Myeloid-Derived Suppressor Cells in the Tumor Microenvironment

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Abstract

Myeloid-derived suppressor cells (MDSCs) represent a heterogenous population of immature myeloid cells capable of modulating immune responses. In the context of cancer, MDSCs are abnormally produced and recruited to the tumor microenvironment (TME) to aid in the establishment of an immunosuppressive TME that facilitates tumor escape. Additionally, MDSCs contribute to non-immunologic aspects of tumor biology, including tumor angiogenesis and metastasis. The clinical significance of MDSCs has recently been appreciated as numerous studies have suggested a correlation between circulating and intratumoral MDSC frequencies and tumor stage, progression, and treatment resistance. In this chapter, we review MDSC characterization, development, expansion, and mechanisms that facilitate immunosuppression and tumor progression. Furthermore, we highlight studies demonstrating the clinical significance of MDSCs in various disease states in addition to strategies that modulate various aspects of MDSC biology for therapeutic gain.

Keywords

Myeloid-derived suppressor cells (MDSC) · Cancer · Tumor microenvironment (TME) · Immunosuppression · Solid tumor · Myelopoiesis · Angiogenesis · Metastasis · Autoimmunity · Therapy resistance · Therapeutic strategies · STAT3 · S100A9 · Transforming growth factor (TGF)-β · Arginase-1 · Regulatory T cells (Treg)

8.1 Introduction

Myeloid-derived suppressor cells (MDSCs) are immature myeloid cells normally produced and secreted by the bone marrow in response to localized inflammatory states such as infection or trauma to try to restrain hyper-inflammation and protect the host from generation of autoimmunity

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[1, 2]. In the setting of cancer, however, MDSCs are abnormally produced and recruited by tumor-derived factors to tumor sites in order to establish a microenvironment around the tumor that can suppress host immunity (via MDSC expression of suppressive cytokines like TGF-β and surface molecules like PDL1 and PDL2), establish new vasculature (via MDSC expression of VEGF and βFGF isoforms), and remodel tissue with tumor-supportive stromal elements (via MDSC arginase-1, iNOS, and MMP-7/MMP-9/MMP-12 activity) [3]. In fact, MDSCs play a central role in controlling and maintaining the suppressive TME in solid tumors [3]. In models where MDSCs are absent or eliminated, the TME breaks down, allowing access and activation of immune effector cells [4, 5]. Recently, these unique suppressive cells of the tumor microenvironment have gained direct clinical significance as increasing evidence has mounted suggesting a correlation between the frequency of circulating and intratumoral MDSCs and cancer stage, disease progression, and resistance to standard chemo- and radiotherapy [6, 7]. Hence, understanding MDSC biology represents an important step in the quest to enhance anticancer immunity. In this chapter, we will review important aspects of MDSC biology, including their characterization, development and expansion, activation, and the suppressive mechanisms that support cancer growth and progression (highlighted in Fig. 8.1). In addition, we will highlight important studies that have attempted to target or manipulate MDSC biology for therapeutic gain. Finally, we will discuss recent trends and potential future directions concerning targeting and use of MDSCs in both oncologic and non-oncologic diseases.

8.2 Defining Human and Murine MDSCs

8.2.1 Defining MDSCs in the Periphery

In general, *murine* MDSCs are defined by a combination of markers utilized for murine myeloid lineages (Gr-1, Ly6, CD11b, CD49d) in combination with suppressive effector function (e.g., suppression of T-cell proliferation). Early studies in mice led to the identification of a suppressive cell population defined as Gr-1^{+}CD11b^{+}. Further characterization of these Gr-1^{+}CD11b^{+} cells revealed two subsets based on their expression of both Gr-1 [8] and the Ly6 superfamily molecules Ly6G and Ly6C [9, 10], which are preferentially expressed on the surface of granulocytes and monocytes, respectively. However, because Gr-1 mAbs bind both Ly6G and Ly6C, double staining of Ly6G and Ly6C is highly recommended to identify the two distinct populations, specifically the polymorphonuclear (PMN-MDSCs), or sometimes referred to as granulocytic (G-MDSCs), and monocytic (M-MDSCs) subsets. PMN-MDSCs are characterized as CD11b^{+}Ly6G^{+}Ly6C$^{low/int}$, whereas M-MDSCs are CD11b^{+}Ly6G^{-}Ly6C^{high}. In addition, PMN-MDSCs, with their high degree of granularity, are high on the side scatter axis in flow cytometric applications, whereas M-MDSCs present as lower on the side scatter axis. Haile et al. identified CD49d as a novel marker to further aid MDSC subset identification, demonstrating that CD11b^{+}CD49d^{+} phenotypically and functionally resembled M-MDSCs [11]. CD11b^{+}CD49d^{-} cells were more granulocytic compared to their CD11b^{+}CD49d^{+} counterparts, thus representing the PMN-MDSC subset.

In 2016, Bronte et al. proposed the minimal phenotypic characteristics of *human* peripheral blood MDSCs [12]. The mouse equivalent of PMN-MDSC is defined as CD11b^{+}CD14^{-}CD15^{+} or CD11b^{+}CD14^{-}CD66^{+}, whereas M-MDSC is CD11b^{+}CD14^{+}CD15^{-}HLA-DR$^{-/low}$. The myeloid marker CD33 can also be used for differentiation, where PMN-MDSCs stain CD33^{dim} and M-MDSCs present CD33$^{+/hi}$. It is also important to include some form of Lineage (Lin) cocktail (CD3, CD19, and CD56) that can differentiate immature MDSC progenitors from PMN-MDSC and M-MDSC, with cells representing Lin^{-}HLA-DR^{-}CD33^{+} defined as early-stage MDSC.

Unfortunately, phenotypic staining analysis of the abovementioned markers alone for both mouse and human MDSC subsets cannot dis-

