *Immunity* 38: 541-554.

54. Ezernitchi, A. V., I. Vaknin, L. Cohen-Daniel, O. Levy, E. Manaster, A. Halabi, E. Pikarsky, L. Shapira, and M. Baniyash. 2006. TCR zeta down-regulation under chronic inflammation is mediated by myeloid suppressor cells differentially distributed between various lymphatic organs. *J. Immunol.* 177: 4763-4772.

55. Sinha, P., V. K. Clements, A. M. Fulton, and S. Ostrand-Rosenberg. 2007. Prostaglandin E2 promotes tumor progression by inducing myeloid-derived suppressor cells. *Cancer Res.* 67: 4507-4513.

56. Talmadge, J. E., K. C. Hood, L. C. Zobel, L. R. Shafer, M. Coles, and B. Toth. 2007. Chemoprevention by cyclooxygenase-2 inhibition reduces immature myeloid suppressor cell expansion. *Int Immunopharmacol* 7: 140-151.

57. Meyer, C., A. Sevko, M. Ramacher, A. V. Bazhin, C. S. Falk, W. Osen, I. Borrello, M. Kato, D. Schadendorf, M. Baniyash, and V. Umansky. 2011. Chronic inflammation promotes myeloid-derived suppressor cell activation blocking antitumor immunity in transgenic mouse melanoma model. *Proc. Natl. Acad. Sci. USA* 108: 17111-17116.

58. Obermajer, N., R. Muthuswamy, J. Lesnock, R. P. Edwards, and P. Kalinski. 2011. Positive feedback between PGE2 and COX2 redirects the differentiation of human dendritic cells toward stable myeloid-derived suppressor cells. *Blood* 118: 5498-5505.

59. Mao, Y., I. Poschke, E. Wennerberg, Y. Pico de Coana, S. Egyhazi Brage, I. Schultz, J. Hansson, G. Masucci, A. Lundqvist, and R. Kiessling. 2013. Melanoma-educated CD14+ cells acquire a myeloid-derived suppressor cell phenotype through COX-2-dependent mechanisms. *Cancer Res.* 73: 3877-3887.

60. Mao, Y., D. Sarhan, A. Steven, B. Seliger, R. Kiessling, and A. Lundqvist. 2014. Inhibition of tumor-derived prostaglandin-e2 blocks the induction of myeloid-derived suppressor cells and recovers natural killer cell activity. *Clinical cancer research : an official journal of the American Association for Cancer Research* 20: 4096-4106.

61. Rodriguez, P. C., C. P. Hernandez, D. Quiceno, S. M. Dubinett, J. Zabaleta, J. B. Ochoa, J. Gilbert, and A. C. Ochoa. 2005. Arginase I in myeloid suppressor cells is induced by COX-2 in lung carcinoma. *J. Exp. Med.* 202: 931-939.

62. Novitskiy, S. V., M. W. Pickup, A. E. Gorska, P. Owens, A. Chytil, M. Aakre, H. Wu, Y. Shyr, and H. L. Moses. 2011. TGF-beta receptor II loss promotes mammary carcinoma progression by Th17 dependent mechanisms. *Cancer Discov.* 1: 430-441.

63. Wu, P., D. Wu, C. Ni, J. Ye, W. Chen, G. Hu, Z. Wang, C. Wang, Z. Zhang, W. Xia, Z. Chen, K. Wang, T. Zhang, J. Xu, Y. Han, X. Wu, J. Wang, W. Gong, S. Zheng, F. Qiu, J. Yan, and J. Huang. 2014. gammadeltaT17 cells promote the accumulation and expansion of myeloid-derived suppressor cells in human colorectal cancer. *Immunity* 40: 785-800.

64. Cheng, P., C. A. Corzo, N. Luetteke, B. Yu, S. Nagaraj, M. M. Bui, M. Ortiz, W. Nacken, C. Sorg, T. Vogl, J. Roth, and D. I. Gabrilovich. 2008. Inhibition of dendritic cell differentiation and accumulation of myeloid-derived suppressor cells in cancer is regulated by S100A9 protein. *J. Exp. Med.* 205: 2235-2249.

65. Sinha, P., C. Okoro, D. Foell, H. H. Freeze, S. Ostrand-Rosenberg, and G. Srikrishna. 2008. Proinflammatory S100 proteins regulate the accumulation of myeloid-derived suppressor cells. *J. Immunol.* 181: 4666-4675.

66. Younis, R. H., K. L. Han, and T. J. Webb. 2016. Human Head and Neck Squamous Cell Carcinoma-Associated Semaphorin 4D Induces Expansion of Myeloid-Derived Suppressor Cells. *J. Immunol.* 196: 1419-1429.

67. Svoronos, N., A. Perales-Puchalt, M. J. Allegrezza, M. R. Rutkowski, K. K. Payne, A. J. Tesone, J. M. Nguyen, T. J. Curiel, M. G. Cadungog, S. Singhal, E. B. Eruslanov, P. Zhang, J. Tchou, R. Zhang, and J. R. Conejo-Garcia. 2017. Tumor Cell-Independent Estrogen Signaling Drives Disease Progression through Mobilization of Myeloid-Derived Suppressor Cells. *Cancer Discov.* 7: 72-85.

68. Markiewski, M. M., R. A. DeAngelis, F. Benencia, S. K. Ricklin-Lichtsteiner, A. Koutoulaki, C. Gerard, G. Coukos, and J. D. Lambris. 2008. Modulation of the antitumor immune response by complement. *Nat. Immunol.* 9: 1225-1235.

