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# Phenotype, development, and biological function of myeloid-derived suppressor cells

Yang Zhao[1], Tingting Wu[1], Steven Shao[1], Bingyi Shi[2,*], and Yong Zhao[1,*]

[1]Transplantation Biology Research Division; State Key Laboratory of Biomembrane and Membrane Biotechnology; Institute of Zoology; Chinese Academy of Sciences; Beijing, China

[2]Organ Transplantation Center of People's Liberation Army; 309 Hospital of Chinese People's Liberation Army; Beijing, China

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**Abbreviations:** APCs, Antigen presenting cells; Arg1, Arginase1; ATRA, All-trans retinoic acid; C/EBPβ, CCAAT/enhancer binding protein beta; C5a, Complement 5a; CK2, Casein kinase 2; CO, Carbon monoxide; COX-1, cyclooxigenase-1; COX-2, cyclooxigenase-2; CXCL5, C-X-C motif chemokine 5; CXCR2, chemokine (C-X-C motif) receptor 2; CXCR4, chemokine (C-X-C motif) receptor 4; DCs, Dendritic cells; ER, Endoplasmic reticulum; FLT3L, Fms-related tyrosine kinase 3 ligand; G-CSF, Granulocyte colony stimulating factor; GM-CSF, Granulocyte-macrophage colony stimulating factor; G-MDSCs, Granulocytic-myeloid-derived suppressor cells; H2O2, hydrogen peroxide; HIF-1α, Hypoxia inducible factor-1; HIF-2α, Hypoxia inducible factor-2; HO-1, Heme oxygenase-1; Hsp72, Heat shock protein 72; IDO, Indoleamine 2,3-dioxygenase; IFN-γ, Interferon-γ; IL-10, Interleukin-10; IL-13, Interleukin-13; IL-1β, Interleukin-1β; IL-2, Interleukin-2; IL-4, Interleukin-4; IL-6, Interleukin-6; iNOS, Inducible nitric oxide synthase; IRF-8, Interferon regulatory factor-8; LNFPIII, Lacto-N-fucopentaose III; LPS, Lipopolysaccharide; MCP-1, Monocyte chemotactic protein-1; M-CSF, Macrophage colony stimulating factor; MDSCs, Myeloid-derived suppressor cells; MIP-2, Macrophage inflammatory protein−2; M-MDSCs, Monocytic-myeloid-derived suppressor cells; MyD88, Myeloid differentiation factor 88; NK, Natural killer cells; NO, Nitric oxide; PBMCs, Peripheral blood mononuclear cells; PD-L1, Programmed death-ligand 1; PGE2, Prostaglandin E2; PI3K, Phosphatidylinositol-3-kinase; PPARγ, Peroxisome proliferator–activated receptor-γ RAGE, Receptor for advanced glycation end products; RNS, Reactive nitrogen species; ROS, Reactive oxygen species; RT-PCR, Reverse transcription-polymerase chain reaction; S100A9, S100 family of proteins containing 2 EF-hand calcium-binding motifs; SCF, Stem cell factor; SCID, Severe combined immunodeficiency; SDF-1, Stroma derived factor 1; SHIP, Src homology 2 domain-containing inositol 59-phosphatase-1; siRNA, Small interfering RNA; Smad3, SMAD family member 3; TCRζ, T cell receptor ζ; TGF-β, Transforming growth factor-β; TGF-βR2, Transforming growth factor-β receptor 2; Th2, T helper cells 2; TLR2, Toll-like receptor; TNF-α, Tumor necrosis factor alpha; TRAIL-R, TNF-related apoptosis-induced ligand receptor; Treg, Regulatory T cells; VEGF, Vascular endothelial growth factor; VEGFR1, Vascular endothelial growth factor receptor 1

$CD11b^+Gr\text{-}1^+$ myeloid-derived suppressor cells (MDSCs) are an important population of innate regulatory cells mainly comprising monocytic MDSCs (M-MDSCs) with a phenotype of $CD11b^+Ly6G^-Ly6C^{high}$ and granulocytic MDSCs (G-MDSCs) with a phenotype of $CD11b^+Ly6G^+Ly6C^{low}$ in mice. They play crucial roles in the pathogenesis of cancers, chronic infections, autoimmune diseases, and transplantation. Various extracellular factors such as lipopolysaccharide (LPS), macrophage colony-stimulating factor (M-CSF), granulocyte macrophage colony-stimulating factor (GM-CSF), stem cell factor (SCF), interleukin (IL)-6, interferon gamma (IFNγ), IL-1β, vascular endothelial growth factor (VEGF), Hsp72, IL-13, C5a, and prostaglandin E2 (PGE2) can induce MDSC differentiation, whereas IL-4 and all-trans-retinoic acid can inhibit this process. For the intracellular signals, signal transducer and activator of transcription (STAT) family members, C/EBPβ and cyclooxigenase-2 (COX-2) promote MDSC function, whereas interferon regulatory factor-8 (IRF-8) and Smad3 downregulate MDSC activity. The immunosuppressive function of MDSCs is mediated through various effector molecules, primarily cellular metabolism-related molecules such as nitric oxide (NO), arginase, reactive oxygen species (ROS), transforming growth factor β (TGFβ), IL-10, indoleamine 2,3-dioxygenase (IDO), heme oxygenase-1 (HO-1), carbon monoxide (CO), and PGE2. In this article, we will summarize the molecules involved in the induction and function of MDSCs as well as the regulatory pathways of MDSCs.

*Correspondence to: Yong Zhao; Email: zhaoy@ioz.ac.cn; Bingyi Shi; Email: shibingyi@medmail.com.cn

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## Introduction

Myeloid-derived suppressor cells (MDSCs) have attracted great interest and prompted intense studies in recent years. MDSCs are characterized by their myeloid origin, immature state, and immunosuppressive ability.[1] This innate cell population remains poorly defined due to inadequate surface markers. It is known, however, that MDSCs belong to a heterogeneous innate cell population that includes the monocytic (M-MDSCs) and granulocytic (G-MDSCs) subsets. MDSCs accumulate in large numbers during many pathological conditions

such as cancer, infectious diseases, trauma, autoimmune diseases, and transplantation.[2-5] Our review will elucidate the molecular pathways responsible for the induction and function of MDSCs.