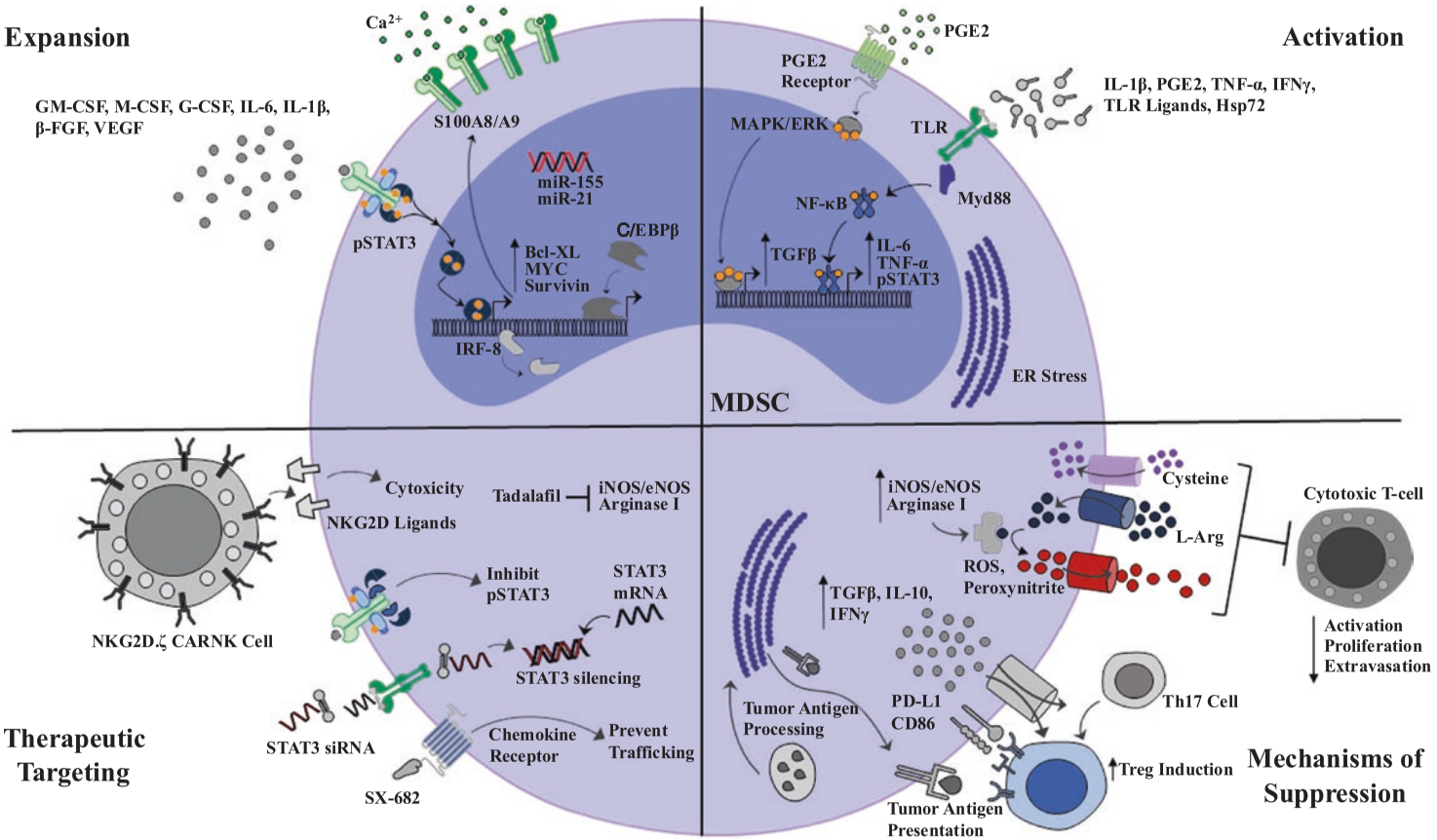

Fig. 8.1 *Expansion*. MDSC expansion is governed by factors secreted by the tumor and tumoral stroma that converge mainly on STAT3 signaling resulting in the upregulation of genes that mediate MDSC survival and proliferation. *Activation*. Once expanded, tumoral stroma secrete factors that converge on MAPK/ERK and NF-κB signaling to upregulate genes that endow MDSCs with their characteristic suppressive capacity. *Mechanisms of suppression*. MDSCs suppress T-cell antitumor responses by sequestering the amino acids cysteine and arginine, in addition to converting arginine to reactive oxygen and nitrogen species that facilitate T-cell suppression. Conversely, MDSCs mediate the induction of Tregs through release of soluble cytokines that further suppress antitumor responses. *Therapeutic targeting*. Efforts to target MDSCs for therapeutic benefit have included inhibiting their expansion and trafficking, inhibiting their suppressive capabilities, and direct elimination of MDSCs from the TME

criminate MDSC subsets from their respective mature cell population. PMN-MDSCs share a common origin with neutrophils and thus present many of the same morphological and phenotypic characteristics. Similarly, M-MDSCs share a common origin with monocytes. In a study that compared $CD11b^{+}Ly6G^{+}Ly6C^{low}$ PMN-MDSCs in tumor-bearing mice to $CD11b^{+}Ly6G^{+}Ly6C^{low}$ neutrophils in tumor-free mice, Youn et al. demonstrated that PMN-MDSCs had significantly higher expression of CD115 and CD244 [13]. In humans, M-MDSC can be differentiated from monocytes by the absence of HLA-DR expression. Recently, the ability to separate PMN-MDSC from neutrophils via a Ficoll gradient separation was described [14]. Despite these proposed differentiating markers, it still is absolutely essential to characterize these MDSC subsets for their immunosuppressive state, both molecularly and in functional suppression assays. MDSCs differ molecularly from mature myelocytes through transcription factors and biochemical signatures associated with immunosuppression [12].

The benchmark suppressive function of MDSCs is their ability to inhibit T-cell proliferation, cytokine production, and cytotoxic functions. MDSCs have demonstrated suppressive effects in vitro on both activated, antigen-specific T cells and naïve, non-specific T cells. Typically, suppression assays examine the ability of purified MDSC populations to suppress either antigen-specific or antigen-non-specific T cells. To induce antigen-independent stimulation, T cells can be stimulated with anti-CD3/CD28 antibodies in the presence of cytokines such as interleukin (IL)-2, IL-7, or IL-15 and assessed for proliferation using ^{3}H-thymidine incorporation, CFSE dilution, or cell trace violet dilution in the presence of MDSCs. Similarly, antigen-specific T cells can be stimulated with cognate antigens or allogeneic leukocytes. MDSC suppressive capacity is also assayed by their ability to inhibit T-cell IFN-γ and IL-2 production. In vivo studies are more technically challenging and include more critical parameters. Marigo et al. described an in vivo mouse protocol where MDSCs are expanded in tumor-bearing mice and tumor control is subsequently assessed to determine MDSC suppressive capacity [15]. In this protocol, the antigen is expressed by the tumor and cross-presented to lymphocytes via dendritic cell vaccination to generate an antigen-specific T-cell population. MDSC suppression was assessed for their ability to inhibit antigen-specific T-cell IFN-γ production and overall tumor control.

8.2.2 Defining MDSCs Within Tumors

Based on the ease of isolation and characterization, most of the early studies defining MDSC subsets focused on circulating MDSCs isolated from blood or spleen. Intratumoral MDSC characterization has been challenging due to the small proportion of MDSCs within a tumor sample as well as the technical challenges of isolating cells from a complex tumor microenvironment (TME) [16]. Comprised of immune infiltrates, stromal cells, connective tissue, and vasculature, the TME facilitates complex, heterotypic interactions that have both acute and chronic impacts on the local components. Further complicating intratumoral MDSC characterization is the presence of both tumor-associated macrophages (TAMs) and neutrophils (TANs) that phenotypically and morphologically resemble M-MDSCs and PMN-MDSCs, respectively. Tissue-resident macrophages are present in all tissues of the body and display high heterotypic and functional diversity [16, 17].

In the mouse, Movahedi et al. demonstrated that $CD11b^{+}Ly6C^{+}$ cells were the exclusive monocytic precursors of TAMs. Further, they demonstrated that tumor-infiltrated myeloid populations could be grouped into at least seven subsets based on their differential expression of MHCII and Ly6C, thus reflecting the high heterogeneity and complexity when characterizing intratumoral suppressive myeloid populations [18]. Similarly, Franklin et al. demonstrated the presence of three intratumoral myeloid populations: tissue-resident macrophages as $CD11b^{+}MHCII^{+}$ and two subsets of TAMs as $CD11b^{low}MHCII^{-}Ly6C^{+}$ or $CD11b^{low}MHCII^{-}Ly6G^{+}$ [19]. Furthermore, TAMs were derived from $CD11b^{+}Ly6C^{+}CCR2^{+}$

circulating monocytes that underwent phenotypic changes characterized by the downregulation of CD11b and Ly6C and upregulation of CD11c, F4/80, and MHCII [19]. MDSCs exhibit plasticity and cross-phenotype skewing with tumor-associated macrophages (TAMs) in the TME in response to tumor-associated hypoxia [20]. Thus, these studies reflect the plasticity of intratumoral myeloid populations and highlight the difficulty in exactly defining and differentiating myeloid subsets within the TME. In the mouse, however, the F4/80 marker has allowed differentiation of murine intratumoral MDSCs, separating PMN-MDSC ($F4/80^-$) from M-MDSC ($F4/80^{low/dim}$) and M-MDSC from TAM ($F4/80^+$) [1].

In *human* intratumoral MDSCs, M-MDSC are defined as $CD11b^+CD33^+CD14^+HLA\text{-}DR^{lo/-}$ and PMN-MDSC as $CD11b^+CD33^+CD15^+CD66^+HLA\text{-}DR^{lo/-}$ [21]. However, analyzing tumor myeloid infiltrates presents the same complexities and challenges as eluded for murine studies: low frequencies of MDSCs within tumor tissue samples and a complex, heterogenous myeloid landscape. Within the TME, human myeloid cells have been classified into four general categories: (1) TAMs, (2) Tie2-expressing monocytes, (3) neutrophils, and (4) MDSCs [21]. PMN-MDSCs share a similar ontogeny with neutrophils and thus complicate clearly characterizing MDSCs within the TME. Indeed, PMN-MDSCs within the TME have been referred to in the literature as pro-tumor, anti-inflammatory neutrophils, and some investigators have proposed that PMN-MDSCs should instead be referred to as "neutrophils with suppressive activity" until further approaches to differentiate these two populations are offered [22]. However, studies have suggested that tumor-associated neutrophils possess both anti-tumor and pro-tumor properties [23]. Thus, differentiating between neutrophils and PMN-MDSCs based simply on suppressive capacity may be misleading. Several differentiating features that can aid in distinguishing intratumoral PMN-MDSCs from neutrophils have been employed. Neutrophils are high density that present with high side scatter axis (SSC) on flow cytometric applications, whereas PMN-MDSCs are lower SSC-density cells [24]. In combination with SSC profiles, CD11b, and CD15, the inclusion of CD16 and CD66b, classic neutrophil markers, has been used to help identify neutrophils [21]. Recently, LOX-1 has also emerged as a marker unique to PMN-MDSC. Condamine et al. demonstrated that $LOX\text{-}1^+$ PMN cells isolated from peripheral blood had a gene expression profile similar to PMN-MDSCs and suppressed T-cell proliferation in vitro [25]. In addition, 15–50% of CD15+ cells isolated from various solid tumors were $LOX\text{-}1^+$.