69. Sinha, P., O. Chornoguz, V. K. Clements, K. A. Artemenko, R. A. Zubarev, and S. Ostrand-Rosenberg. 2011. Myeloid-derived suppressor cells express the death receptor Fas and apoptose in response to T cell-expressed FasL. *Blood* 117: 5381-5390.

70. Ostrand-Rosenberg, S., P. Sinha, O. Chornoguz, and C. Ecker. 2012. Regulating the suppressors: apoptosis and inflammation govern the survival of tumor-induced myeloid-derived suppressor cells (MDSC). *Cancer Immunol. Immunother.* 61: 1319-1325.

71. Parker, K. H., P. Sinha, L. A. Horn, V. K. Clements, H. Yang, J. Li, K. J. Tracey, and S. Ostrand-Rosenberg. 2014. HMGB1 enhances immune suppression by facilitating the differentiation and suppressive activity of myeloid-derived suppressor cells. *Cancer Res.* 74: 5723-5733.

72. Beury, D. W., K. A. Carter, C. Nelson, P. Sinha, E. Hanson, M. Nyandjo, P. J. Fitzgerald, A. Majeed, N. Wali, and S. Ostrand-Rosenberg. 2016. Myeloid-Derived Suppressor Cell Survival and Function Are Regulated by the Transcription Factor Nrf2. *J. Immunol.* 196: 3470-3478.

73. Bronte, V., and P. Zanovello. 2005. Regulation of immune responses by L-arginine metabolism. *Nat. Rev. Immunol.* 5: 641-654.

74. Condamine, T., I. Ramachandran, J. I. Youn, and D. I. Gabrilovich. 2015. Regulation of tumor metastasis by myeloid-derived suppressor cells. *Annu. Rev. Med.* 66: 97-110.

75. Gabrilovich, D., S. Ostrand-Rosenberg, and V. Bronte. 2012. Coordinated regulation of meyloid cells by tumours. *Nat. Rev. Immunol.* 12: 253-268.

76. Kumar, V., S. Patel, E. Tcyganov, and D. I. Gabrilovich. 2016. The Nature of Myeloid-Derived Suppressor Cells in the Tumor Microenvironment. *Trends Immunol.* 37: 208-220.

77. Ostrand-Rosenberg, S. 2016. Immune suppressive myeloid-derived suppressor cells in cancer. In *Encyclopedia of Immunology*. M. Ratcliffe, ed. Oxford: Academic Press, Elsevier. 5122-5525.

78. Srivastava, M. K., P. Sinha, V. K. Clements, P. Rodriguez, and S. Ostrand-Rosenberg. 2010. Myeloid-derived suppressor cells inhibit T-cell activation by depleting cystine and cysteine. *Cancer Res.* 70: 68-77.

79. Sinha, P., V. K. Clements, S. K. Bunt, S. M. Albelda, and S. Ostrand-Rosenberg. 2007. Cross-talk between myeloid-derived suppressor cells and macrophages subverts tumor immunity toward a type 2 response. *J. Immunol.* 179: 977-983.

80. Ostrand-Rosenberg, S. 2010. Myeloid-derived suppressor cells: more mechanisms for inhibiting antitumor immunity. *Cancer Immunol. Immunother.* 59: 1593-1600.

81. Elkabets, M., V. S. Ribeiro, C. A. Dinarello, S. Ostrand-Rosenberg, J. P. Di Santo, R. N. Apte, and C. A. Vosshenrich. 2010. IL-1beta regulates a novel myeloid-derived suppressor cell subset that impairs NK cell development and function. *Eur. J. Immunol.* 40: 3347-3357.

82. Schlecker, E., A. Stojanovic, C. Eisen, C. Quack, C. S. Falk, V. Umansky, and A. Cerwenka. 2012. Tumor-infiltrating monocytic myeloid-derived suppressor cells mediate CCR5-dependent recruitment of regulatory T cells favoring tumor growth. *J. Immunol.* 189: 5602-5611.

83. Huang, B., P. Y. Pan, Q. Li, A. I. Sato, D. E. Levy, J. Bromberg, C. M. Divino, and S. H. Chen. 2006. 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 Res.* 66: 1123-1131.

84. Serafini, P., S. Mgebroff, K. Noonan, and I. Borrello. 2008. Myeloid-derived suppressor cells promote cross-tolerance in B-cell lymphoma by expanding regulatory T cells. *Cancer Res.* 68: 5439-5449.

85. Hanson, E. M., V. K. Clements, P. Sinha, D. Ilkovitch, and S. Ostrand-Rosenberg. 2009. Myeloid-derived suppressor cells down-regulate L-selectin expression on CD4+ and CD8+ T cells. *J. Immunol.* 183: 937-944.

86. Ku, A. W., J. B. Muhitch, C. A. Powers, M. Diehl, M. Kim, D. T. Fisher, A. P. Sharda, V. K. Clements, K. O'Loughlin, H. Minderman, M. N. Messmer, J. Ma, J. J. Skitzki, D. A. Steeber, B. Walcheck, S. Ostrand-Rosenberg, S. I. Abrams, and S. S. Evans. 2016. Tumor-induced MDSC act via remote control to inhibit L-selectin-dependent adaptive immunity in lymph nodes. *Elife* 5.