## The Phenotypes of MDSCs

Myeloid-derived suppressor cells in mice were initially defined as cells of the $CD11b^{+}Gr\text{-}1^{+}$ phenotype. Morphologically, MDSCs consist of M-MDSCs and G-MDSCs with various markers in mice (**Table 1**) and in humans (**Table 2**). M-MDSCs display a phenotype of $CD11b^{+}Ly6G^{-}Ly6C^{high}$, and G-MDSCs are $CD11b^{+}Ly6G^{+}Ly6C^{low}$.[6–7] Studies using each population separately showed that $CD11b^{+}Gr\text{-}1^{low}$ cells are the most immunosuppressive, $CD11b^{+}Gr\text{-}1^{int}$ cells are less immunosuppressive, and $CD11b^{+}Gr\text{-}1^{high}$ cells, mostly granulocytes, are least immunosuppressive.[8] Other markers have been used to distinguish these cells from other myeloid cells such as tumor-associated macrophages, which express higher F4/80 and lower Gr-1 than MDSCs.[1] In addition to the use of CD11b and Gr-1 surface markers and the measure of cell maturity and suppressive activity, other strategies have also been used to identify MDSCs.[9,10] Immature MDSCs express CD31 (adhesion molecular PECAM-1), which are present on the progenitors and myeloid blast cells. Low expression of major histocompatibility complex class II (MHC-II) is an indication of immature MDSCs.[11,12] For example, approximately 30% of $CD11b^{+}Gr\text{-}1^{+}$ splenocytes express CD31 and less than 3% of $CD11b^{+}Gr\text{-}1^{+}$ splenocytes express MHC-II in a sepsis model.[13] In addition, the suppressive activity of MDSCs is associated with multiple markers including CD115 (M-CSF receptor) and CD124 (IL-4Rα).[10] Compared to G-MDSCs, M-MDSCs express higher levels of F4/80, CD115, and CCR2, which suppress $CD8^{+}$ T cells via an inducible nitric oxide (iNOS)-mediated pathway.[7,14] In mice bearing C26 colon carcinoma transduced to release mouse granulocyte-macrophage colony-stimulating factor (GM-CSF), a population of circulating $CD11b^{+}IL\text{-}4R\alpha\ (CD124)^{+}$ monocytes with immunosuppressive function is elicited by growing tumors and activated by IFNγ released by T cells, whereas $CD11b^{+}IL\text{-}4R\alpha^{-}$ cells comprising granulocytes at different stages of differentiation are not immunosuppressive.[10] $Gr\text{-}1^{+}CD115^{+}$ M-MDSCs isolated from bone marrow can inhibit T-cell immune response in a model of chemically induced colon carcinoma.[15] However, studies with tumor-bearing mouse models of 10 different tumor cells did not detect the selective expression of CD124, CD115, CD80, programmed cell death ligand 1 (PD-L1), and PD-L2 on MDSCs, indicating that their phenotype may vary from the tumor types and animal models used.[7] It was reported that the expression of CXCR2 is much higher on $CD11b^{+}Gr\text{-}1^{+}$ MDSCs from G-CSF-treated mice,[16] supporting the hypothesis that the systemic recruitment and trafficking result in increased frequencies at multiple peripheral sites. Movahedi et al. presented genetic evidence that the loss of CXCR2 dramatically suppresses chronic colonic inflammation and colitis-associated tumorigenesis by inhibiting MDSC infiltration into the colonic mucosa and tumors in a mouse model of colitis-associated cancer.[17] Rhabdomyosarcoma induces robust expansion of $CXCR2^{+}CD11b^{+}Ly6G^{hi}$ MDSCs, and CXCR2 deficiency prevents $CD11b^{+}Ly6G^{hi}$ MDSC tumor trafficking.[18] Thus, CXCR2 is identified as a novel target for modulating tumor immune escape and $CXCR2^{+}CD11b^{+}Ly6G^{hi}$ MDSCs are an important suppressive

**Table 1.** Phenotypes of mouse MDSC subsets

| Molecules | M-MDSCs | G-MDSCs |
|---|---|---|
| Gr-1 | ++ | + |
| CD11b | + | + |
| Ly6G | – | + |
| Ly6C | High | Low |
| F4/80 | + | – |
| CD2 (LFA-2) | + | – |
| CD11a (LFA-1) | + | + |
| CD14 | – | – |
| $CD16^{low}$ | + | + |
| CD31 (PECAM-1) | + | – |
| CD34 | – | – |
| CD43 | + | + |
| CD44 | + | + |
| CD49d | + | – |
| CD54 (ICAM-1) | High | Low |
| CD62L | + | + |
| CD71 (transferrin receptor) | + | – |
| CD80 (B7–1) | + | + |
| CD115 (M-CSFR) | + | – |
| CD117 (C-kit) | – | – |
| CD162 (PSGL-1) | + | + |
| CD124(IL-4Rα) | + | + |
| CD204 (SR-A) | – | – |
| B7H1 (PD-L1) | | + |
| B7H1 (PD-L1) | | ± |
| MHC II | Low | Low |
| MHC I | + | + |
| HLA-DR- | – | – |
| CCR2 | High | Low |
| CXCR2 | + | + |
| CXCR4 | + | + |
| TIE2 | – | – |
| Integrin α4β1 | Subset | – |
| VEGF2 | + | + |
| VEGF1 | + | + |

**Table 2.** Phenotypes of human MDSC subsets

| Molecules | M-MDSCs | G-MDSCs |
|---|---|---|
| CD11b | + | + |
| Gr-1 | – | – |
| CD13 | Subset | Subset |
| CD14 | + | – |
| CD15 | – | + |
| $CD16^{low}$ | Subset | Subset |
| CD33 | + | + |
| CD34 | Subset | Subset |
| CD38 | Subset | Subset |
| CD39 | + | + |
| CD66b | – | + |
| MHC II | Low | Low |
| $Lin^{-}$ | + | + |

myeloid subset in certain tumors and inflammatory diseases. In addition, the expression of CD49d (also known as intengrin-α4) has been used to differentiate between M-MDSCs and G-MDSCs. $CD49d^+$MDSCs appear to be M-MDSCs with immunosuppressive activity, while $CD49d^-$MDSCs represent a granulocytic phenotype with little T–cell-inhibitory activity.[19]

In humans, the phenotype of MDSCs is more elusive (**Table 2**). In the late 1990s, human MDSCs were classified by CD34 expression and immunosuppressive activity in patients with GM–CSF-secreting cancers.[4,20,21] These MDSCs are $Lin^-$ ($CD3^-CD19^-CD13^-CD56^-$) $CD34^+$ cells. The lack of human leukocyte antigen (HLA)-DR expression is also a phenotype of human MDSCs.[22] In human blood, MDSCs can be divided into 2 subpopulations: $CD11b^+CD14^-CD15^+$ G-MDSCs and $CD11b^+CD14^+HLA\text{-}DR^{low/neg}$ M-MDSCs.[1,23,24] In other studies of cancer patients, MDSCs express the myeloid cell markers CD33 and CD11b and granulocyte markers.[22,25,26] MDSCs detected in the peripheral blood of tumor patients express the common myeloid marker CD33 but lack the markers of mature myeloid cells like HLA-DR.[22,27,28] Based on the expression of granulocytic marker CD15, MDSCs are divided into at least 2 subsets. The $CD15^+$ MDSCs are granulocytic like mouse $Gr\text{-}1^{high}$ MDSCs, whereas the $CD15^-$ subset of monocytic MDSCs has been shown to suppress T-cell function in patients with kidney cancer through arginase and/or reactive oxygen species (ROS)-dependent mechanisms.[24,29] The clinical studies showed that human MDSCs in the peripheral blood of patients with renal cell carcinoma are a subpopulation of activated polymorphonuclear cells expressing high levels of CD66b, CD11b, and vascular endothelial growth factor receptor 1 (VEGFR1), and low levels of CD62L and CD16.[23] The circulating $CD14^+HLA\text{-}DR^{-/low}$ MDSCs in advanced malignant melanoma patients display overexpression of CD80, CD83, and DC-Sign.[30] The proportion of $CD45^+CD13^+CD33^+CD14^-CD15^-$ MDSCs is significantly increased in primary breast cancer tissues and the peripheral blood of patients.[31] Therefore, the phenotypes of MDSCs may be distinct in patients with different diseases. Nevertheless, to avoid any confusion, it may be necessary to clearly define each subpopulation of MDSCs, including G-MDSCs and M-MDSCs, in mice and humans besides using CD11b and Gr-1 markers. Identification of new surface markers for G-MDSCs and M-MDSCs still needs to be intensively explored.