8.3 MDSC Development and Expansion

In healthy individuals, hematopoietic stem cells (HSCs) in the bone marrow give rise to common myeloid progenitor cells (MPCs) and immature myeloid cells (IMCs) that then differentiate into mature macrophages, dendritic cells, and granulocytes in peripheral organs. During acute inflammatory stimuli, normal activation of these mature cell populations results in marked phagocytosis and release of pro-inflammatory signals, in addition to remodeling of tissues after the inflammatory state is resolved [26]. Thus, normal myelopoiesis and myeloid differentiation is a tightly regulated process that controls and limits inflammatory responses. However, in pathological conditions such as cancer, chronic inflammatory signals secreted by the tumor microenvironment reprogram myelopoiesis and serve to exacerbate tumor progression [27]. Tumor-derived factors in the form of growth factors, cytokines, chemokines, and other inflammatory mediators facilitate the expansion of an immature myeloid population characterized by defective antigen presentation and secretion of several factors that suppress the resultant antitumor response [27]. Thus, myeloid-derived suppressor cells (MDSCs) were so named to encompass both their characteristic immature state and ability to suppress antitumor responses [28].

The conversion of HSCs into MDSCs remains a process that is incompletely understood but is proposed to be mediated by two general signals. The first signal facilitates the expansion and

impaired differentiation of myeloid cells and includes factors that are produced primarily by tumor and bone marrow stromal cells. The second signal activates MDSCs and results in their suppressive capabilities [29, 30].

8.3.1 Signal 1

The same factors that govern normal myelopoiesis are also proposed to induce the mobilization and expansion of MDSCs. These include granulocyte/macrophage colony-stimulating factor (GM-CSF), macrophage CSF (M-CSF), granulocyte CSF (G-CSF), IL-6, IL-1β, beta-fibroblast growth factor (β-FGF), and vascular endothelial growth factor (VEGF) [31–33]. Tumor and stromal cells produce and secrete these factors resulting in autocrine and paracrine loops that further drive tumor progression [33]. The colony-stimulating factors represent a group of cytokines essential in modulating myelopoiesis. GM-CSF functions on a more global level, displaying pleiotropic and widespread effects on hematopoietic cells, whereas G-CSF and M-CSF are relatively more lineage specific [34]. In particular, GM-CSF has repeatedly demonstrated to be a key mediator of MDSC expansion both in vitro and in vivo [35–40]. Interestingly, preclinical and clinical evidence suggests that the effect of GM-CSF is dose-dependent. In vitro, murine bone marrow cells cultured in high GM-CSF concentrations generated MDSCs along with neutrophils in 3–4 days, whereas lower concentrations of GM-CSF required 8–10 days to generate MDSCs [37]. Results of clinical trials studying GM-CSF as an adjuvant in cancer vaccination were reviewed by Parmiani et al. [41]. At low concentrations, GM-CSF potentiated a vaccine-induced antitumor response, whereas at higher doses, an immunosuppressive effect was observed [41]. In addition to dose-specific effects, GM-CSF preferentially expanded highly suppressive M-MDSCs in a mammary tumor mouse model [8]. In the same study, G-CSF preferentially expanded PMN-MDSCs that resulted in a less immunosuppressive environment [8].

Vascular endothelial growth factor (VEGF), a key mediator of angiogenesis in tumors, is also a potent inducer of MDSC expansion. Gabrilovich et al. were the first to demonstrate that VEGF produced by breast and colon cancer cells significantly affected the functional maturation of progenitor stem cells [42]. Subsequent studies revealed that activation of VEGF receptor (VEGFR) in the bone marrow of mice leads to myeloid expansion [43]. In this same study, neutralizing GM-CSF activity via antibodies, or by use of GM-CSF-null hematopoietic cells, inhibited VEGFR-mediated myeloid progenitor activity. More recently, Horikawa et al. demonstrated that patient high-grade serious ovarian cancer IHC samples that presented high levels of VEGF upregulated genes associated with myeloid cell chemoattractants and matrix metalloproteases (MMPs) [44]. Furthermore, a mouse ovarian tumor cell line (ID8) modified to overexpress *Vegf* induced an increase in intratumoral MDSCs and decrease in effector T cells [44].

In addition to key myeloid-specific growth factors, IL-1β and IL-6 are potent inducers of MDSC expansion. Mammary carcinoma cells transfected to overexpress IL-1β in mice exhibited decreased survival times in addition to elevated levels of splenic MDSCs [45]. Similarly, stomach-specific overexpression of human IL-1β in transgenic mice subsequently leads to spontaneous gastric inflammation and cancer that correlated with recruitment of MDSCs [46]. It is proposed that IL-1β skews MDSC expansion to that of PMN-MDSCs [47]. Due to the pleiotropic nature of IL-1β, it is believed that IL-1β acts to stimulate MDSCs both directly [46, 47] and indirectly via stimulation of growth factors and cytokines [48], including IL-6. Mice deficient in IL-1R exhibited reduced MDSC numbers that were rescued by re-expression of IL-6 [49]. In a hormone-resistant prostate cancer mouse model, IL-6 correlated with both aggressive tumor growth and MDSC recruitment that could be diminished via an IL-6-silencing shRNA [50]. Moreover, IL-6 blocking mAbs resulted in significantly less accumulation of MDSCs in a mouse model of squamous cell carcinoma [51].

In response to these growth factors and cytokines, myeloid progenitor cells initiate a complex transcriptional network that enables their expansion and prevents their differentiation. Many of the abovementioned factors converge on the activation of signal transducer and activator of transcription (STAT) 3 [52]. For this reason, STAT3 is generally considered to be a master transcription factor regulating both the expansion and activation of MDSCs [1]. In general, STAT proteins are intracellular transcription factors that mediate a wide variety of cell functions, including proliferation, apoptosis, and differentiation. In response to appropriate stimuli, transmembrane receptors dimerize and induce *trans*-phosphorylation of their associated Janus kinases (JAKs). The phosphorylated JAKs can subsequently activate downstream targets, including STATs. Upon activation by phosphorylation, STAT proteins form homo- or hetero-dimers with other STAT family members and translocate to the nucleus, where they bind DNA and induce the transcription of multiple gene targets. STAT3 upregulates genes essential for myeloid progenitor cell survival and proliferation, including Bcl-XL, MYC, survivin, and cyclin D1 [1, 53]. Furthermore, STAT3 activation drives cell surface expression of the S100A8/S100A9 dimer, a calcium- and zinc-binding complex that regulates a variety of inflammatory immune responses and serves as a phenotypic and functional marker for MDSCs. Cheng et al. demonstrated mice lacking S100A9 elicited potent antitumor immune responses, which could be reversed by adoptive transfer of wild-type MDSCs from tumor-bearing mice into S100A9-deficient mice [54]. In contrast, overexpression of S100A9 in cultured embryonic stem cells and transgenic mice inhibited the differentiation of DCs and macrophages and resulted in accumulation of MDSCs [54]. As a clinical correlate, an interaction between S100A9 and the common myeloid marker CD33 facilitated the expansion of MDSC in myelodysplastic syndrome patients. STAT3 activation was also shown to modulate interferon regulatory factor-8 (IRF-8), an integral transcriptional factor regulating myeloid terminal differentiation [55]. *Irf8*-deficient mice generated phenotypically and functionally similar immature myeloid populations compared to tumor-induced MDSCs. In contrast, IRF-8 overexpression in mice facilitated a reduction in MDSC levels in the spleen, bone marrow, and tumor site, suggesting an important negative regulatory role for IRF-8 in MDSC accumulation and differentiation [55]. CCAAT-enhancer-binding proteins (C/EBPs) are a family of transcription factors implicated downstream of STAT3 in blocking terminal differentiation as well as expansion of MDSCs. C/EBPs comprise a family of six basic-region leucine zipper transcription factors that homo- or hetero-dimerize with other C/EBPs and transcription factors that enable binding to target DNA. Specifically, C/EBPα and C/EBPε mediate differentiation and maturation of myeloid progenitors, whereas C/EBPβ is only important in regulating emergency myelopoiesis [56, 57]. Mackert et al. demonstrated that C/EBPα was significantly reduced in MDSCs from tumor-bearing mice compared to non-tumor-bearing hosts [58]. Similarly, myeloid lineage-specific deletion of C/EBPα resulted in significantly enhanced MDSC proliferation and expansion as well as increased myeloid progenitors and decreased mature cells [58]. Conversely, Marigo et al. demonstrated that in a myeloid lineage-specific mouse model of C/EBPβ deletion, there was a significant loss of an MDSC-like population and reversal of a tolerogenic state exhibited by tumor-specific T cells [15]. These data suggest that chronic signals secreted via the tumor skew the normal balance of C/EBPs to a profile that sustains myeloid proliferation while blocking terminal differentiation.