87. Ortiz, M. L., V. Kumar, A. Martner, S. Mony, L. Donthireddy, T. Condamine, J. Seykora, S. C. Knight, G. Malietzis, G. H. Lee, M. Moorghen, B. Lenox, N. Luetteke, E. Celis, and D. Gabrilovich. 2015. Immature myeloid cells directly contribute to skin tumor development by recruiting IL-17-producing CD4+ T cells. *J. Exp. Med.* 212: 351-367.

88. Peng, D., T. Tanikawa, W. Li, L. Zhao, L. Vatan, W. Szeliga, S. Wan, S. Wei, Y. Wang, Y. Liu, E. Staroslawska, F. Szubstarski, J. Rolinski, E. Grywalska, A. Stanislawek, W. Polkowski, A. Kurylcio, C. Kleer, A. E. Chang, M. Wicha, M. Sabel, W. Zou, and I. Kryczek. 2016. Myeloid-Derived

Suppressor Cells Endow Stem-like Qualities to Breast Cancer Cells through IL6/STAT3 and NO/NOTCH Cross-talk Signaling. *Cancer Res.* 76: 3156-3165.

89. Cui, T. X., I. Kryczek, L. Zhao, E. Zhao, R. Kuick, M. H. Roh, L. Vatan, W. Szeliga, Y. Mao, D. G. Thomas, J. Kotarski, R. Tarkowski, M. Wicha, K. Cho, T. Giordano, R. Liu, and W. Zou. 2013. Myeloid-derived suppressor cells enhance stemness of cancer cells by inducing microRNA101 and suppressing the corepressor CtBP2. *Immunity* 39: 611-621.

90. Otvos, B., D. J. Silver, E. E. Mulkearns-Hubert, A. G. Alvarado, S. M. Turaga, M. D. Sorensen, P. Rayman, W. A. Flavahan, J. S. Hale, K. Stoltz, M. Sinyuk, Q. Wu, A. Jarrar, S. H. Kim, P. L. Fox, I. Nakano, J. N. Rich, R. M. Ransohoff, J. Finke, B. W. Kristensen, M. A. Vogelbaum, and J. D. Lathia. 2016. Cancer Stem Cell-Secreted Macrophage Migration Inhibitory Factor Stimulates Myeloid Derived Suppressor Cell Function and Facilitates Glioblastoma Immune Evasion. *Stem Cells* 34: 2026-2039.

91. Condamine, T., J. Mastio, and D. I. Gabrilovich. 2015. Transcriptional regulation of myeloid-derived suppressor cells. *J. Leukoc. Biol.* 98: 913-922.

92. Nefedova, Y., M. Huang, S. Kusmartsev, R. Bhattacharya, P. Cheng, R. Salup, R. Jove, and D. Gabrilovich. 2004. Hyperactivation of STAT3 is involved in abnormal differentiation of dendritic cells in cancer. *J. Immunol.* 172: 464-474.

93. Gabrilovich, D. I., and S. Nagaraj. 2009. Myeloid-derived suppressor cells as regulators of the immune system. *Nat. Rev. Immunol.* 9: 162-174.

94. Chalmin, F., S. Ladoire, G. Mignot, J. Vincent, M. Bruchard, J. P. Remy-Martin, W. Boireau, A. Rouleau, B. Simon, D. Lanneau, A. De Thonel, G. Multhoff, A. Hamman, F. Martin, B. Chauffert, E. Solary, L. Zitvogel, C. Garrido, B. Ryffel, C. Borg, L. Apetoh, C. Rebe, and F. Ghiringhelli. 2010. Membrane-associated Hsp72 from tumor-derived exosomes mediates STAT3-dependent

immunosuppressive function of mouse and human myeloid-derived suppressor cells. *J Clin Invest* 120: 457-471.

95. Abad, C., H. Nobuta, J. Li, A. Kasai, W. H. Yong, and J. A. Waschek. 2014. Targeted STAT3 disruption in myeloid cells alters immunosuppressor cell abundance in a murine model of spontaneous medulloblastoma. *J. Leukoc. Biol.* 95: 357-367.

96. Ko, J. S., P. Rayman, J. Ireland, S. Swaidani, G. Li, K. D. Bunting, B. Rini, J. H. Finke, and P. A. Cohen. 2010. Direct and differential suppression of myeloid-derived suppressor cell subsets by sunitinib is compartmentally constrained. *Cancer Res.* 70: 3526-3536.

97. Yuan, H., P. Cai, Q. Li, W. Wang, Y. Sun, Q. Xu, and Y. Gu. 2014. Axitinib augments antitumor activity in renal cell carcinoma via STAT3-dependent reversal of myeloid-derived suppressor cell accumulation. *Biomed. Pharmacother.* 68: 751-756.

98. Nefedova, Y., S. Nagaraj, A. Rosenbauer, C. Muro-Cacho, S. M. Sebti, and D. I. Gabrilovich. 2005. Regulation of dendritic cell differentiation and antitumor immune response in cancer by pharmacologic-selective inhibition of the janus-activated kinase 2/signal transducers and activators of transcription 3 pathway. *Cancer Res.* 65: 9525-9535.

99. Xin, H., C. Zhang, A. Herrmann, Y. Du, R. Figlin, and H. Yu. 2009. Sunitinib inhibition of Stat3 induces renal cell carcinoma tumor cell apoptosis and reduces immunosuppressive cells. *Cancer Res.* 69: 2506-2513.

100. Corzo, C. A., M. J. Cotter, P. Cheng, F. Cheng, S. Kusmartsev, E. Sotomayor, T. Padhya, T. V. McCaffrey, J. C. McCaffrey, and D. I. Gabrilovich. 2009. Mechanism regulating reactive oxygen species in tumor-induced myeloid-derived suppressor cells. *J. Immunol.* 182: 5693-5701.