## The Immunosuppressive Function of MDSCs

### T cells

Myeloid-derived suppressor cells remarkably suppress the proliferation and cytokine secretion of T cells and induce their apoptosis.[32–34] The presence of activated granulocytes in the peripheral blood of tumor patients correlates with the reduction of TCRζ chain expression and cytokine production by T cells.[25] It was reported that decreased TCRζ chain expression on $CD8^+$ T cells is strongly associated with increased MDSCs in patients with chronic hepatitis C and is restored by L-arginine treatment *in vitro*.[35] $Gr\text{-}1^+CD11b^+$ MDSCs are able to take up soluble proteins *in vivo*, process them, and present antigenic epitopes on their surface and induce antigen (Ag)-specific T-cell anergy,[36] implicating their possible role in tumor-associated $CD8^+$ T-cell tolerance. $CD14^+HLA\text{-}DR^{-/low}$ cells isolated from hepatocellular carcinoma patients can induce the development of $CD4^+CD25^+Foxp3^+$ regulatory T cells (Tregs) when co-cultured with autologous T cells.[28] Thus, MDSCs may suppress immunity through the induction of T-cell anergy and Tregs.

### Natural killer cells

Myeloid-derived suppressor cells from liver cancer-bearing mice significantly inhibit the cytotoxicity, NKG2D expression, and interferon gamma (IFNγ) production of natural killer (NK) cells as well as induce anergy of NK cells.[37] The depletion of MDSCs rescues the function of impaired hepatic NK cells. In 1 study, MDSCs from chronic inflammation cause T- and NK-cell dysfunction associated with TCR ζ chain (CD247) downregulation. CD247 is a key factor for the initiation of immune responses and is affected by the immunosuppressive milieu generated.[38] Furthermore, membrane-bound transforming growth factor β 1 (TGFβ1) on MDSCs is responsible for MDSC-mediated suppression of NK cells.[37] Therefore, cancer-induced MDSCs, acting through membrane-bound TGF-β1, are the main negative regulators of hepatic NK cells in tumor-bearing hosts.

### B cells

The fundamental B-cell response seems susceptible to MDSC regulation because MDSC inhibition of both antigen-specific T-cell responses and broad non-major histocompatibility complex (MHC)-restricted responses to polyclonal activators and mitogens have been widely reported.[39] LP-BM5 retrovirus infection in mice, which causes acquired immunodeficiency, can induce highly immunosuppressive $CD11b^+Gr\text{-}1^+Ly6C^+$ MDSCs capable of suppressing both T and B cells via nitric oxide (NO) signaling.[39]

### Dendritic cells

Myeloid-derived suppressor cells and dendritic cells (DCs) are both important regulators of immune responses against tumors and infections. The combined treatment of bone marrow-derived MDSCs with LPS and IFNγ inhibits the development of DCs and enhances MDSC suppressive activity.[40] MDSCs can decrease the efficacy of DC vaccines. MDSC frequency does not affect the yield or viability of the DCs produced, but induces a dose-dependent decrease in DC maturation. High frequencies of $CD14^+HLA\text{-}DR^{-/low}$ cells can inhibit DC maturation and engender impaired DC function, which are important for vaccine success.[41] Thus, the balance between MDSCs and DCs may play an important role in tumor and infection therapy.

## The Induction of MDSCs

Several studies have shown that emergency myelopoiesis induces the production and accumulation of MDSCs in mice and