Lastly, microRNAs (miRNAs) have also been implicated in facilitating the induction and expansion of MDSCs. miRNAs are endogenous, small non-coding RNAs that modulate gene expression. miRNAs have been proposed to function as either oncogenes or tumor suppressors [59]. More specific to MDSCs, miRNAs can affect the development and differentiation of HSCs to lineage-specific cells [60]. Recently, miR-155 and miR-21 were identified as the two miRNAs highly upregulated during the induction of MDSC from the bone marrow cells via GM-CSF and IL-6 [61]. miR-155 and miR-21

miRNAs down-modulated the expression of the negative regulator of myeloid cell proliferation, SHIP-1, and the tumor suppressor gene PTEN. Down-modulation of either SHIP-1 or PTEN leads to an increase in STAT3 activation. Thus, miR-155 and miR-21 may synergistically enhance the induction of MDSCs via down-modulation of negative regulators, in addition to increasing STAT3 activation [61].

8.3.2 Signal 2

After immature myeloid cell expansion and accumulation, it is thought that these cells then receive an "activation signal" that endows them with suppressive functionality. This activation is mediated by tumoral stroma factors that include IL-1β, PGE_2, TNF-α, toll-like receptor (TLR) ligands, and IFN-γ [29]. Similar to the importance of STAT3 in MDSC induction and expansion, the NF-κB pathway is an essential factor mediating MDSC activation [30]. NF-κB proteins encompass a family of dimeric transcription factors that regulate the expression of genes governing a broad range of immunological processes. In general, stimuli activate an IKK complex, which subsequently phosphorylates IκB proteins that exist in complex with NF-κB proteins. Upon phosphorylation, IκB is ubiquitinated and degraded, freeing the NF-κB proteins to enable translocation to the nucleus, where they bind target DNA sequences and facilitate gene transcription. IL-1β activates MDSCs recruited to tumor sites via an NF-κB-dependent pathway, evidenced by an increase in tumor microenvironment TNF-α and IL-6 [46]. Similarly, it was demonstrated in a mammary tumor mouse model that IL-1β-induced inflammation increased MDSC production of IL-6 and TNF-α via the TLR4/CD14 pathway, which signals through the NF-κB pathway [62]. The timing of myeloid cell expansion in relation to these "activation signals" is not understood and is an area of active investigation.

Liu et al. demonstrated that tumor-derived exosomes (TDEs) were also potent inducers of IL-6 and TNF-α in MDSCs [63]. In this study, TDE-mediated activation was dependent on MyD88, an important adapter protein in the TLR signaling pathway. Analogously, another study demonstrated that TDE membrane-associated heat shock protein 72 (Hsp72) activated MDSC suppressive functions through TLR2/MyD88-dependent mechanisms [64]. Both studies confirmed dependence on the MyD88 pathway with an associated increase in phosphorylated STAT3, suggesting synergy between NF-κB and STAT3 signaling. More recently, Achyut et al. demonstrated the importance of NF-κB signaling in MDSC function within a mouse model of glioblastoma [64]. Conditional deletion of p65 in myeloid cells in this model resulted in decreased intratumoral MDSCs with increased dendritic cells and T cells, further suggesting a role for NF-κB in MDSC expansion.

PGE_2 has also been implicated in the activation of MDSCs. Activation of MDSC functions by PGE_2 exposure requires contact or close proximity between monocytes and melanoma cells and was dependent on COX2 [65]. Cancer patient-derived M-MDSCs treated with PGE_2 resulted in the activation of the p38 MAPK/ERK pathway and an increase in TGF-β secretion, leading to potent suppression of T and NK cell function in vitro [66]. Furthermore, silencing of COX2 via shRNA resulted in reduced MDSC numbers in the spleen and an increase in the number of NK cells in an in vivo model. PGE_2 has also been implicated in MDSC expansion. Sinha et al. demonstrated that bone marrow stem cells stimulated with agonists of the prostaglandin EP2 receptor (EP2R) induced differentiation into murine $Gr\text{-}1^+CD11b^+$ MDSCs [67]. Additionally, EP2R knockout mice exhibited reduced MDSC numbers to wild type, suggesting that EP2 partially mediates MDSC induction and expansion. Blocking PGE_2 production by COX2 inhibitors also reduced MDSC numbers in these models.

In order for MDSCs to exert their tumor-promoting suppressive functions at sites of tumor, they must be able to function within tumor microenvironments that present hostile conditions, including hypoxia, low pH, and oxidative stress. In most cells of the body, these conditions disrupt the protein-folding capacity of the endoplasmic

reticulum (ER) and result in activation of the ER stress response pathway, which when overwhelmed leads to cell dysfunction or death [68]. However, persistent ER stress within tumors paradoxically facilitates tumor progression through effects on both malignant cells and infiltrating cells, such as MDSCs [68]. In tumor-bearing mice, Lee et al. demonstrated that repeat administration of the ER stress inducer thapsigargin resulted in increased mRNA levels of the immunosuppressive factors ARG1, iNOS, and NOX2 in tumor-infiltrating MDSCs [69]. Thus, the TME reinforces MDSC-mediated immunosuppression, though it still may eventually influence MDSC in vivo life span [70].

8.4 Mechanisms of MDSC Suppression

Peripheral MDSCs, such as those in circulation or localized to secondary lymphoid organs, differ in their mechanisms of suppression compared to MDSCs localized to the tumor. In the periphery, MDSC immunosuppression is governed by multiple mechanisms, including production of immunosuppressive metabolites. For example, MDSC suppression has been affiliated with the metabolism of L-arginine. Local L-arginine depletion results in the proliferation arrest of activated T lymphocytes, as well as downregulation of the CD3ζ chain [71, 72]. Indeed, using arginase-1 inhibitors, either in vitro or in tumor-bearing mice, restored T-cell function and resulted in immune-mediated antitumor responses [73]. Similar to arginine, cysteine also serves as an essential amino acid for T lymphocytes, which rely on antigen-presenting cells (APCs) to export soluble cysteine into the milieu. MDSCs harbor a cystine transporter but lack a cysteine transporter. Thus, MDSCs can sequester extracellular cystine from APCs without returning cysteine back to the milieu [74], starving T and NK lymphocytes of this essential amino acid. Oxidative stress via the production of reactive oxygen and nitrogen species by NO synthases also contributes to peripheral T-cell inhibition. Raber et al. demonstrated that PMN-MDSCs and M-MDSCs utilize different NO synthases for the suppression of T lymphocytes [75]. PMN-MDSC inhibited T lymphocyte proliferation via peroxynitrites dependent on endothelial nitric oxide synthase (eNOS), whereas M-MDSCs elicited their effects via the release of NO by iNOS. Molecular mechanisms of reactive oxygen and nitrite species-mediated T lymphocyte suppression include loss of TCR ζ-chain expression [76] and inhibiting T-cell activation [77]. In addition, peroxynitrites impede extravasation of lymphocytes from circulation, thus decreasing the pool of antitumor lymphocytes available at tumor sites [78, 79].