101. Lu, T., R. Ramakrishnan, S. Altiok, J. I. Youn, P. Cheng, E. Celis, V. Pisarev, S. Sherman, M. B. Sporn, and D. Gabrilovich. 2011. Tumor-infiltrating myeloid cells induce tumor cell resistance to cytotoxic T cells in mice. *J. Clin. Invest.* 121: 4015-4029.

102. Nagaraj, S., K. Gupta, V. Pisarev, L. Kinarsky, S. Sherman, L. Kang, D. L. Herber, J. Schneck, and D. I. Gabrilovich. 2007. Altered recognition of antigen is a mechanism of CD8+ T cell tolerance in cancer. *Nat. Med.* 13: 828-835.

103. Waight, J. D., C. Netherby, M. L. Hensen, A. Miller, Q. Hu, S. Liu, P. N. Bogner, M. R. Farren, K. P. Lee, K. Liu, and S. I. Abrams. 2013. Myeloid-derived suppressor cell development is regulated by a STAT/IRF-8 axis. *J. Clin. Invest.* 123: 4464-4478.

104. Stewart, T. J., K. M. Greeneltch, J. E. Reid, D. J. Liewehr, S. M. Steinberg, K. Liu, and S. I. Abrams. 2009. Interferon regulatory factor-8 modulates the development of tumour-induced CD11b+Gr-1+ myeloid cells. *J. Cell. Mol. Med.* 13: 3939-3950.

105. Netherby, C. S., and S. I. Abrams. 2017. Mechanisms overseeing myeloid-derived suppressor cell production in neoplastic disease. *Cancer Immunol. Immunother.*

106. Netherby, C. S., M. N. Messmer, L. Burkard-Mandel, S. Colligan, A. Miller, E. Cortes Gomez, J. Wang, M. J. Nemeth, and S. I. Abrams. 2017. The Granulocyte Progenitor Stage Is a Key Target of IRF8-Mediated Regulation of Myeloid-Derived Suppressor Cell Production. *J. Immunol.* 198: 4129-4139.

107. Nam, S., K. Kang, J. S. Cha, J. W. Kim, H. G. Lee, Y. Kim, Y. Yang, M. S. Lee, and J. S. Lim. 2016. Interferon regulatory factor 4 (IRF4) controls myeloid-derived suppressor cell (MDSC) differentiation and function. *J. Leukoc. Biol.* 100: 1273-1284.

108. Marigo, I., E. Bosio, S. Solito, C. Mesa, A. Fernandez, L. Dolcetti, S. Ugel, N. Sonda, S. Bicciato, E. Falisi, F. Calabrese, G. Basso, P. Zanovello, E. Cozzi, S. Mandruzzato, and V. Bronte. 2010. Tumor-induced tolerance and immune suppression depend on the C/EBPbeta transcription factor. *Immunity* 32: 790-802.

109. Condamine, T., V. Kumar, I. R. Ramachandran, J. I. Youn, E. Celis, N. Finnberg, W. S. El-Deiry, R. Winograd, R. H. Vonderheide, N. R. English, S. C. Knight, H. Yagita, J. C. McCaffrey, S. Antonia, N.

Hockstein, R. Witt, G. Masters, T. Bauer, and D. I. Gabrilovich. 2014. ER stress regulates myeloid-derived suppressor cell fate through TRAIL-R-mediated apoptosis. *J. Clin. Invest.* 124: 2626-2639.

110. Lee, B. R., S. Y. Chang, E. H. Hong, B. E. Kwon, H. M. Kim, Y. J. Kim, J. Lee, H. J. Cho, J. H. Cheon, and H. J. Ko. 2014. Elevated endoplasmic reticulum stress reinforced immunosuppression in the tumor microenvironment via myeloid-derived suppressor cells. *Oncotarget* 5: 12331-12345.

111. Thevenot, P. T., R. A. Sierra, P. L. Raber, A. A. Al-Khami, J. Trillo-Tinoco, P. Zarreii, A. C. Ochoa, Y. Cui, L. Del Valle, and P. C. Rodriguez. 2014. The stress-response sensor chop regulates the function and accumulation of myeloid-derived suppressor cells in tumors. *Immunity* 41: 389-401.

112. Liu, Y., X. Xiang, X. Zhuang, S. Zhang, C. Liu, Z. Cheng, S. Michalek, W. Grizzle, and H. G. Zhang. 2010. Contribution of MyD88 to the tumor exosome-mediated induction of myeloid derived suppressor cells. *Am. J. Pathol.* 176: 2490-2499.

113. Bunt, S. K., V. K. Clements, E. M. Hanson, P. Sinha, and S. Ostrand-Rosenberg. 2009. Inflammation enhances myeloid-derived suppressor cell cross-talk by signaling through Toll-like receptor 4. *J. Leukoc. Biol.* 85: 996-1004.

114. Hong, E. H., S. Y. Chang, B. R. Lee, Y. S. Kim, J. M. Lee, C. Y. Kang, M. N. Kweon, and H. J. Ko. 2013. Blockade of Myd88 signaling induces antitumor effects by skewing the immunosuppressive function of myeloid-derived suppressor cells. *Int. J. Cancer* 132: 2839-2848.