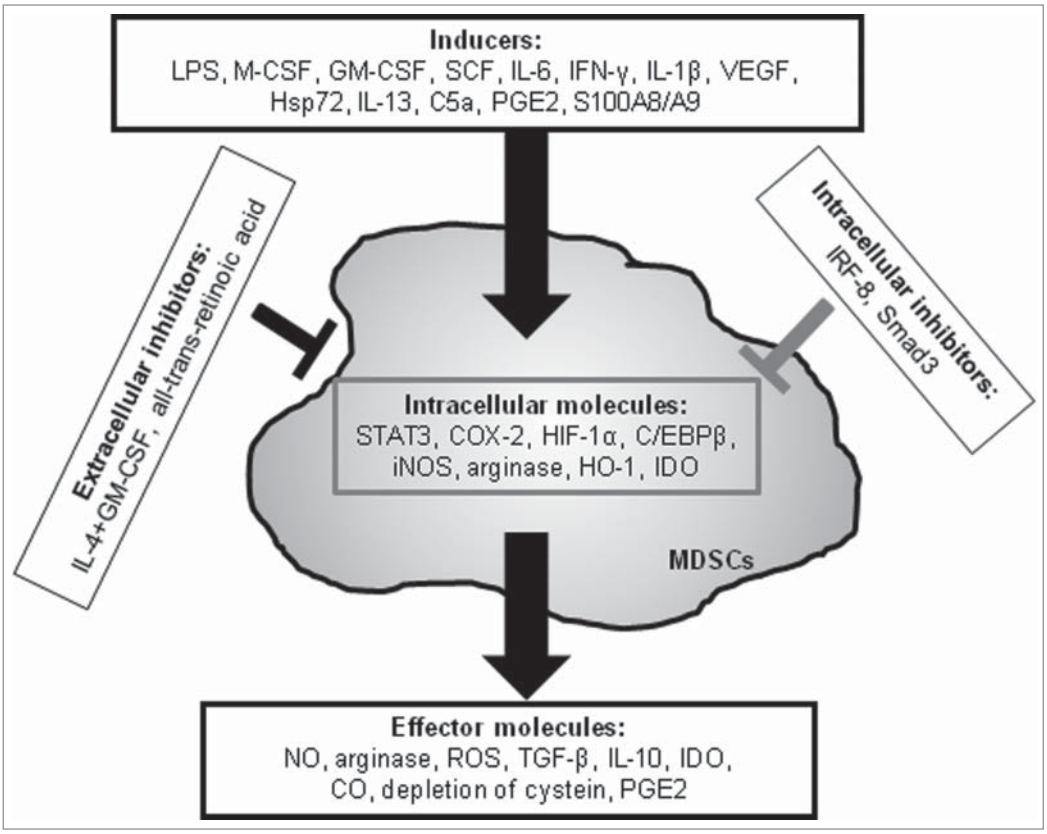


**Figure 1.** Molecules involved in the induction and function of MDSCs. Numerous factors can induce the development and differentiation of MDSCs. These factors include, but are not limited to, lipopolysaccharide (LPS); macrophage-colony stimulating factor (M-CSF); granulocyte macrophage-colony stimulating factor (GM-CSF); stem cell factor (SCF); interleukin 6 (IL-6); interferon gamma (IFNγ); IL-1β; vascular endothelial growth factor (VEGF); Hsp72; IL-13; C5a; and prostaglandin E 2 (PGE2). IL-4/GM-CSF and all-trans-retinoic acid may inhibit the development of MDSCs. Some of the intracellular molecules involved in MDSC function include Stat3, COX-2, hypoxia-inducible factor 1a (HIF-1α), C/EBPβ, inducible nitric oxide synthase (iNOS), arginase, heme oxygenase 1 (HO-1), and IDO. The known effector molecules of MDSCs include: (1) arginase-1, which induces arginine deprivation and causes CD3ζ nitrosylation and downmodulation; (2) iNOS, which induces nitric oxide (NO) production and leads to T-cell apoptosis and inhibition of T-cell proliferation; (3) NOX2, which inhibits T-cell proliferation through reactive oxygen species (ROS) production, CD3ζ and major histocompatibility complex (MHC)-I nitration; (4) the enzyme HO-1, which leads to inhibition of T-cell proliferation through carbon monoxide (CO) production; (5) MDSCs that prevent antigen-presenting cells (APCs) from providing sufficient cysteine to T cells for glutathione (GSH) production, thus inhibiting T-cell proliferation; (6) membrane-bound TGFβ1, which promotes NK cell anergy and induced regulatory T cells (iTreg); (7) IL-10 promotes Th2 deviation and type 2 macrophage polarization; (8) ADAM metallopeptidase domain 17 (ADAM17) activity leads to cleavage of L-selectin (CD62L) on T cells resulting in inhibition of T-cell homing to lymph nodes and sites of inflammation.

humans (**Fig. 1**). Intraperitoneal injection of Lacto-N-fucopentaose III (LNFPIII)-dextran results in rapid expansion of Gr-$1^+$F4/$80^+$CD$11b^+$ peritoneal cells that can suppress naive $CD4^+$ T-cell proliferation.[42] Notably, LNFPIII-dextran also induces functional immunosuppressive Gr-$1^+$ macrophages in T/B cell-deficient SCID mice,[42] demonstrating that an adaptive immune system is dispensable for the induction of MDSCs in this particular model.

**Inflammatory cytokines and growth factors**

The granulocyte macrophage colony-stimulating factor (GM-CSF) was reported to induce the differentiation of mouse bone marrow cells into immunosuppressive $CD11c^-$Ly-$6C^+$Ly-$6G^{low}CD11b^+CD31^+$ER-$MP58^+$asialo$GM1^+F4/80^+$ cells *in vitro*.[12] The administration of GM-CSF-based vaccines in metastatic melanoma patients markedly increases $CD14^+$HLA-$DR^{-/low}$ MDSCs in the peripheral blood.[43] The administration of recombinant G-CSF or IL-2 in mice results in the accumulation of Gr-$1^+CD11b^+$ MDSCs and Treg cells in the peripheral lymphoid organs, which significantly delays allogeneic donor skin rejection.[16] It was reported that IL-1R deficiency delays MDSC accumulation in tumor-bearing mice.[44] In contrast, excessive inflammation in IL-1R antagonist-deficient mice promotes the accumulation of MDSCs with enhanced immunosuppressive activity.[44] Mice bearing tumor cells with a siRNA knockdown of stem cell factor (SCF) exhibit significantly reduced MDSC expansion.[45] Injection of IL-6 increases the accumulation of MDSCs in tumor-bearing IL-1R-deficient mice, indicating that IL-6 is a mediator for the induction of MDSCs.[44] Cytokines GM-CSF, G-CSF, and IL-6 produced by various tumors can allow rapid generation of MDSCs from precursors present in mouse and human bone marrow. Furthermore, MDSCs induced by GM-CSF+IL-6 possess high tolerogenic activity, as revealed by their ability to inhibit $CD8^+$ T-cell proliferation and allow long-term acceptance of pancreatic islet allografts.[46] Recent studies found that adding poly(I:C) to the standard DC polarizing condition in which DCs are generated in culture with GM-CSF and IL-4 can accumulate MDSCs after extended stimulation.[47] This indicates that poly(I:C) favors the development and expansion of MDSCs. The proinflammatory molecule S100A9 interacts with its receptor CD33 to induce bone marrow accumulation of MDSCs in both mice and humans with myelodysplastic syndromes. Further studies showed

that S100A9/CD33 activates immunoreceptor tyrosine-based inhibition motif (ITIM) to induce secretion of the immunosuppressive cytokines IL-10 and TGFβ by immature myeloid cells.[48] The proinflammatory proteins S100A8/A9 have the ability to induce Gr-1$^{high}$CD11b$^{high}$F4/80$^{-}$CD80$^{+}$IL-4Rα$^{+/-}$Arginase$^{+}$ MDSCs.[49] Studies with transgenic and gene knockout mice showed that tumor-induced upregulation of S100A9 protein is essential for the accumulation of MDSCs.[50] S100A8/A9 proteins interact with carboxylated N-glycans on the cell surface glycoprotein receptors of MDSCs to activate the NF-kB pathway and promote MDSC accumulation.[49] Inhibition of S100A8/A9 binding to MDSCs using an anti-carboxylated glycan antibody reduces MDSC levels in the blood and secondary lymphoid organs of mice with metastatic diseases.[49]