Another major mechanism mediating peripheral immunosuppression is the recruitment and induction of other suppressive or regulatory cells, such as thymus-derived natural T regulatory (nTreg) cells and local tumor-induced Treg (iTreg) cells. In healthy individuals, Tregs are a subset of T cells that play critical roles in immune modulation, specifically maintaining peripheral tolerance and preventing autoimmunity. However, in the context of cancer, Tregs contribute to an immunosuppressive periphery and TME that facilitates tumor escape [80]. Because the TME allows close proximity between MDSCs and Tregs, considerable cross-talk exists that serves to modulate both populations. Indeed, Ghiringhelli et al. demonstrated that immature myeloid cells induced by tumor progression selectively promoted the proliferation of Tregs in a TGF-β-dependent manner in vivo [81]. Huang et al. also demonstrated that MDSCs induce the development of Treg cells in vitro and in tumor-bearing mice and that Treg induction was dependent on MDSC-secreted IL-10 and IFN-γ [82]. Further, MDSCs upregulate ligands for several costimulatory molecules (specifically, CD86 and PD-L1) that additionally provided signals for Treg development. In a mouse model of B-cell lymphoma, MDSCs demonstrated the ability to uptake tumor-associated antigens and present them to facilitate the expansion of tumor-specific Tregs [83]. In addition to inducing the development of Tregs, Hoechst et al. also showed that MDSCs were capable of inducing the transdifferentiation of Th17 T cells into Tregs [84]. Conversely, Tregs can also modulate MDSC

expansion and function. In a mouse model of colitis, Tregs potentiated both the expansion of MDSCs and suppressive functions through a TGF-β-dependent mechanism [85]. Thus, factors secreted by both MDSCs and Tregs exist in positive feedback loops to facilitate the expansion of each population and reinforce the suppressive environment.

In addition to the cross-talk between MDSCs and Tregs, MDSCs have also demonstrated effects on macrophages. In a mammary carcinoma mouse model, MDSCs decreased IL-12 production by macrophages and facilitated the polarization of macrophages to a tumor-promoting M2 phenotype [86]. Beury et al. sought to further define the cross-talk that occurs between macrophages and MDSCs in the context of murine tumor cell lines [87]. They found that IL-6, IL-10, IL-12, TNF-α, and NO are modulated within the cross-talk between macrophages, MDSCs, and tumor that create an immunosuppressive environment.

In contrast to the mechanisms employed by MDSCs in the periphery, MDSCs localized to tumor tissues exhibit several key differences. Studies directly comparing MDSCs from spleens (peripheral tissue) and tumors of the same mouse demonstrated that tumor MDSCs acquire a more suppressive phenotype in the TME characterized by high amounts of NO, arginase-1, and immunosuppressive cytokines. In a mouse model of prostate cancer, Haverkamp et al. demonstrated that MDSCs derived from tumor tissue possessed immediate ability to inhibit T-cell function, whereas MDSCs isolated from the spleens and liver were not suppressive without additional in vitro exposure to suppressive cytokines [88]. Maenhout et al. showed that both PMN-MDSC and M-MDSC isolated from the tumor had much stronger suppressive capacity compared to MDSCs isolated from the spleen, associated with higher nitrogen dioxide production and arginase-1 [89]. Another study examining the metabolic characteristics of tumor-infiltrating MDSCs (TI-MDSCs) reported that TI-MDSCs increased fatty acid uptake and activated fatty acid oxidation (FAO) [90]. In this study, MDSCs isolated from the site of the tumor were able to inhibit antigen non-specific T-cell proliferation, whereas splenic MDSCs did not.

The suppressive mechanisms employed by different MDSC subsets likely depend on multitude of factors that includes the preferential expansion of the MDSC subset and local inflammatory milieu. The relative suppressive capacity of the major MDSC subsets, M-MDSCs and PMN-MDSCs, is a matter of debate, with the literature suggesting mixed results. Traditionally, it has been proposed that M-MDSCs are more suppressive due to the increased levels and higher half-life of suppressive mediators [91]. However, in certain tumor subtypes, PMN-MDSCs represented the more suppressive subset. For example, in a mouse model of pancreatic adenocarcinoma, PMN-MDSCs were preferentially expanded and represented the majority of MDSCs in the bone marrow, blood, spleen, and pancreas [92]. Targeted depletion of PMN-MDSCs in this model resulted in restored antitumor immunity and a reduction in tumor size. In a study analyzing the peripheral blood of patients with head and neck and urological cancers, a higher frequency of PMN-MDSCs was associated with a significant decrease in survival [93]. Additionally, PMN-MDSCs were more suppressive in in vitro T-cell suppression assays compared to the M-MDSCs isolated from the same patients [93]. Similarly, in advanced-stage melanoma patients, PMN-MDSCs isolated from peripheral blood suppressed stimulated T cells more strongly than M-MDSCs and negatively correlated with survival rate [94]. When compared to healthy controls, lung cancer patients had elevated levels of circulating M-MDSCs but not PMN-MDSCs [95]. However, a significant increase in both intratumoral M-MDSCs and PMN-MDSCs was evident compared to peripheral levels in tumor patients. In a breast cancer patient cohort, both PMN-MDSC and M-MDSC peripheral levels were elevated to similar levels when compared to each other and healthy controls [96]. Thus, a tumor-specific understanding of the relevant MDSC subsets in the periphery and TME can inform of potential mechanisms of suppression.

8.5 Non-immunologic Functions of MDSCs

In addition to their immunosuppressive effects, MDSCs also support non-immunologic aspects of tumor biology including tumor angiogenesis and metastasis [33]. During tumor growth, especially solid tumors, the tumor microenvironment requires angiogenesis to provide adequate oxygen and nutrients, as well as the removal of waste products, both of which facilitate optimal growth [97]. Yang et al. demonstrated in murine tumor models that Gr-1$^+$CD11b$^+$ cells co-injected with tumor cells increased tumor angiogenesis and vasculature maturation compared to tumor cells alone [98]. Additionally, Gr-1$^+$CD11b$^+$ cells were found directly incorporated into the new vessel endothelium and that they drastically upregulated endothelial markers. In a mouse model of glioblastoma, intratumoral accumulation of CD11b$^+$ myeloid cells promoted angiogenesis [99]. Interestingly, in both studies, MMP-9 activity was necessary to facilitate angiogenesis. In a murine model of multiple myeloma, PMN-MDSCs, but not M-MDSCs, induced a pro-angiogenic effect using the chick chorioallantoic membrane assay [100]. Furthermore, PMN-MDSCs demonstrated an upregulation of pro-angiogenic factors, including VEGF. Parihar et al. also demonstrated that in several xenograft mouse models including neuroblastoma and rhabdomyosarcoma, co-inoculation of M-MDSC with tumor cells facilitated an increase in TME microvasculature and vascular leakiness compared to tumor alone [101]. Bv8 (also known as prokineticin-2), a VEGF homologue [102], is upregulated in MDSCs and promoted tumor angiogenesis in murine xenograft [103] and transgenic models [104].