115. Strauss, L., S. Sangaletti, F. M. Consonni, G. Szebeni, S. Morlacchi, M. G. Totaro, C. Porta, A. Anselmo, S. Tartari, A. Doni, F. Zitelli, C. Tripodo, M. P. Colombo, and A. Sica. 2015. RORC1 Regulates Tumor-Promoting "Emergency" Granulo-Monocytopoiesis. *Cancer Cell* 28: 253-269.

116. Sahakian, E., J. J. Powers, J. Chen, S. L. Deng, F. Cheng, A. Distler, D. M. Woods, J. Rock-Klotz, A. L. Sodre, J. I. Youn, K. V. Woan, A. Villagra, D. Gabrilovich, E. M. Sotomayor, and J. Pinilla-Ibarz.

2015. Histone deacetylase 11: A novel epigenetic regulator of myeloid derived suppressor cell expansion and function. *Mol. Immunol.* 63: 579-585.

117. Whiteside, T. L. 2016. Tumor-Derived Exosomes and Their Role in Cancer Progression. *Adv. Clin. Chem.* 74: 103-141.

118. Whiteside, T. L. 2016. Exosomes and tumor-mediated immune suppression. *J. Clin. Invest.* 126: 1216-1223.

119. Xiang, X., A. Poliakov, C. Liu, Y. Liu, Z. B. Deng, J. Wang, Z. Cheng, S. V. Shah, G. J. Wang, L. Zhang, W. E. Grizzle, J. Mobley, and H. G. Zhang. 2009. Induction of myeloid-derived suppressor cells by tumor exosomes. *Int. J. Cancer* 124: 2621-2633.

120. Burke, M., W. Choksawangkarn, N. Edwards, S. Ostrand-Rosenberg, and C. Fenselau. 2014. Exosomes from myeloid-derived suppressor cells carry biologically active proteins. *J. Proteome Res.* 13: 836-843.

121. Adams, K., S. Chauhan, V. Clements, Y. Wang, N. Edwards, S. Ostrand-Rosenberg, and C. Fenselau. 2017. Ubiquitin conjugation probed by inflammation in MDSC exosomes. *in review*.

122. Burke, M. C., M. S. Oei, N. J. Edwards, S. Ostrand-Rosenberg, and C. Fenselau. 2014. Ubiquitinated proteins in exosomes secreted by myeloid-derived suppressor cells. *J. Proteome Res.* 13: 5965-5972.

123. Chauhan, S., S. Danielson, V. Clements, N. Edwards, S. Ostrand-Rosenberg, and C. Fenselau. 2017. Surface Glycoproteins of Exosomes Shed by Myeloid-Derived Suppressor Cells Contribute to Function. *J. Proteome Res.* 16: 238-246.

124. Chao, M. P., I. L. Weissman, and R. Majeti. 2012. The CD47-SIRPalpha pathway in cancer immune evasion and potential therapeutic implications. *Curr. Opin. Immunol.* 24: 225-232.

125. Geis-Asteggiante, L. 2017. Quantitative analysis of intact proteins and RNAs carried by immunosuppressive exosomes. *Ph.D. thesis* University of Maryland.

126. De Henau, O., M. Rausch, D. Winkler, L. F. Campesato, C. Liu, D. H. Cymerman, S. Budhu, A. Ghosh, M. Pink, J. Tchaicha, M. Douglas, T. Tibbitts, S. Sharma, J. Proctor, N. Kosmider, K. White, H. Stern, J. Soglia, J. Adams, V. J. Palombella, K. McGovern, J. L. Kutok, J. D. Wolchok, and T. Merghoub. 2016. Overcoming resistance to checkpoint blockade therapy by targeting PI3Kgamma in myeloid cells. *Nature* 539: 443-447.

127. Meyer, C., L. Cagnon, C. M. Costa-Nunes, P. Baumgaertner, N. Montandon, L. Leyvraz, O. Michielin, E. Romano, and D. E. Speiser. 2014. Frequencies of circulating MDSC correlate with clinical outcome of melanoma patients treated with ipilimumab. *Cancer Immunol. Immunother.* 63: 247-257.

128. Kim, K., A. D. Skora, Z. Li, Q. Liu, A. J. Tam, R. L. Blosser, L. A. Diaz, Jr., N. Papadopoulos, K. W. Kinzler, B. Vogelstein, and S. Zhou. 2014. Eradication of metastatic mouse cancers resistant to immune checkpoint blockade by suppression of myeloid-derived cells. *Proc. Natl. Acad. Sci. U S A* 111: 11774-11779.

129. Kostlin, N., K. Hofstadter, A. L. Ostermeir, B. Spring, A. Leiber, S. Haen, H. Abele, P. Bauer, J. Pollheimer, D. Hartl, C. F. Poets, and C. Gille. 2016. Granulocytic Myeloid-Derived Suppressor Cells Accumulate in Human Placenta and Polarize toward a Th2 Phenotype. *J. Immunol.* 196: 1132-1145.

130. Kostlin, N., H. Kugel, B. Spring, A. Leiber, A. Marme, M. Henes, N. Rieber, D. Hartl, C. F. Poets, and C. Gille. 2014. Granulocytic myeloid derived suppressor cells expand in human pregnancy and modulate T-cell responses. *Eur. J. Immunol.* 44: 2582-2591.

131. Ostrand-Rosenberg, S., P. Sinha, C. Figley, R. Long, D. Park, D. Carter, and V. K. Clements. 2017. Frontline Science: Myeloid-derived suppressor cells (MDSCs) facilitate maternal-fetal tolerance in mice. *J. Leukoc. Biol.* 101: 1091-1101.