**Tumor-derived factors**

In female transgenic mice that spontaneously develop mammary carcinomas from the expression of rat oncogene c-erbB-2, MDSCs can be induced by the release of VEGF but not GM-CSF.[51] Administration of all-trans retinoic acid (ATRA) dramatically reduced the presence of MDSCs in mouse tumor models.[52] ATRA at effective concentrations (>150 ng/mL blood) significantly decreases MDSC numbers in the peripheral blood of patients with metastatic renal cell carcinoma.[53] Prostaglandin E2 (PGE2) and other cyclooxigenase-2 (COX-2) activators like lipopolysaccharide (LPS), IL-1β and IFNγ induce CD11b$^{+}$Gr-1$^{+}$ MDSCs by promoting COX-2 expression in monocytes and blocking their differentiation into mature DCs,[54,55] thereby supporting the central role of COX-2-PGE2 feedback in the induction and persistence of MDSCs. High PGE-producing tumor cells constitutively expressing COX-1 and COX-2 induce arginase I expression in MDSCs via PGE2 receptor E-prostanoid 4.[56,57] Genetic and pharmacological inhibition of COX-2, but not COX-1, block arginase I induction *in vitro* and *in vivo* and elicit a lymphocyte-mediated antitumor response.[56] These results demonstrate a novel pathway for prostaglandin-induced immune dysfunction and suggest a new mechanism for the cancer-prevention effects of COX-2 inhibitors. IFNγ can drive circulating CD11b$^{+}$IL-4Rα$^{+}$ MDSCs responsive to IL-13 and immunosuppressive factors.[54] Hsp72 was proven to be essential for the expansion, activation, and suppressive function of mouse and human MDSCs through a Stat3 signaling pathway.[58] The tumor-derived exosome-associated Hsp72 determines the suppressive activity of the MDSCs via activation of Stat3 in a TLR2/MyD88-dependent manner.[58] Several tumor-derived factors such as TGFβ, IL-3, IL-6, IL-10, platelet-derived growth factors, and GM-CSF can also induce ROS production by MDSCs.[59] Gr-1$^{+}$CD11b$^{+}$ myeloid cells are recruited into mammary carcinomas with type II TGFβ receptor gene deletion and directly promote tumor metastasis.[60] This may be explained by increased TGFβ1 in tumors with TGFβR2 deletion and enhanced SDF-1/CXCR4 and CXCL5/CXCR2 chemokine axes.[60] Tumor-secreted growth factors not only induce myelopoiesis and chemokines that recruit MDSCs but also regulate MDSC development and maturation. For example, TNFα impairs MDSC maturation[38] by regulating RAGE and its ligands S100A8 and S100A9.[50] In addition, overexpression of fms-like tyrosine kinase 3 ligand in tumor-bearing mice results in increased MDSCs that inhibit the antitumor activity of effector immune cells.[61] Complement anaphylatoxin C5a increases tumor-infiltrating MDSCs with an immunosuppressive activity through ROS and reactive nitrogen species (RNS) regulation.[62]

**The factors mediating the apoptosis and proliferation of MDSCs**

Besides soluble factors, MDSCs are controlled by their expression of Fas which leads to cell apoptosis after associating with Fas-L on activated T cells.[63] In lupus-prone MRL-Fas$^{lpr}$ mice, CD11b$^{+}$Gr-1$^{low}$ cells, which can suppress CD4$^{+}$ T-cell proliferation via Arg1, significantly increase in percentage in the kidneys and blood during disease progression.[64] This indicates that the Fas pathway may be involved in the regulation of MDSCs in mice.

Recently, it has been reported that endoplasmic reticulum (ER) stress can regulate MDSC fate through TNF-related apoptosis-induced ligand receptor (TRAIL-R)-mediated apoptosis.[65] MDSCs in tumor-bearing mice are less viable and have shorter half-lives compared with normal monocytes and neutrophils. The reduced MDSC viability is due to increased apoptosis mediated by the expression of TRAIL-Rs on these cells. Thus, TRAIL-Rs may be considered as potential targets for selective inhibition of MDSCs.

Additionally, 1 study using microRNA (MiR) microarray and TaqMan probe–based quantitative real-time polymerase chain reaction (RT-PCR) assay identified miR-155 and miR-21 as the 2 most transcribed miRNAs during the induction of MDSCs from bone marrow cells by GM-CSF and IL-6.[66] Overexpression of miR-155 and miR-21 enhances the frequency of cytokine-induced MDSCs and induces the expansion of both monocytic and granulocytic MDSCs.[66] Accordingly, depletion of miR-155 and miR-21 has the opposite effect. These results demonstrate a novel miR-155/miR-21–based regulatory mechanism that modulates functional MDSC induction.

As previously mentioned, a plethora of growth factors and inflammatory cytokines regulates the development of MDSCs. However, in an immune reconstitution mouse model, the adoptive transfer of Gr-1$^{+}$CD115$^{+}$ M-MDSCs derived from CD40-deficient mice fails to induce tolerance and Treg cell development *in vivo*.[67] This suggests that the immune stimulatory receptor CD40 is essential for MDSC-mediated immune suppression and tumor-specific Treg cell expansion. Other plausible co-stimulatory molecules involved in the regulation of MDSC development should be further explored. In addition, whether the development of G-MDSCs and M-MDSCs requires similar or different stimulating factors needs to be addressed in the near future.

## The Intracellular Molecular Regulation of MDSCs

**Stat family**

Studies in a mouse sepsis model showed that signaling through MyD88 is required for the expansion of CD11b$^{+}$Gr-1$^{+}$

MDSCs expressing high IL-10.[13] Tumor-derived exosomes-associated Hsp72 triggers Stat3 activation in MDSCs in a TLR2/MyD88-dependent manner and determines their immunosuppressive activity.[58] Increased levels of Stat3 in an active state were noted in $CD14^{+}HLA\text{-}DR^{-/low}$ MDSCs of advanced malignant melanoma patients.[30] Importantly, inhibition of Stat3 relieves the suppressive activity of MDSCs almost completely.[30] Given the redox imbalance in MDSCs, the authors speculated that Stat3-dependent oxidative stress through the regulation of NADH oxidase might play an important role in MDSCs-mediated T-cell suppression.[30] Markedly enhanced functions of DCs, T cells, NK cells, and neutrophils are observed in tumor-bearing mice with Stat3-deficient haematopoietic cells.[68] Targeting Stat3 with a small-molecule drug induces T- and NK–cell-dependent growth inhibition of established tumors in mice.[68] These results indicate that Stat3 signaling restricts host natural tumor immune surveillance and that inhibiting haematopoietic Stat3 in tumor-bearing hosts efficiently triggers therapeutic anti-tumor immunity. Culture of peripheral blood mononuclear cells (PBMCs) with pancreatic stellate cell supernatants for 7 d promotes PBMC differentiation into a $CD11b^{+}CD33^{+}$MDSC phenotype and a subpopulation of polymorphonuclear $CD11b^{+}CD33^{+}CD15^{+}$ cells, which functionally suppresses autologous T-cell proliferation.[69] The supernatant of the cultured pancreatic stellate cells leads to Stat3 but not Stat1 or Stat5 phosphorylation in co-cultured PBMCs, and Stat3 inhibitor FLLL32 abrogates the induced MDSC differentiation.[69] Thus, pancreatic cancer cells induce MDSCs in a Stat3-depdendent manner. Indoleamine 2,3-dioxygenase (IDO) expression and Stat3 phosphorylation are significantly upregulated in MDSCs isolated from fresh human breast cancer tissues, which strongly correlates with increased infiltration of $Foxp3^{+}$ Treg cells in tumors and lymph node metastasis in patients.[31] These MDSCs inhibit IL-2 and anti-CD3/CD28 mAb-induced T-cell amplification and Th1 polarization in an IDO-dependent manner. IDO inhibitor 1-methyl-L-tryptophan or Stat3 antagonist JSI-124 blocks MDSCs' immunosuppressive activity on T cells,[31] indicating that Stat3-dependent IDO expression mediates the immunosuppressive effects of MDSCs in breast cancer. However, $F4/80^{+}$ tumor-associated macrophages are able to inhibit T–cell-mediated immune response *in vitro* via induction of T-cell apoptosis through arginase- and NO-independent manners.[70] Using Stat knockout (KO) mice, Kusmartsev et al. determined that Stat1 but not Stat3 or Stat6 is responsible for the immunosuppressive activity.[70] Although Stat3 is considered the central transcription factor for MDSC expansion, Stat3 inhibition or ablation does not abolish but augments Flt3L-mediated MDSC expansion.[71] In contrast, the Flt3L-mediated DC expansion is reduced when Stat3 is inhibited or deleted. Thus, Stat3 has opposite effects on Flt3L-induced immunosuppressive MDSCs and immunostimulatory DC expansion. The roles of Stat family members in MDSCs require further clarification.