Clinical correlative data support the notion of MDSC-mediated tumor metastasis in breast cancer [105], non-small cell lung cancer (NSCLC) [106], melanoma [107], and prostate cancer [108]. Indeed, MDSCs have been readily implicated in tumor invasion. Clark et al. demonstrated in a mouse model of pancreatic ductal adenocarcinoma that MDSCs suppressed early antitumor immune responses that facilitated invasive tumor lesions [109]. The establishment of a pre-metastatic niche that accepts and protects circulating tumor cells in secondary organs is widely accepted to be a key determinant of tumor metastasis [110]. MDSCs have been shown to remodel the secondary organ local microenvironment through secretion of pro-angiogenic and growth factors in addition to inflammatory cytokines [111, 112]. Recruitment of tumor metastases to secondary sites is largely facilitated through chemokines and integrins. In an orthotopic murine model of colorectal carcinoma, VEGF secreted by primary tumor cells stimulated TAMs to produce CXCL1 that subsequently is released into circulation [113]. In response to CXCL1, circulating CXCR2$^+$ MDSCs were then recruited to the liver to establish a pre-metastatic niche that expedited liver metastasis. Similarly, in a mouse model of breast cancer, primary breast tumor-derived chemokine CCL2 resulted in the accumulation of PMN-MDSCs in the lungs [114], resulting in establishment of a pre-metastatic niche. Once at the site of the secondary organ, MDSCs contribute to the remodeling of the extracellular matrix (ECM) to make the local microenvironment more permissive for the seeding of circulating tumor cells. MDSC-mediated factors include chemokines, cytokines, growth factors, and extracellular vesicles, including TGF-β [115], VEGFA [116], S100A8/A9 [117], and MMP-9 that aid in pre-metastatic niche formation [110].

8.6 Clinical Significance of MDSCs

With the recent success of immunotherapies (checkpoint blockage, CAR-T cells) in treating a limited number of cancer types, efforts to translate this success to other tumors have intensified. Because the TME helps evade and inhibit antitumor responses, investigators have begun to assess peripheral and intratumoral MDSCs within the context of clinical trials (reviewed in Table 8.1). Elevated levels of circulating and intratumoral MDSC correlate with poor prognosis in various types of cancer and thus are postulated to correlate to immune escape. In

Table 8.1 Examples of completed and recruiting clinical trials where the frequency and/or suppressive capacity of MDSCs were assessed as primary or secondary outcome measures

Clinical trial number	Study title	Condition(s)	Interventions	Status	Primary/secondary outcome measure (of how many measures)
NCT00499122	NOV-002, Doxorubicin, Cyclophosphamide, and Docetaxel in Women with Newly Diagnosed Stage II or IIIC Breast Cancer	Breast cancer	Cyclophosphamide, Docetaxel, Doxorubicin, NOV 002	Completed, with results	Secondary
NCT00843635	Tadalafil in Treating Patients Undergoing Surgery for Cancer of the Oral Cavity or Oropharynx	HNSCC	Tadalafil	Completed, with results	Primary (1 of 3)
NCT02403778	Ipilimumab and All-Trans Retinoic Acid Combination Treatment of Advanced Melanoma	Advanced melanoma	VESANOID, Ipilimumab	Completed, with results	Primary (2 of 3)
NCT01425749	Study to Assess Safety and Immune Response of Stage IIB–IV Resected Melanoma After Treatment with MAGE-A3 ASCI (Mel55)	Melanoma	recMAGE-A3, AS15 ASCI	Completed, with results	Secondary
NCT01876212	Dendritic Cell Vaccines + Dasatinib for Metastatic Melanoma	Metastatic melanoma	DC vaccine, Dasatinib	Completed, with results	Secondary
NCT01581970	Potentiation of Cetuximab by Tregs Depletion With CSA in Advanced Head and Neck Cancer	HNC, HNSCC	Cyclophosphamide, Cetuximab	Completed, results submitted	Secondary
NCT03188276	The Relationship Between MDSCs and NK Cells Activity of CHC Patient Treated by DAAs	Chronic hepatitis C	Ledipasvir-Sofosbuvir, Daclatasvir-Sofosbuvir	Completed, no results posted	Primary (1 of 1), secondary
NCT02332642	Immunomonitoring of Patients With Metastatic Melanoma (AJCC Stadium IV) Under Chemotherapy (ImmunoPAX)	Metastatic melanoma	Immunomonitoring	Completed, no results posted	Primary
NCT02275039	p53MVA Vaccine and Gemcitabine Hydrochloride in Treating Patients With Recurrent Ovarian Epithelial Cancer	Recurrent ovarian epithelial, fallopian tube, and peritoneal carcinoma	Modified vaccinia virus ankara vaccine expressing p53, gemcitabine hydrochloride	Completed, no results posted	Secondary
NCT02718443	VXM01 Phase I Pilot Study in Patients With Operable Recurrence of a Glioblastoma	Glioblastoma	VXM01	Completed, no results posted	Secondary
NCT01653249	A Phase I Clinical Trial of an HPV Therapeutic Vaccine	Human papillomavirus	Vaccine consisting of four HPV-16 E6 peptides in combination with Candin®	Completed, no results posted	Secondary

NCT04022616	Myeloid-Derived Suppressor Cell Function in Breast Cancer Patients	Breast cancer	Specimen collection	Recruiting	Primary (1 of 1), secondary
NCT02827344	PDL-1 Expression on Circulating Tumor Cells in Non-small Cell Lung Cancer (IMMUNO-PREDICT)	Lung cancer	Blood sample collection for circulating tumor cells and MDSC analysis	Recruiting	Secondary
NCT02669173	Capecitabine + Bevacizumab in Patients With Recurrent Glioblastoma	Glioblastoma	Capecitabine, Bevacizumab	Recruiting	Primary (1 of 1), secondary
NCT02916979	Myeloid-Derived Suppressor Cells and Checkpoint Immune Regulators' Expression in Allogeneic SCT Using FluBuATG (FluBuATG)	Various hematological malignancies	Fludarabine, Busulfan, Rabbit ATG, Methotrexate	Recruiting	Secondary
NCT03486119	A Study for Identification of Predictive Immune Biomarker in Peripheral Blood for Nivolumab Therapy in NSCLC Patients	Non-small cell lung cancer	Nivolumab	Recruiting	Primary (1 of 6)

patients with advanced hepatocellular carcinoma (HCC), frequencies of circulating M-MDSCs were significantly higher compared to early-stage HCC patients [118]. Additionally, patients who underwent curative radiotherapy and had higher frequencies of M-MDSCs posttreatment had significantly shortened relapse-free and overall survival [118]. Similarly, frequencies of peripheral MDSCs increased with advanced cancer stage in patients with breast cancer [96]. In a meta-analysis of 16 different studies involving 1864 cancer patients with GI, HCC, NKT lymphoma, and melanoma tumors, Ai et al. found that increased MDSC frequencies were associated with poor prognoses and decreased overall survival [119]. MDSCs have also been proposed to serve as a prognostic marker in informing the best option for treatment. One such example is the immune checkpoint inhibitor ipilimumab approved for the treatment of metastatic melanoma. Ipilimumab has demonstrated efficacy in some cases, but overall clinical response rates remain low, with 3-year survival rates of about 20% [120]. Hence, efforts to understand factors that govern whether a patient responded or not are needed to enhance ipilimumab efficacy. Meyer et al. reported that metastatic melanoma patients who responded to ipilimumab treatment had significantly lower circulating frequencies of MDSCs compared to nonresponders [121]. In a similar study that aimed to identify baseline peripheral prognostic markers in metastatic melanoma that correlated with clinical outcome following ipilimumab treatment, MDSC frequencies were the strongest stand-alone predictor of clinical response to treatment [122]. This finding was also demonstrated in several other melanoma studies [123, 124] in addition to prostate cancer [125]. Thus, the clinical significance of MDSCs has inspired efforts to therapeutically target this population that could enhance the efficacy of antitumor immunity.

8.7 Therapeutic Targeting of MDSCs

Efforts to therapeutically target MDSCs have attempted to do so by (1) inhibiting MDSC expansion and trafficking, (2) differentiating MDSCs into mature and less suppressive myeloid cells, (3) inhibiting MDSC immunosuppressive function, and (4) depleting MDSCs from the TME [126].