132. Xia, S., H. Sha, L. Yang, Y. Ji, S. Ostrand-Rosenberg, and L. Qi. 2011. Gr-1+ CD11b+ myeloid-derived suppressor cells suppress inflammation and promote insulin sensitivity in obesity. *J. Biol. Chem.* 286: 23591-23599.

133. Clements, V., T. Long, R. Long, C. Figley, and S. Ostrand-Rosenberg. 2017. High fat diet and leptin promote tumor progression by inducing myeloid-derived suppressor cells. *J. Leukoc. Biol.* in revision.

Footnotes

[1] The authors were supported by NIH RO1CA84232, RO1CA115880, RO1GM021248, and OD019938.

[2]Abbreviations

Arg1	arginase 1
CAF	cancer-associated fibroblast(s)
CHOP	C/EBPβ homologous protein
COX_2	cyclooxygenase 2
CSFR	colony stimulating factor receptor
DC	dendritic cell(s)
G-CSF	granulocyte colony stimulating factor
GM-CSF	granulocyte-macrophage colony stimulating factor
HDAC	histone deacetylase
HIF	hypoxia inducible factor
HMGB1	high mobility group box protein 1
IDO	indoleamine 2,3 dioxygenase
iNOS	inducible nitric oxide synthase
IRF	interferon-inducible regulatory factor
LOX1	lectin-type oxidized low-density lipoprotein receptor
MIF	macrophage migration inhibitory factor
M-MDSC	monocytic myeloid-derived suppressor cell(s)
NOX2	NADPH oxidase
Nrf2	nuclear factor erythroid2-related factor 2
NSCLC	non-small cell lung cancer

PGE2	prostaglandin E2
PMN-MDSC	polymorphonuclear MDSC
RAGE	receptor for advanced glycation endproducts
RORC1/RORγ	retinoic acid related orphan receptor
ROS	reactive oxygen species
Sema4D	semaphorin4D
STAT	signal transducer activator of transcription
TAMs	tumor-associated macrophages
TANs	tumor-associated neutrophils
TSP	thrombospondin
VEGF	vascular endothelial growth factor

Figure Legends

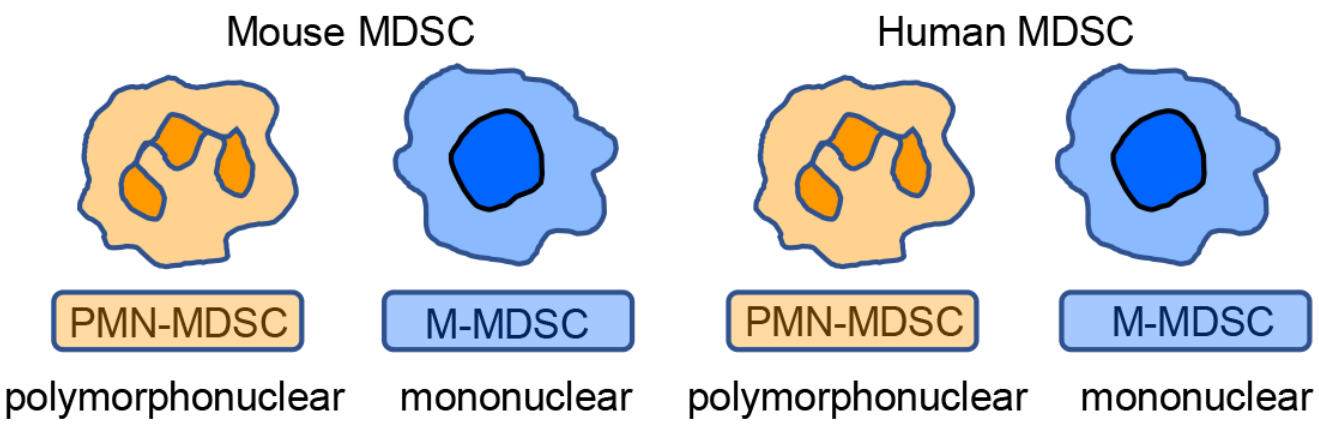
Figure 1. Phenotype and immune suppressive functions of mouse and human monocytic (M-MDSC) and polymorphonuclear (PMN-MDSC) MDSC. Lin^- indicates cells are negative for CD3, CD19, CD20, and CD56.

Figure 2. Cytokines, immune regulatory molecules, and transcription factors control the development, accumulation, suppressive potency, and survival of MDSC. Growth factors, hormones, and transcription factors that regulate myelopoiesis induce the expansion of MDSC in bone marrow. Within the pro-inflammatory tumor microenvironment a variety of cytokines and non-cytokine regulatory proteins are produced by tumor cells and tumor-infiltrating host cells (e.g. DCs, lymphocytes, macrophages, mast cells, and fibroblasts) and increase MDSC suppressive potency by activating transcription factors and signal transduction pathways in MDSC. Survival of MDSC is mediated by many of the same factors/conditions that induce the accumulation of MDSC plus cell surface receptors and genes that prevent/limit apoptosis.

Figure 3. MDSC use a variety of immune and non-immune mechanisms to promote tumor progression, but have beneficial effects in other settings. In individuals with cancer, MDSC inhibit adaptive antitumor immunity by suppressing $CD4^+$ and $CD8^+$ T cell activation and function, and by driving and recruiting T regulatory cells. They inhibit innate immunity by polarizing macrophages towards a type 2 tumor-promoting phenotype and by inhibiting NK-mediated cytotoxicity. MDSC also promote cancer cell stemness and facilitate angiogenesis and drive tumor invasion and metastasis. Beneficial effects of MDSC include their lowering of blood glucose levels and reduction of insulin tolerance in

obese individuals, and their maintenance of maternal-fetal tolerance and embryo implantation during pregnancy.