**C/EBPβ**

Studies using mice with C/EBPβ deletion in all haematopoietic lineage cells showed that the induced MDSCs and the immunosuppressive activity of both tumor-induced and bone marrow-derived MDSCs are entirely dependent on the transcription factor C/EBPβ.[46] Adoptive transfer of tumor antigen-specific $CD8^{+}$ T cells results in tumor clearance only in mice lacking C/EBPβ in the myeloid compartment.[46] These data suggest that C/EBPβ is a critical regulator in the development of MDSCs.

**Hypoxia-inducible factor**

HIF-1α was found to be primarily responsible for MDSC differentiation and function in the tumor microenvironments.[72] Hypoxia causes a rapid, dramatic, and selective upregulation of PD-L1 on splenic MDSCs in tumor-bearing mice.[73] Furthermore, the upregulation of PD-L1 is dependent on HIF1-α, but not HIF-2α. Blockade of PD-L1 under hypoxia enhances MDSC-mediated T-cell activation and is accompanied by the downregulation of IL-6 and IL-10. Thus, the blockade of PD-L1, accompanied by the inhibition of HIF-1α, may be a novel approach in cancer immunotherapy. The tyrosine kinase inhibitor sunitinib-based therapy has the potential to modulate antitumor immunity by reversing tumor-induced MDSC-mediated immunosuppression.[29]

**Interferon regulatory factor-8**

Myeloid-derived suppressor cell-inducing factors G-CSF and GM-CSF facilitate IRF-8 downregulation via Stat3- and Stat5-dependent pathways.[74] IRF-8–deficient mice generate myeloid populations highly homologous to tumor-induced MDSCs with respect to their phenotype, function, and gene expression profile, whereas IRF-8 overexpression in mice attenuates MDSC accumulation.[74] These results implicate that IRF-8 may be a negative regulator of MDSC development and a potential molecular target for modulating MDSCs in patients.

**Smad3**

Using an allogeneic skin-graft mouse model, we discovered more $CD11b^{+}Gr\text{-}1^{+}$ myeloid cell infiltration and less T-cell infiltration in allografts occur in Smad3-deficient recipients.[33] Notably, the increased $CD11b^{+}Gr\text{-}1^{+}$myeloid cells in Smad3-deficient mice are immunosuppressive and responsible for the delayed allograft rejection, largely via an NO-dependent pathway.[33] Thus, our study pinpoints Smad3 as an intrinsic negative regulator that critically inhibits the differentiation and function of immunosuppressive $CD11b^{+}Gr\text{-}1^{+}$ MDSCs. However, whether Smad3 is activated by TGFβ or other factors in MDSCs should be studied in the future.

**Ras**

Ras can promote tumor growth by altering tumor microenvironment. In a pancreatic cancer-bearing mouse model, overexpression of constitutively active Kras leads to the induction of cytokines like MIP-2 and MCP-1, promoting the recruitment of macrophages and MDSCs into the tumor stroma to suppress antitumor immune response.[75] Similarly, in a Kras-driven lung cancer model, the immune response is attenuated by the presence of MDSCs in the tumor stoma.[76] Thus, the overexpression of

Ras in cancer cells, which facilitates MDSC recruitment and promotes tumor growth, may be an additional pathway that links Ras with tumor development.[77]

**Peroxisome proliferator-activated receptor-γ**

Peroxisome proliferator-activated receptor-γ (PPARγ) is an anti-inflammatory receptor. dnPPAR overexpression that leads to the upregulation of IL-1β, IL-6, and TNFα in the blood plasma. As a result, $CD11b^{+}Ly6G^{+}$ cells are systemically increased.[78] Myeloid cells can directly differentiate into MDSCs in PPARγC knockout mice.[78] Therefore, PPARγ plays a key role in controlling MDSC expansion and immunosuppression in myeloid-lineage cells.

**Src homology 2 domain-containing inositol 59-Phosphatase-1**

Src homology 2 domain-containing inositol 59-phosphatase-1 (SHIP-1) influences phosphatidylinositol-3-kinase (PI3K) signaling events, which regulate immune homeostasis. One study showed that myeloid-specific ablation of SHIP leads to the expansion of both MDSCs and Treg cell numbers, indicating a SHIP-dependent regulation of Treg cells by a myeloid cell type.[79] Meanwhile, G-CSF levels are profoundly increased in $SHIP^{-/-}$ mice, suggesting that this myelopoietic growth factor can promote MDSC expansion in a cell-extrinsic manner. Thus, SHIP controls MDSC numbers, in part, by limiting production of the myelopoietic growth factor G-CSF.[79] In addition, SHIP-1-regulated MDSC expansion and function may contribute to pancreatic tumor progression.[80]

**MyD88**

The activation of MyD88-NF-kB signaling is closely associated with MDSC expansion in infection and LPS shock models. Toll-like receptors (TLRs) and IL-1R can regulate MDSCs by activating NF-kB signaling.[13] TLR ligation upregulates the triggering receptor expression on myeloid cells via MyD88-activated NF-kB signaling.[81] MDSCs deficient in MyD88 develop into stimulatory cells.[82] Furthermore, $CD4^{+}$ T cells residing in tumor-draining lymph nodes of $MyD88^{-/-}$ mice secret more TNFα than those of wild-type mice. Finally, the blockade of MyD88 signaling with MyD88 inhibitory peptides during later tumor stages drastically restricts the growth of immunogenic tumors.[83] Overall, these data suggest that signaling through the MyD88 adaptor molecule is critical for the direct suppressive function of MDSCs, and blocking MyD88-mediated signaling in MDSCs might effectively inhibit the immunosuppressive function of MDSCs.