8.7.1 Inhibiting MDSC Expansion and Trafficking

As discussed, MDSC generation occurs by way of abnormal myelopoiesis stimulated via tumor-derived factors. Thus, efforts to modulate or correct this abnormal myelopoiesis could prove beneficial in preventing MDSC accumulation. As discussed previously, STAT3 activation has been demonstrated to play a crucial role in the mobilization and expansion of MDSCs. Hence, agents that can block STAT3 activation in MDSCs could prove a viable option in preventing the expansion of MDSCs. Sunitinib, a small-molecule multitargeted receptor tyrosine kinase inhibitor with antitumor and anti-angiogenic effects [127], has proven a useful agent in targeting STAT3 signaling in MDSCs. In patients with renal cell carcinoma (RCC), treatment with sunitinib resulted in reduced numbers of circulating MDSCs [128]. Xin et al. later demonstrated in mouse models of RCC that sunitinib inhibited STAT3 activity and concomitantly resulted in a significant reduction of MDSCs and Tregs at the site of the tumor [129]. Pretreatment with sunitinib in RCC patients resulted in improved tumor-infiltrating lymphocytes that inversely correlated with intratumoral MDSC numbers [130]. Bevacizumab, an anti-VEGF mAb, has also shown to have effects on MDSC expansion. Rather than having a direct effect on MDSCs, bevacizumab inhibits VEGF at the site of the tumor, thus preventing VEGF as an inducer of MDSC expansion. In a mouse model of RCC, bevacizumab resulted in a decrease of peripheral $CD11b^+$ myeloid cells [131]. Similarly, in patients with metastatic colorectal carcinoma, bevacizumab administered with the chemotherapy drugs 5-fluorouracil and oxaliplatin resulted in a decrease in PMN-MDSC in 15 out of 25 evaluable patients [132]. Given that broad inhibition of STAT signaling may have off-target implications that restrain overall antitumor responses, other methods to more specifically target STAT3

have included the use of STAT3 siRNA or antisense oligonucleotides synthetically attached to a toll-like receptor 9 (TLR9) agonist that selectively targets TLR9+ myeloid cells [133]. STAT3 siRNA directed at TLR9+ cells elicited a potent antitumor response in vivo [133].

In addition to STAT3, targeting PGE_2 and COX2 has reduced MDSC numbers in mouse models. Sinha et al. first demonstrated that the COX2 inhibitor SC58236 reduced MDSC accumulation and delayed primary tumor growth in a mouse model of breast cancer [67]. Subsequently, the COX2 inhibitor celecoxib was effective in reducing MDSC numbers in mouse models of mesothelioma [134] and glioma [135]. Currently, a clinical trial (NCT02432378) is recruiting patients with recurrent ovarian cancer to examine the effects of celecoxib in addition with cisplatin. A secondary outcome measure in this study will be the change in the number of MDSCs in the peritoneal fluid of the patients.

Preventing MDSC trafficking and localization to the tumor may prevent accumulation and subsequent pro-tumor effects within the TME. Studies have implicated the chemokine receptor CXCR2 as a key mediator of MDSC trafficking that facilitates both tumorigenesis and metastasis [113, 136–138]. Highfill et al. demonstrated in a murine model of rhabdomyosarcoma that tumor cells expressing CXCL1 and CXCL2 facilitated trafficking of CXCR2+ MDSCs to the tumor [139]. In mice with a CXCR2-deficient bone marrow compartment, the percentage and absolute numbers of MDSC recruited to the tumor were significantly decreased [139]. Similarly, in mouse models of lung carcinoma, the small-molecule CXCR1/2 inhibitor SX-682 abrogated PMN-MDSC recruitment to the tumor that resulted in potentiated T-cell activation and antitumor immunity [140]. A clinical trial (NCT03161431) is currently evaluating the efficacy of SX-682 to block MDSC recruitment in metastatic melanoma. In addition to CXCR2, CXCR5 can also recruit MDSCs to intratumoral sites [141, 142]. Fusion proteins (CCR5-Ig) directed at all three CCR5 ligands demonstrated reduced MDSC infiltrates in mouse models of melanoma [141] and prostate cancer [142].

8.7.2 Differentiating MDSCs into Mature Cells

As MDSCs represent an immature myeloid population, efforts have been directed at understanding the requirements for their differentiation that may then attenuate their suppressive functions. To this end, all-*trans* retinoic acid (ATRA) has demonstrated a potent differentiator of MDSCs. ATRA is a natural metabolite of vitamin A oxidation and is a well-known regulator of cell differentiation, including the terminal differentiation of promyelocytes into mature neutrophils in patients with acute promyelocytic leukemia [143], and thus is a commonly used antineoplastic in chemotherapy regimens for this disease. Almand et al. demonstrated the utilization of ATRA to differentiate MDSCs into dendritic cells [144] and that differentiation eliminated the inhibitory function of MDSCs in vitro [145]. The mechanism of ATRA-dependent differentiation appears to be neutralization of ROS production in MDSCs via the accumulation of glutathione, both in patients and mice [146]. Subsequent clinical trials have demonstrated the potential of ATRA alone [146] or in combination with other therapies to reduce the number of circulating MDSCs [147, 148]. Although the clinical efficacy of ATRA has been demonstrated in a multitude of other trials given its use in common chemotherapy regimens, because MDSCs were not evaluated in these trials, the effect of ATRA could not be attributed to MDSC reduction [149]. Vitamin D3 has also demonstrated the ability to induce the differentiation of immature myelocytes. In patients with head and neck squamous cell carcinoma (HNSCC), CD34+ progenitor cells believed to be responsible for intratumoral immunosuppression were isolated and cultured in the presence of vitamin D3 and various cytokines [150]. CD34+ cells cultured with the combination of vitamin D3 and cytokines resulted in increased numbers of cells phenotypically similar to mature dendritic cells. In addition, these cells present antigen more efficiently to autologous T cells. In a study examining the clinical efficacy of vitamin D3 in reducing immature CD34+ at tumor sites, Kulbersh et al. found that

patients treated with vitamin D3 displayed reduced immature CD34⁺ numbers, whereas levels of intratumoral dendritic cells increased [151]. HNSCC patients treated with vitamin D3 had increased numbers of intratumoral T cells and had a longer relapse-free survival compared with the controls [152]. In patients with chronic lymphocytic leukemia (CLL), significantly higher levels of circulating M-MDSCs were present in patients with low vitamin D levels compared to those of CLL patients with high vitamin D levels [153]. Tumor-conditioned CLL exosomes facilitated the induction of healthy donor-derived monocytes to MDSCs that was reversed with pretreatment of the CLL exosomes with vitamin D3 [153].

8.7.3 Inhibiting MDSC Suppressive Function

Phosphodiesterase (PDE) inhibitors, such as sildenafil and tadalafil, are pharmacologic agents that increase the intracellular concentrations of secondary messenger molecules cAMP and cGMP. PDE inhibitors have been used clinically with widespread use in non-malignant conditions such as erectile dysfunction. Their utilization for inhibition of MDSC suppressive functions was demonstrated by Serafini et al. [154], where in multiple tumor models, the administration of sildenafil downregulated arginase-1 and NOS expression. This resulted in enhanced intratumoral T-cell infiltration and activation with resultant reduced tumor growth [154]. Subsequent studies demonstrated modulation of the suppressive function of MDSCs with improved antitumor immunity after treatment with PDE inhibitors in mouse models of colonic inflammation-induced tumorigenesis [155] and melanoma [156]. In the clinic, tadalafil has demonstrated safety and efficacy in reducing peripheral and intratumoral MDSC numbers. In patients with HNSCC, tadalafil significantly reduced arginase-1 and iNOS activity compared to controls, in addition to enhancing antitumor immunity [157]. A significant reduction in MDSC numbers was observed in patients treated with tadalafil, suggesting that arginase-1 and iNOS inhibition could interfere with autocrine and paracrine feedback loops that facilitate MDSC myelopoiesis [157]. Similarly, in a phase II clinical trial, tadalafil significantly reduced both MDSC and Treg numbers in HNSCC patients with an increase in CD8⁺ T cells [158]. A study in patients with metastatic melanoma demonstrated that tadalafil achieved stable disease as best response in 25% (3/12) evaluable patients. Moreover, in the responders, M-MDSC peripheral numbers decreased, and NO production was reduced in MDSC-infiltrated metastatic lesions [159].