Figure 1



Phenotype

	Mouse PMN-MDSC	Mouse M-MDSC	Human PMN-MDSC	Human M-MDSC
	CD11b ⟵ ⟶		CD33$^+$ ⟵ ⟶	
	Gr1 ⟵ ⟶		CD11b$^+$ ⟵ ⟶	
			HLA-DR$^-$ ⟵ ⟶	
			Lin$^-$ ⟵ ⟶	
	Ly6G$^+$	Ly6G$^-$	CD15$^+$	CD15$^-$
	Ly6C$^{low/-}$	Ly6C$^+$	CD14$^-$	CD14$^+$
	SSC^{hi}	SSC^{how}	CD66b$^+$	CD66b$^-$
	iNOS$^-$	iNOS$^+$	IL-4Rα$^-$	IL-4Rα$^+$
	F4/80$^{low/-}$	F4/80$^{med/hi}$		
	CD115$^{low/-}$	CD115$^{med/hi}$		
	ROS^{hi}	ROS^{med}		
	Arg1$^+$	Arg1$^+$		

Functional assessment

Mouse PMN-MDSC	Mouse M-MDSC	Human PMN-MDSC	Human M-MDSC
• Suppress Ag-specific T cell activation • Suppress αCD3/CD28 T cell activation		• Suppress αCD3/CD28 T cell activation • Increased αCD3/CD28 T cell activation following depletion of MDSC • Suppress MLR	