**Notch**

It is well known that Notch tightly regulates the progressive lineage commitment of haematopoietic stem cells.[84] Recently, it was demonstrated that the increased activity of casein kinase 2 (CK2) observed in MDSCs might be responsible for the phosphorylation of Notch and downregulation of Notch signaling. Moreover, inhibition of CK2 restores Notch signaling in myeloid cells and substantially restores their ability to differentiate both *in vitro* and *in vivo*.[85] Overall, this may prompt a new perspective on the pharmacological regulation of MDSC differentiation in cancer.

Based on the current studies, Stat, C/EBPβ, Flt3-Flt3L, PPARγ, SHIP, Ras, NF-kB, and Notch signaling pathways are inducers of MDSCs. On the other hand, IRF8 and Smad3 are negative regulators for the differentiation of MDSCs.

**Effector Molecules of MDSCs**

The underlying mechanisms of MDSC-mediated immune-regulation are clearly defined. In general, G-MDSCs primarily use ROS as the mechanism of immunosuppression. In contrast, M-MDSCs primarily use upregulation of iNOS, arginase, and an array of immunosuppressive cytokines to inhibit various immune functions (**Fig. 2**). Studies implicated that pathways involving arginase 1, iNOS, ROS, and induction of IDO are the major mediators of immunosuppression by MDSCs. $CD11b^{+}CD14^{-}CD15^{+}$ G-MDSCs with a polymorphonuclear granulocyte morphology and increased arginase activity in human cancer patients cause low levels of arginine and high levels of ornithine in the plasma as well as immunosuppression.[26] PMNs may induce arginase1-dependent immunosuppression through concomitant exocytosis of gelatinase and azurophil granules.[86] The important role of the arginase 1 and iNOS pathways, which has been thoroughly reviewed,[1,24,87,88] will be omitted from our discussion.

Repetitive injections of LPS efficiently induce $CD11b^{+}Gr\text{-}1^{+}$ MDSCs, which suppress T-cell proliferation and Th1 and Th2 cytokine production *in vitro*.[89] Adoptive transfer of these induced $CD11b^{+}$ MDSCs into untreated recipients significantly prolonged allo-skin-graft survival.[89] MDSCs induced with this protocol produce large amounts of IL-10 and highly express heme oxygenase-1 (HO-1), a stress-responsive enzyme with immunoregulatory and cytoprotective properties critically involved in the immunosuppressive ability in the allo-skin graft mouse model.[89] This study reveals that HO-1 is one of the key mediators of MDSC-associated suppression mechanism in transplantation.

$CD14^{+}HLA\text{-}DR^{-/low}$ MDSCs induced by the administration of GM-CSF-based vaccines in metastatic melanoma patients direct immunosuppression via a TGFβ-dependent pathway.[43] $CD14^{+}HLA\text{-}DR^{-/low}$ MDSCs, increased in the blood and tumor of hepatocellular carcinoma patients, have high arginase activity, are immunosuppressive, and induce $CD4^{+}CD25^{+}Foxp3^{+}$ Treg cells in co-culture with host $CD4^{+}$ T cells.[28] $CD11b^{+}Gr\text{-}1^{+}$ MDSCs of liver cancer-bearing mice can inhibit NK cell function and induce anergy of NK cells via membrane-bound TGF-β1.[37] In a model of chemically-induced colon carcinoma, either $Gr\text{-}1^{+}CD115^{+}$ or $Gr\text{-}1^{+}F4/80^{+}$ M-MDSCs isolated from the bone marrow can inhibit T-cell immune response via inducible nitric oxide synthase 2 (NOS2).[15] These MDSCs are also able to trigger $CD25^{+}$ Treg cell induction *in vivo* by the secretion of IL-10 and TGF-β.[15] It was reported that $Gr\text{-}1^{+}CD11b^{+}$ MDSCs in a sepsis mouse model contribute to poor T-cell response and preferential Th2 polarization through IL-10.[13] In addition, TGF-β can regulate MDSC function indirectly by altering microRNA expression. In

tumor bearing mice, TGFβ can regulate MDSC proliferation by inducing miRNA494 expression. Deletion of miRNA494 in MDSCs enhances tumor growth and metastasis.[90]

In mice bearing GM-CSF–transduced C26 colon carcinoma, circulating $CD11b^{+}IL\text{-}4R\alpha^{+}$ monocytes produce IL-13 and IFNγ to trigger the molecular pathways that inhibit antigen-activated $CD8^{+}$ T cells.[10] It was found that i.p. injection of Lacto-N-fucopentaose III (LNFPIII)-dextran results in rapid expansion of the subpopulation of $Gr\text{-}1^{+}F4/80^{+}CD11b^{+}$ peritoneal cells, which can suppress anti-CD3/CD28-induced proliferation of naive $CD4^{+}$ T cells. Addition of the iNOS inhibitor N(G)-monomethyl-L-arginine and anti-IFNγ antibody restore the ability of $CD4^{+}$ T cells to proliferate in vitro, indicating that these induced MDSCs mediated immunosuppression through NO- and IFNγ-dependent manners.[42]

Induction of IDO, the rate-limiting enzyme in tryptophan degradation in the kynurenine pathway, acts as a potent immunoregulatory loop by MDSCs. Compared to healthy donor cells, $CD14^{+}HLA\text{-}DR^{-/low}$ MDSCs in allogeneic haematopoietic stem cell transplanted patients are $pStat1^{low}$ and $IDO^{high}$.[91] Importantly, dysfunction of the patients' T cells including reduced proliferative capacity and CD3ζchain expression is rescued by blocking IDO activity in $CD14^{+}HLA\text{-}DR^{-/low}$ MDSCs.[91] It was reported that the inhibitory effects of tumor-induced G-MDSCs on Treg differentiation from naive $CD4^{+}$ T cells depend on ROS and IDO but not arginase 1, iNOS, NO, cystine/cysteine depletion, PD-1/PD-L1, or COX-2.[92] IDO expression is significantly upregulated in $CD45^{+}CD13^{+}CD33^{+}CD14^{-}CD15^{-}$ MDSCs in primary breast cancer tissues and the patients' peripheral blood.[31] Further studies showed that these MDSCs inhibit IL-2 and anti-CD3/CD28 monoclonal antibody (mAb)-induced T-cell proliferation and Th1 polarization in an IDO-dependent manner.[31] Thus, blocking IDO to reverse MDSC-induced T-cell suppression may represent a novel approach for cancer immunotherapy.