8.7.4 Depleting MDSCs from the TME

Efforts have also focused on directly eliminating MDSCs at the tumor site to overcome their suppressive effects. Observations in mouse models of various tumors found that cytotoxic agents such as cisplatin [160], 5-fluorouracil [161], gemcitabine [161, 162], and oxaliplatin [163] were effective at selectively reducing MDSC numbers. However, due to their non-specific cytotoxicity and adverse side effects, other groups have developed novel approaches to selectively target intratumoral MDSCs. One such approach is the use of liver X receptor (LXR) agonists. LXR agonism significantly suppressed tumor growth and metastasis in vitro and in vivo [164]. Thus, Tavazoie et al. sought to define the antitumor mechanisms of LXR agonism in various cancer models [165]. LXR agonist RGX-104 resulted in significant tumor growth suppression in an array of cancer animal models, including lung, breast, ovarian, and colon cancer [165]. Tumor suppression was due to RGX-104-mediated reduction of MDSCs. In a phase I dose-escalation trial (NCT02922764) evaluating the safety of RGX-104, peripheral PMN-MDSC and M-MDSC numbers were decreased by an average of 85% in the first cohort of evaluable patients [165]. Another novel approach developed by Parihar et al. is the use of natural killer (NK) cells modified to express a chimeric version of the NK cell-activating receptor NKG2D, herein referred

to as NKG2D.ζ, that targets MDSCs specifically within the TME, as intratumoral MDSCs upregulated activating ligands for NKG2D [101]. Tumors escape wild-type NKG2D-mediated NK cell cytotoxicity by down-modulating the wild-type NKG2D cytotoxic adapter molecule DAP10 [166]. Thus, a synthetic NKG2D construct expressed on the surface of NK cells that bypasses the need for DAP10 could overcome the suppressive effects of the TME and rescue NKG2D-mediated cytotoxicity. Indeed, NKG2D.ζ expressing NK cells exhibited cytotoxicity against autologous and allogeneic MDSCs in vitro and in vivo. In a mouse model of neuroblastoma that recapitulated the suppressive TME, co-injection of NKG2D.ζ NK cells and neuroblastoma-directed chimeric antigen receptor (CAR)-T cells increased the antitumor activity of CAR-T cells when compared to CAR-T cells alone. Thus, NKG2D.ζ NK cells as a combination treatment could enhance current immunotherapeutic treatment modalities by altering the TME to one more permissive of antitumor immunity [101].

8.8 MDSCs in Non-oncologic Conditions

Though studies regarding MDSCs have largely focused on their implications in cancer, it is now being appreciated the role MDSCs might play in non-oncologic settings, such as autoimmunity. Because studies investigating the involvement of MDSCs in autoimmunity are relatively new, much is yet to be understood about the potential role that MDSCs have in either promoting or inhibiting autoimmune disease [167]. Similar to cancer studies, MDSCs in autoimmunity demonstrate the same plasticity and heterogeneity that make definitive characterization difficult. Thus, efforts to compare and contrast studies characterizing MDSCs in autoimmunity have been extremely challenging [167]. In a mouse model of experimental autoimmune encephalitis (EAE) that closely resembles the pathology of multiple sclerosis, adoptive transfer of PMN-MDSCs reversed EAE by suppressing the expansion of autoreactive T cells through PD-L1 [168]. In addition, circulating PMN-MDSC frequency was increased in the periphery of human subjects with MS [168], indicating MDSCs might also play a role in facilitating MS. Conversely, Cantoni et al. found that overall MDSC numbers were decreased in the periphery of MS patients compared to healthy controls. In a study examining MDSCs over the course of MS disease progression, M-MDSCs isolated from patients diagnosed with secondary progressive MS were impaired in their ability to suppress autologous $CD3^+$ T-cell proliferation [169]. Thus, rather than working to eliminate MDSCs or inhibit their suppressive function, potentiating the suppressive capacity of MDSCs in autoimmune settings could prove an efficacious option. A single injection of IFN-β at the clinical onset of EAE enhanced the presence of and promoted the immunosuppressive activity of MDSCs, limiting the severity of EAE [170].

This approach can also be applied in organ transplantation, where host immune rejection of the donor graft limits the effectiveness of transplant. Patients with circulating MDSC frequencies >10% who received a kidney transplant demonstrated 1- and 5-year graft survival rates of 93% and 79%, respectively, whereas patients with MDSC frequencies <10% had 1- and 5-year survival rates of 68% and 36% [171]. Of note, the levels of circulating MDSC in these patients also positively correlated with the levels of Tregs, indicating a generalized state of immune tolerance. Similarly, in models of hematopoietic stem cell transplantation (HSCT), MDSCs have proven useful in limiting graft-versus-host disease (GVHD) and inducing host immune tolerance. Highfill et al. demonstrated that in a fully MHC-mismatched model of HSCT, MDSCs transferred into mice suppressed donor T-cell activation, resulting in reduced GVHD lethality [172]. In 62 patients who received a haploidentical HSCT, donor grafts that displayed higher absolute counts of M-MDSCs and PMN-MDSCs resulted in lower incidences of acute and chronic GVHD [173]. However, because adoptive transfer of MDSCs failed to induce allograft tolerance in recipients [174], research has focused on inducing MDSC expansion within the host as

a means of promoting immune tolerance [175]. Yang et al. demonstrated that murine isolated bone marrow cells could be induced in vitro into M-MDSCs via M-CSF and IFN-γ and that the adoptive transfer of either recipient- or donor-induced MDSCs significantly prolonged allo-skin graft survival in mice [176]. Taken together, these studies highlight the potential to exploit the suppressive capacity of MDSCs for therapeutic benefit in non-oncologic conditions.

8.9 Trends and Future Directions

Data presented in this chapter have highlighted that the expansion and activation of MDSCs in tumor-bearing hosts contribute to multiple aspects of tumor progression, through the inhibition of endogenous and adoptive antitumor responses as well as by promoting tumor vascularization and metastasis. Because MDSCs are highly plastic with variations depending on tumor type, stage, and disease phase, research efforts over the last decade have been directed at defining their phenotype and characterizing their tumor-promoting functions in these varying disease states. Studies highlighted in this chapter emphasize the difficulty in defining MDSCs through cell surface markers alone. Conventional fluorophore-based flow cytometric applications have been limited by the low number of phenotypic markers able to be detected. The relatively recent advent of high-throughput methods, e.g., "-omics" approaches, heavy metal- or synthetic fluorophore-based cytometry, and mass spectrometry (CyTOF), should help further define cell surface markers as well as signaling and gene networks that are characteristic of MDSCs. High-throughput methods are already being utilized to investigate MDSCs in various cancers [177, 178]. In addition, consortiums dedicated to defining and characterizing suppressive myeloid cells within tumor microenvironments have been established. A current example is the Mye-EUNITER consortium (http://www.mye-euniter.eu/), made up of researchers with the primary goal of establishing gold standard protocols and guidelines for defining and characterizing myeloid regulatory cells in cancer, infection, and inflammation. Insights gained from these more global approaches and types of studies will hopefully facilitate a clear consensus on MDSC definition and functional characterization that can be applied across disease and tumor types.

Only recently have the therapeutic implications of MDSCs been expanded, as technology to allow MDSC genetic modification and reprogramming is being developed. As discussed above, efforts to therapeutically target MDSCs have been directed to modulate both the expansion and inhibit the suppressive capacities of MDSCs in the context of cancer. MDSCs have also demonstrated to be prognostic markers that correlate with disease stage and progression, in addition to providing clinicians predictive insight on potential microenvironment-directed treatment options such as checkpoint blockade. Oncologists have also utilized metronomic dosing of chemotherapy that is based on frequent, lower doses of traditional chemotherapeutic agents (as opposed to toxic high-dose chemotherapy) that facilitate a reduction in suppressive immune subsets, including MDSCs. Conversely, potentiating the suppressive capacity of MDSCs for therapeutic gain in conditions such as autoimmunity and stem cell transplant has also been explored, and studies highlighted here have demonstrated the potential MDSCs have to alleviate these disease pathologies [179]. Current efforts are being directed at further understanding and exploiting the suppressive potential of MDSCs in diseases such as multiple sclerosis, GVHD, and other autoimmune disorders. Finally, utilizing MDSCs as a cell platform for genetic manipulation and therapy is also now being explored, given the advent of technology that allows successful genetic reprogramming of primary cells of the myeloid lineage [180].

Ultimately, future efforts directed to further understand MDSC biology should focus on a deeper understanding of the mechanisms behind their pathological expansion, activation, pro-angiogenic capabilities, and immune regulatory mechanisms. Insights gained from these studies will likely provide prognostic markers and novel

therapeutic targets to either inhibit or potentiate their suppressive capacity.

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