Figure 2

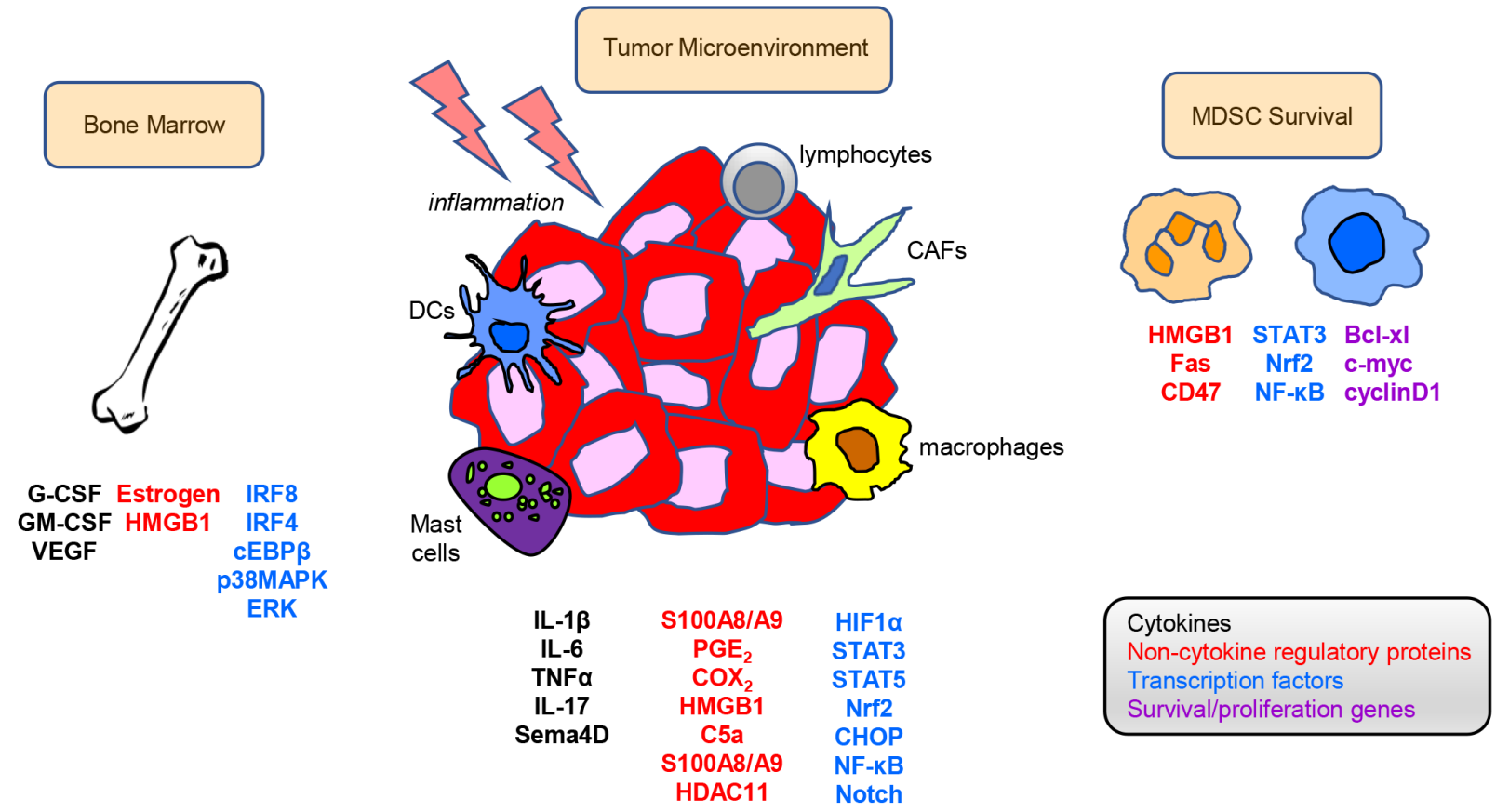
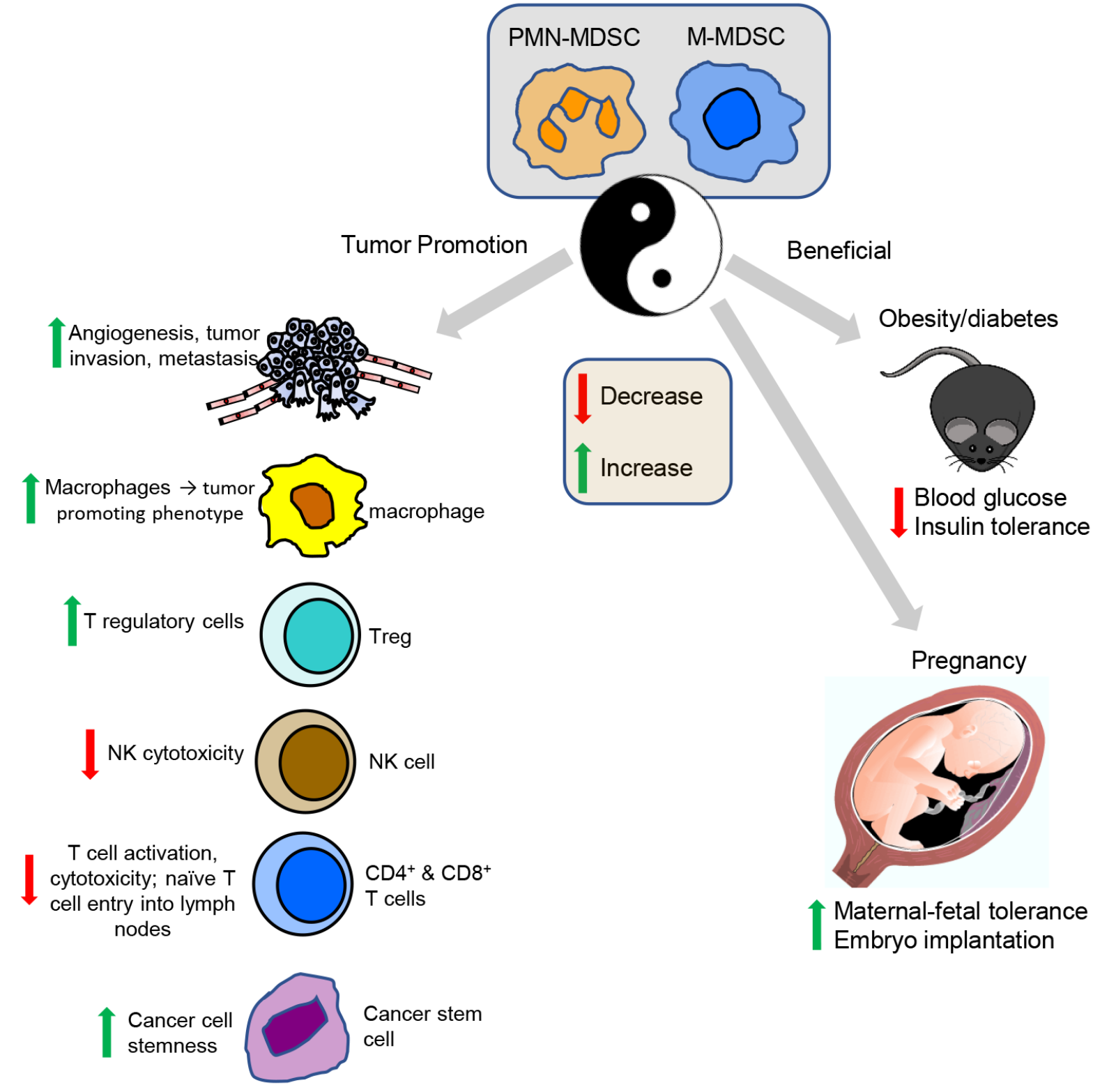

Bone Marrow
Tumor Microenvironment
MDSC Survival
inflammation
lymphocytes
CAFs
DCs
macrophages
Mast cells
G-CSF Estrogen IRF8
GM-CSF HMGB1 IRF4
VEGF cEBPβ
p38MAPK
ERK
IL-1β S100A8/A9 HIF1α
IL-6 PGE2 STAT3
TNFα COX2 STAT5
IL-17 HMGB1 Nrf2
Sema4D C5a CHOP
S100A8/A9 NF-κB
HDAC11 Notch
HMGB1 STAT3 Bcl-xl
Fas Nrf2 c-myc
CD47 NF-κB cyclinD1
Cytokines
Non-cytokine regulatory proteins
Transcription factors
Survival/proliferation genes

Figure 3


PMN-MDSC
M-MDSC
Tumor Promotion
Beneficial
Decrease
Increase
Angiogenesis, tumor invasion, metastasis
Macrophages → tumor promoting phenotype
macrophage
T regulatory cells
Treg
NK cytotoxicity
NK cell
T cell activation, cytotoxicity; naïve T cell entry into lymph nodes
CD4+ & CD8+ T cells
Cancer cell stemness
Cancer stem cell
Obesity/diabetes
Blood glucose
Insulin tolerance
Pregnancy
Maternal-fetal tolerance
Embryo implantation