Cysteine is an essential amino acid for T-cell activation because T cells lack cystathionase, which converts methionine into cysteine. MDSCs express the xc-transporter to import cystine but not the ASC transporter to export cysteine. Thus, MDSCs compete with APCs for extracellular cystine and limit the extracellular pool of cysteine, thereby depriving T cells of the cysteine they require for activation and function. This study presents a new mechanism for the MDSC-mediated immunosuppression of T cells, namely the deprivation of essential amino acids in their microenvironment.[93]

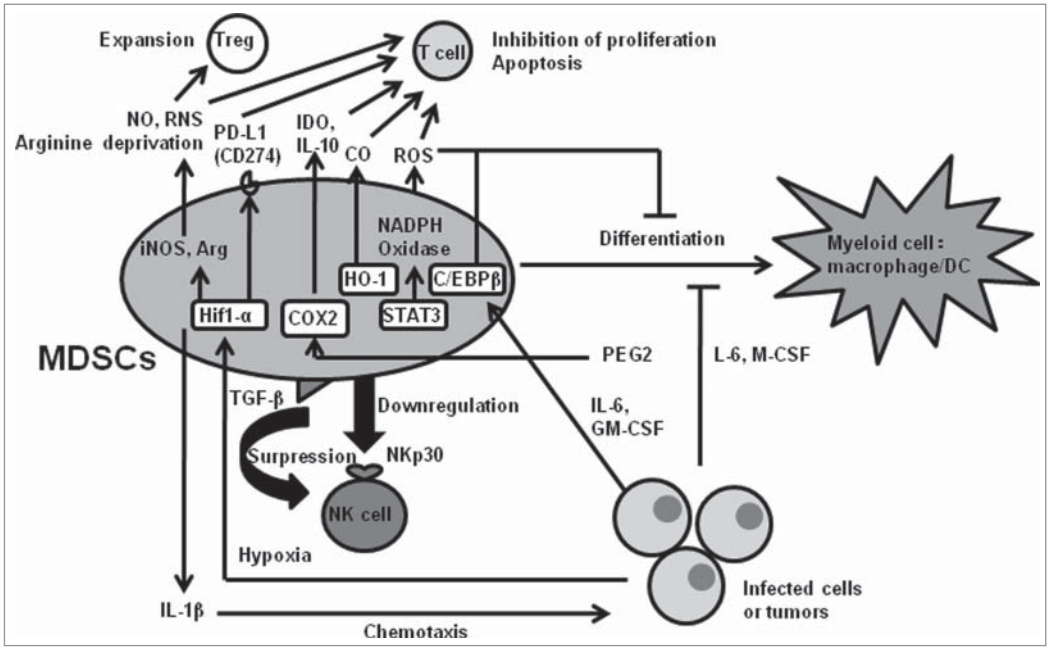


**Figure 2.** Molecules that mediate MDSC immunosuppressive ability. MDSCs suppress T cells, NK cells, and the differentiation of myeloid cells via different mechanisms. The classic mechanism for MDSCs to inhibit T-cell proliferation and promoting Treg cell expansion is through arginine deprivation, nutrition depletion, or high levels of NO production to inhibit T-cell function. Upregulation of HIF1-α expression under hypoxic conditions can enhance the inhibitory function of MDSCs via the mentioned pathways. Signal transducer and activator of transcription 3 (Stat3), a key factor in MDSCs, can increase ROS production by MDSCs, which inhibits T-cell proliferation and myeloid differentiation. In addition, infected cells or tumors can produce GM-CSF and IL-6, both of which increase C/EBPβ expression and promote MDSC differentiation. Furthermore, PGE2, IL-6, and M-CSF from inflammatory cells can produce IDO, IL-10, and CO via regulating COX2 and HO-1 to prevent T-cell proliferation and myeloid cell differentiation. However, MDSC-mediated NK cell inhibition occurs by TGFβ or downregulation of the NK–cell-activating receptor, Nkp30. IL, interleukin; M-CSF, macrophage colony-stimulating factor; PGE2, prostaglandin E2; COX-2, cyclooxygenase-2; IDO, indoleamine 2,3-dioxygenase; HIF-1α, hypoxia-inducible factor-1α; NOS, nitric oxide synthase; Stat3, signal transducer and activator of transcription 3; ROS, reactive oxygen species; TGFβ, transforming growth factor-β.

The inhibitory role of G-MDSCs on TCRζ chain expression and cytokine production by T cells is abrogated by the addition of a hydrogen peroxide ($H_2O_2$) scavenger, catalase.[25] This implicates that G-MDSC–derived $H_2O_2$ is a major effector molecule in the severe systemic T-cell suppression in tumor patients. Antigen-specific $CD8^{+}$ T-cell tolerance, induced by MDSCs, is 1 of the main mechanisms of tumor escape. Using *in vivo* models, Kusmartsev et al. showed that MDSCs directly disrupt the binding of antigen peptide–MHC dimers to $CD8^{+}$ T cells through nitration of tyrosines in the TCR–CD8 complex caused by the hyperproduction of ROS and peroxynitrite during direct cell–cell contact.[36] These molecular changes might affect the conformational flexibility of TCR-CD8 and its interaction with peptide-MHC dimers,

eventually inducing T-cell anergy and tolerance.[36] These findings identify a new mechanism of T-cell tolerance associated with the accumulation of MDSCs in cancer and other pathological microenvironments.

Activated T cells are sensitive to Fas-mediated apoptosis, which is important in the regulation of immune responses and maintenance of self-tolerance. However, the immunosuppressive effect of MDSCs on $CD8^+$ T cells is not dependent on the FasR (CD95)-FasL pathway in mice immunized with viral immunogens.[34] On the other hand, MDSCs likely downregulate L-selectin on naive T cells through their plasma membrane expression of ADAM17 (a disintegrin and metalloproteinase domain 17), an enzyme that cleaves the ectodomain of L-selectin.[94] Therefore, this study presents another process in which MDSCs inhibit immunity by downregulating L-selectin on T cells and disrupting T-cell homing to lymph nodes and sites of inflammation.

All in all, recent studies have identified several new mechanisms of MDSC-mediated immunosuppression. These include upregulation of COX-2 and PGE2, production of TGFβ, depletion of cystein, and downregulation of L-selectin expression on T cells and induction of Tregs. It is apparent that the immunosuppressive function of MDSCs is highly pleiotropic and the associated pathways are highly dependent on the microenvironment. Understanding the immunosuppressive mechanisms of MDSCs will be essential to designing effective immunotherapies.

## Conclusion

Numerous factors like LPS, M-CSF, GM-CSF, SCF, IL-6, IFNγ, IL-1β, VEGF, Hsp72, IL-13, C5a, and PGE2 induce MDSC differentiation, while IL-4 and ATRA inhibit this process. Stat, C/EBPβ, Flt3-Flt3L、PPARγ, SHIP, Ras, NF-kB, COX-2, and Notch signaling pathways promote MDSC differentiation and function, whereas IRF-8 and Smad3 downregulate MDSC activity. The immunosuppressive function of MDSCs is mainly mediated by NO, arginase, ROS, TGFβ, IL-10, IDO, HO-1, CO, and PGE2 as well as depletion of cysteine. It is clear that we have limited knowledge of MDSCs and their biological function. Comprehensive classification of MDSC subpopulations may be essential to avoiding confusion and inconsistency, as the current MDSCs are extremely heterogeneous. Understanding the molecular regulatory networks of MDSC development and function may offer novel therapeutic approaches for patients with cancer, chronic infection, autoimmune disease, and transplantation.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.


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